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*7,809 volunteer abstracts, 18 symposium and workshop abstracts.

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CHRONOLOGICAL LIST OF SESSIONS

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TUESDAY

THURSDAY

Thematic List of Sessions

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311.	Differentiation and development V	Slide	Wed PM
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414.	Differentiation and development VII	Poster	Thu AM
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401.	Motor systems I	Poster	Thu AM
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55.	Ion channels: calcium channels II	Poster	Mon AM
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440.	Presynaptic mechanisms: modulators	Poster	Thu PM
30.	Presynaptic mechanisms: toxins	Poster	Mon AM
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479.	Transmitter uptake, storage, secretion and metabolism III	Slide	Fri AM
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392.	Cardiovascular regulation VII	Poster	Thu AM
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177.	Hypothalamic-pituitary-gonadal regulation II	Poster	Tue AM
431.	Hypothalamic-pituitary-gonadal regulation III	Slide	Thu PM
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93.	Neural control of immune system I	Poster	Mon PM
304.	Neural control of immune system II	Slide	Wed PM
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256.	Neuroendocrine controls: pituitary III	Poster	Wed AM
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201.	Sensory systems: auditory systems III	Poster	Tue PM

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264.	Sensory systems: auditory systems V	Poster	Wed AM
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286.	Somatosensory cortex II	Poster	Wed AM
306.	Somatosensory cortex III	Slide	Wed PM
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497.	Motor systems and sensorimotor integration: cerebellum III	Poster	Fri AM
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208.	Motor systems and sensorimotor integration: cortex III	Poster	Tue PM
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320.	Motor systems and sensorimotor integration: oculomotor system II	Poster	Wed PM
386.	Motor systems and sensorimotor integration: oculomotor system III	Poster	Thu AM
387.	Motor systems and sensorimotor integration: oculomotor system IV	Poster	Thu AM
28.	Motor systems and sensorimotor integration: posture and movement I	Poster	Mon AM
29.	Motor systems and sensorimotor integration: posture and movement II	Poster	Mon AM

105.	Motor systems and sensorimotor integration: posture and movement III	Poster	Mon PM
106.	Motor systems and sensorimotor integration: posture and movement IV	Poster	Mon PM
192.	Motor systems and sensorimotor integration: posture and movement V	Slide	Tue PM
384.	Motor systems and sensorimotor integration: posture and movement VI	Poster	Thu AM
385.	Motor systems and sensorimotor integration: posture and movement VII	Poster	Thu AM
496.	Motor systems and sensorimotor integration: posture and movement VIII	Poster	Fri AM
74.	Motor systems and sensorimotor integration: vestibular system I	Slide	Mon PM
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138.	Motor systems and sensorimotor integration: vestibular system III	Poster	Tue AM
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427.	Spinal cord and brainstem	Slide	Thu PM
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139.	Spinal cord and brainstem: immunocytochemistry	Poster	Tue AM
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472.	Brainstem systems	Poster	Thu PM
23.	Cerebral metabolism and blood flow I	Poster	Mon AM
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399.	Cerebral metabolism and blood flow III	Poster	Thu AM
488.	Cerebral metabolism and blood flow IV	Slide	Fri AM
26.	Comparative neuroanatomy: amphibians, reptiles, birds	Poster	Mon AM
504.	Comparative neuroanatomy: cerebral cortex	Poster	Fri AM
25.	Comparative neuroanatomy: fish	Poster	Mon AM
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100.	Hippocampus and amygdala II	Poster	Mon PM
343.	Hippocampus and amygdala III	Poster	Wed PM
470.	Hypothalamus I	Poster	Thu PM
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181.	**New Insights into the Functions of the Basal Forebrain Cholinergic System**	**Symp.**	**Tue PM**

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Session Number	Session Title	Type	Day and Time
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281.	Alcohol III	Poster	Wed AM

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22.	Psychotherapeutic drugs I	Poster	Mon AM
87.	Psychotherapeutic drugs II	Poster	Mon PM
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259.	Alzheimer's disease: amyloid	Poster	Wed AM
437.	Alzheimer's disease: neuropathology	Poster	Thu PM
361.	Alzheimer's disease: protein	Slide	Thu AM
493.	Alzheimer's disease: transmitters	Poster	Fri AM
505.	Behavioral disorders	Poster	Fri AM
80.	Cerebral ischemia I	Poster	Mon PM
205.	Cerebral ischemia II	Poster	Tue PM
327.	Cerebral ischemia III	Poster	Wed PM
398.	Cerebral ischemia IV	Poster	Thu AM
429.	Cerebral ischemia V	Slide	Thu PM
503.	Cerebral ischemia VI	Poster	Fri AM
141.	Clinical CNS neurophysiology I	Poster	Tue AM
312.	Clinical CNS neurophysiology II	Poster	Wed PM
7.	Degenerative disease: Parkinson's	Slide	Mon AM
525.	Degenerative disease: Parkinson's (nonprimates)	Poster	Fri AM
157.	Degenerative disease: Parkinson's (primates)	Poster	Tue AM
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526.	Degenerative disease: other II	Poster	Fri AM
468.	Developmental disorders	Poster	Thu PM
6.	Epilepsy I	Slide	Mon AM
102.	Epilepsy II	Poster	Mon PM
194.	Epilepsy III	Slide	Tue PM
345.	Epilepsy IV	Poster	Wed PM
412.	Epilepsy: GABA and benzodiazepines	Poster	Thu AM
227.	Epilepsy: hippocampus and neocortex I	Poster	Tue PM
351.	Epilepsy: hippocampus and neocortex II	Poster	Wed PM
411.	Epilepsy: peptides	Poster	Thu AM
228.	Epilepsy: second messengers and mRNA	Poster	Tue PM
459.	Epilepsy: substantia nigra and amygdala	Poster	Thu PM
195.	Genetic models I	Slide	Tue PM
332.	Genetic models II	Poster	Wed PM
520.	Infectious diseases	Poster	Fri AM
273.	Neuromuscular diseases	Poster	Wed AM
135.	Neurotoxicity I	Slide	Tue AM
313.	Neurotoxicity II	Poster	Wed PM
436.	Neurotoxicity III	Poster	Thu PM
491.	Neurotoxicity IV	Poster	Fri AM
492.	Neurotoxicity V	Poster	Fri AM
357.	**New Genes from Old Diseases**	**Symp.**	**Thu AM**
253.	Trauma I	Slide	Wed AM
460.	Trauma II	Poster	Thu PM

2

SYMPOSIUM: INTERLEUKIN-1: IMMUNE AND NEURAL MODULATOR. D. Felten, Univ. of Rochester, (Chairperson), C.A. Dinarello*, Tufts Univ., C. Blatteis, Univ. of Tenn., C. River, Salk Inst., C.B. Saper, Univ. of Chicago, M. Schultzberg, Univ. of Stockholm.

Interleukin-1 (IL-1) is a lymphokine that is secreted by monocytes and macrophages during inflammation, resulting in a variety of neurally mediated responses including fever, sleepiness, and corticosteroid secretion. This symposium will explore the recent advances in understanding the role of IL-1 in modulating interactions between the immune and nervous system.

D. Felten will address the field of immune-neural interactions, and place the role of IL-1 into perspective. C.A. Dinarello will then describe the basic cellular and molecular biology of IL-1.

C. Blatteis will describe the effects of IL-1 on hypothalamic neurons involved in thermoregulation and sleep. C. Rivier will focus on the effects of IL-1 on neuroendocrine function in the hypothalamus.

The final two speakers will examine the possibility that IL-1 may serve as a neuromodulator as well as a circulating hormone. C.B. Saper will describe the organization of IL-1β immunoreactive neurons in the central nervous system, and M. Schultzberg will address the distribution of IL-1α immunoreactivity in the peripheral nervous system.

The twin roles of IL-1 as a circulating hormone and as a neuromodulator underscore the close coordination of immune and neural function. The dual role for this peptide may reflect a general organizing principle of the nervous system.

3

WORKSHOP. FUNCTIONAL PROPERTIES OF SOMATIC SENSORY "BARREL FIELD" CORTEX. F.F. Ebner, Brown Univ (Chairperson); B.W. Connors, Brown Univ; M. Armstrong-James*, Univ of London; D.J. Simons, Univ of Pittsburgh; R.B. Masterton, Florida State Univ.

The "barrel field" region of SI cortex that receives sensory information from the contralateral mystacial vibrissae has emerged as an important model system for studying 1) normal mechanisms of cortical function, 2) the effects of sensory deprivation and 3) the response of cortex to central and peripheral injury. This workshop will provide a forum for discussion of recent physiological and behavioral results on barrel field cortex.

Dr. Connors will describe techniques for the study of the barrel thalamocortical system *in vitro*. He will discuss thalamic activation of cortical circuits as well as the mechanisms and functions of multiple forms of intrinsic GABAergic inhibition. Dr. Armstrong-James will discuss the receptive field organization of barrel field neurons, comparing 1) features that depend upon thalamic inputs with those dependent upon cortical circuitry and 2) the responses of cells in the barrel to those of cells in the septa. In addition, he will explore the role of NMDA-type amino acid receptor mechanisms in regulating cortical cell excitability. Dr. Simons will discuss the thalamocortical response transformation in normal and neonatally sensory deprived animals: the anatomical relationship of a thalamic barreloid with its corresponding cortical barrel, the role of GABAergic neurons in shaping the spatial and temporal aspects of receptive field properties and the effect of sensory deprivation on the development of these properties. Dr. Ebner will discuss the response of barrel field cortex to direct injury and/or infraorbital nerve damage; whether such lesions affect the innervation and subsequent sensory response properties of embryonic neurons implanted into the cortical lesion site. Dr. Masterton will illustrate the distinction between barrel field-dependent and -independent vibrissal discriminations. He will summarize recent results on the agnosia-like deficits produced by aspiration of barrels and their use as a behavioral assay of barrel field function. An open panel discussion of issues raised during the sessions will follow the last presentation.

LEARNING AND MEMORY: ANATOMY I

4.1

LAYER IV ENTORHINAL PATHOLOGY DISRUPTS HIPPOCAMPAL-CORTICAL FEEDBACK IN ALZHEIMER'S DISEASE. W.G. Tourtellotte, G.W. Van Hoesen, B.T. Hyman and A.R. Damasio. Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242.

In Alzheimer's disease (AD) laminar specific neurofibrillary tangles are a consistent feature in the entorhinal cortex (EC) of the parahippocampal gyrus. In addition to layer II, the pyramidal neurons of layer IV (nomenclature of Lorente de Nó, 1933) are selectively affected. Little is known regarding the extent of their involvement or the functional relationships they have with hippocampal processing. Neurons in this region were examined for disease related involvement in 18 AD cases and 5 age compatible nondemented controls utilizing thioflavin S to detect neurofibrillary tangles (NFT) and Alz-50 to detect AD related immunoreactivity. In all cases studied, these neurons were extensively involved throughout the rostral-caudal axis of the EC. In addition, Alz-50 neuropil staining in the layer IV zone was noted in many cases. This was particularly prominent in cases with extensive subicular and CA1 involvement and suggest strongly that the subicular-entorhinal pathway may be compromised as well.

To highlight some of the connections established by these neurons, 6 rhesus monkeys were injected with neural tracing compounds. One case injected with ^{3}H leucine/lysine highlights the subicular-entorhinal pathway terminating in layer IV. Retrograde fluorescent tracers demonstrate direct projections from layer IV to rostral superior temporal, anterior cingulate, and posterior parahippocampal gyri as well as the temporal polar cortex. Labeled neurons were topographically distributed within the EC with very few double labeled in those cases receiving two tracers. Finally, a retrograde tracer placed in the superficial layers of the EC demonstrates intrinsic transport to layer IV.

These data demonstrate extensive involvement of layer IV neurons in AD and experimental studies demonstrate projections to some unimodal and multimodal association areas as well as an intrinsic entorhinal pathway. It is expected that all may be compromised in AD. Focal pathology of this nature may be devastating for hippocampal feedback to higher order association areas. (Supported by NS 14944, PO NS 19632, and the Mathers Foundation.)

4.2

LIMBIC CORTICAL PROJECTIONS TO SENSORY AND MULTI-MODAL ASSOCIATION AREAS IN THE OLD-WORLD MONKEY. S.E. Arnold*, D.R. Brady, G.W. Van Hoesen, B.T. Hyman and A.R. Damasio. Depts. of Neurology and Anatomy, Univ. of Iowa, Iowa City, IA 52242.

It is well-known that the entorhinal cortex (EC) is a convergence zone for limbic and association cortex projections that can influence the hippocampal formation via the perforant pathway. Less well-appreciated is the fact that hippocampal output, from the subiculum and CA1 zone is directed toward layer IV of the EC (nomenclature of Lorente de Nó, 1933) and that neurons of this layer project widely to cingulate, temporal polar, perirhinal and posterior parahippocampal cortices. We have investigated the cortical projections of temporal cortical areas that receive direct projections from layer IV of the EC using autoradiographic methodology. Although topographical differences exist, the cortical projection of these areas are remarkably similar, and two or more project to the following cortical areas: medial frontal; orbitofrontal; anterior cingulate; anterior insular; superior temporal; inferior temporal; and inferior peristriate. The posterior parahippocampal cortex projects in addition to the inferior parietal and dorsolateral prefrontal cortices. Together these connections form a host of cortical neural systems via which hippocampal-entorhinal output feeds back to widespread limbic and association cortices. (Supported by NS 14944 and PO NS 19632.)

4.3

LONG-TERM EFFECTS OF NEONATAL TEMPORAL CORTICAL AND LIMBIC LESIONS ON HABIT AND MEMORY FORMATION IN RHESUS MONKEYS. J. Bachevalier and M. Mishkin. Lab. Neuropsychol., NIMH, Bethesda, MD 20892.

Assessment of the effects of neonatal damage to temporal cortical area TE and limbic structures on the early development of habits and memories pointed to greater compensatory potential after the neonatal cortical removals (Bachevalier and Mishkin, Soc. Neurosci. 12:22, 1986). To determine the long-term effects of these early lesions, we retested 3 animals with neonatal area TE lesions and 3 others with neonatal limbic lesions at the age of 4 years on the same behavioral tasks, i.e. object discrimination learning with 24-hr ITIs to measure habit formation, and delayed nonmatching-to-sample with trial-unique objects to measure memory formation. The performance of the animals was compared to that of 4-year-olds that received the same lesions in adulthood. At 4 years of age, neonatal damage to area TE yielded a significant sparing of both habit and memory formation that was of the same magnitude as that found earlier when the animals were 3 to 12 months of age. By contrast, at 4 years of age, both neonatal and adult limbic lesions left habit formation intact but severely impaired memory formation. Apparently compensatory mechanisms operate early to promote permanent recovery from neonatal temporal cortical lesions but not from neonatal limbic lesions.

4.4

PLASTICITY OF VISUAL MEMORY CIRCUITS IN DEVELOPING MONKEYS. M.J. Webster, J. Bachevalier, and L.G. Ungerleider. Lab Neuropsychol., NIMH, Bethesda, MD 20892.

Object recognition, as measured by delayed nonmatching-to-sample, is severely impaired in monkeys that receive lesions of temporal cortical area TE as adults but not in those that receive the same lesions as infants. This sparing of function in animals with neonatal lesions appears to be permanent since such animals were found to perform well not only at one but also at four years postsurgically. Because object recognition normally depends on interactions between area TE and limbic structures, we investigated whether other visual cortical areas connected with limbic structures but not necessary for object recognition become necessary if area TE is removed in infancy. Thus, areas PG, TF, TEO, and STP were ablated in a 2-year-old rhesus monkey that had undergone neonatal area TE removal and had shown sustained recovery of object recognition. Following the secondary removals, the monkey demonstrated significant impairment, which became progressively greater with increasing delays and list lengths. Apparently, one or more areas removed in the secondary lesion had assumed a critical role in object recognition as a result of the neonatal TE lesion. Presumably, such compensation is possible because visual cortical areas are not yet fully mature at birth and possess significant functional plasticity.

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

4.5

FUNCTIONAL MATURATION OF INFERIOR TEMPORAL CORTEX IN INFANT RHESUS MONKEYS. C. Hagger, J. Bachevalier, K.A. Macko*, C. Kennedy, L. Sokoloff, and M. Mishkin. Lab. of Neuropsychology, and Lab. of Cerebral Metabolism, NIMH, Bethesda, MD 20892.

Although lesions of area TE in adults yield severe impairment in visual discrimination learning and recognition memory, the same lesions in neonates result in significant sparing of function, suggesting that area TE is still immature early in life (Bachevalier and Mishkin, Soc. Neurosci., 12:22, 1986). Support for this conclusion was provided by tracing the functional development of area TE by use of the 2-deoxyglucose method. Before local cerebral glucose utilization (LCGU) was measured, eight infant monkeys were subjected to unilateral optic tract section and forebrain commissurotomy at 1 day, 1 week, 3 weeks, and 1, 2, 3, and 5 months of age. The weighted average of LCGU for area TE was calculated by means of a computerized image-processing system. Differences in rates of glucose utilization between the "seeing" and "blind" hemispheres were small until 3 months of age and reached adult levels only at about 6 months. These results also help account for the recent finding that electrophysiological activity in area TE does not show the adult pattern before about 4 to 6 months of age (Rodman et al., Soc. Neurosci., 14, 1988).

4.6

DEVELOPMENTAL TIME COURSE AS WELL AS NATURE OF SOCIO-EMOTIONAL DISTURBANCES IN RHESUS MONKEY FOLLOWING NEONATAL LIMBIC LESIONS RESEMBLE THOSE IN AUTISM. P. M. Merjanian, J. Bachevalier, K. D. Pettigrew, and M. Mishkin. NIMH, Bethesda, MD 20892.

To investigate further the development of socio-emotional disturbances in animals with limbic lesions, we analyzed behavior at 2 months of age and compared it with a previously reported analysis of behavior at 6 months of age in the same group of 18 monkeys (Soc. Neurosci. Abstr., 12, 1986). Six monkeys had received combined amygdalo-hippocampal lesions (AH) neonatally, 6 were normals (N/AH) reared with monkeys from Group AH and 6 were normals reared with other normals (N/N). At both ages, each animal from Group AH was paired in a play cage with its age-matched control (N/AH), and their behavior was observed for 12, 5-minute intervals on 6 days. Similarly, each N/N was paired with another N/N. At 2 months only, the animals of Group AH had more temper tantrums when first placed in the novel play cage, showed more passive behavior, and manipulated objects less than the controls. On the other hand, Group AH animals at 2 months of age did not display the more striking pathology they exibited at 6 months, namely, lack of social contact, extreme submissiveness including active withdrawal, and gross motor stereotypies. The developmental time course and the nature of these disturbances in animals with limbic lesions resemble those seen in autism.

4.7

ROLE OF THE AMYGDALA AND HIPPOCAMPUS IN VISUAL-VISUAL ASSOCIATIVE MEMORY IN RHESUS MONKEYS. E.A. Murray, D. Gaffan, and M. Mishkin, Lab. of Neuropsychology, NIMH, Bethesda, MD 20892, and Dept. of Expt. Psychology, Oxford University, Oxford OX1 3UD, UK.

Naive normal monkeys (Macaca mulatta) were trained in an automated apparatus at the rate of 100 trials per day to associate in memory a set of 10 pairs of visual stimuli, i.e. 10 paired associates. Each trial began with the presentation in the middle of a TV screen of a single visual stimulus which disappeared when the monkey touched it. 0.5 s later, two other stimuli appeared on the sides of the screen, one the paired associate, and the other a member of a different pair. The monkey could obtain a food reward only by touching the paired associate. Following training to a criterion of 3 consecutive days of 90% correct responses, the monkeys received bilateral amygdalectomy (A), hippocampectomy (H), or both (AH), or were left unoperated (C), and were then retrained. Groups C and H relearned almost immediately ($\bar{X} \doteq 80$ errors each), Group A was retarded in relearning ($\bar{X} \doteq 800$ errors), whereas Group AH did not relearn within the training limit of 5000 trials ($\bar{X} \doteq 2000$ errors). The results suggest that the retrieval of recently learned visual-visual associations as well as their reacquisition are mediated by both the amygdala and the hippocampus.

4.8

PERFORMANCE ON A NONSPATIAL SELF-ORDERED TASK AFTER SELECTIVE LESIONS OF THE PRIMATE FRONTAL CORTEX. M.Petrides.Dept.Psychology and Montreal Neurological Institute,McGill Univ.,Montreal, Quebec,Canada.

It has been shown that patients with damage to the frontal cortex are impaired on tasks in which they have to monitor the execution of a series of self-initiated responses (Petrides and Milner, Neuropsychologia,20:249,1982). The frontal cortex is a structurally heterogeneous region of the brain. To establish the critical area within the dorsolateral frontal cortex for the performance of such responses,a nonspatial self-ordered task modelled on those that had been used with the patients was developed for monkeys. Nine monkeys (macaca nemestrina) were trained on this task. Three monkeys were subjected to a bilateral excision of the dorsolateral frontal cortex lying within,around and dorsal to the sulcus principalis (areas 46 and 9),three monkeys had the posterior dorsolateral frontal cortex within and around the arcuate sulcus removed (area 8 and rostral area 6),and three animals served as unoperated controls. Postoperatively,the monkeys with excisions of areas 46 and 9 were impaired,whereas those with lesions of areas 6 and 8 performed as well as the normal control animals.

4.9

MNEMONIC CAPACITY IN SPLIT-BRAIN MACAQUES. J.D. Lewine and R.W. Doty, U. of Rochester, Roch., N.Y.

Two macaques, each with transected optic chiasma and forebrain commissures, were tested on a probe recognition task. When required to determine if a monocularly viewed probe image is a member of a previously viewed target list, for which all targets were presented to the probed eye and hemisphere, the accuracy of the evaluation decreases as a function of the number of initial targets. For example, animal ART, working with left eye and hemisphere and right hand, performs at 90% with 2 targets, 83% with 4 targets, and 77% with 6 targets. As expected, ART performs at chance levels when classifying monocular probes on the basis of target information initially presented to only the contralateral hemisphere. For all animals, when the target list is divided between the hemispheres, the accuracy of monocular probe evaluations still reflects the total memory-load rather than the load of the probed hemisphere (e.g., When ART is given 2 targets to the left hemisphere, 4 to the right, and probed on the left with a positive probe matching one of the left hemisphere's targets, performance is only 75% rather than 90%). Thus, while the performance deficit caused by an increase in memory-load cannot be attributed to direct interference between memory traces, transection of the commissures is not sufficient to completely dissociate the mnemonic systems of the two hemispheres. The data suggest that performance depends upon a limited resource, allocated by the intact brainstem.

4.10

DOUBLE-DISSOCIATION OF EGOCENTRIC AND ALLOCENTRIC SPACE FOLLOWING MEDIAL PREFRONTAL AND PARIETAL CORTEX LESIONS IN THE RAT. R.P. Kesner, Dept. of Psychology, University of Utah, Salt Lake City, Utah 84112.

Animals with medial prefrontal cortex, parietal cortex lesions, or sham-operated and nonoperated controls were tested for the acquisition of an adjacent arm tas that accentuated the importance of egocentric spatial localization and a cheese board task that accentuated the importance of allocentric spatial localization. Results indicated that relative to controls and animals with parietal cortex lesions, animals with medial prefrontal cortex lesions are impaired on the adjacent arm task, but displayed facilitation on the cheese board task. In contrast, relative to controls and animals with medial prefrontal cortex lesions, rats with parietal cortex lesions are impaired on the cheese board task, but show no impairment on the adjacent arm task. The data suggest a double dissociation of function between medial prefrontal cortex and parietal cortex in terms of coding of egocentric versus allocentric spatial information.

4.11

REFLEX MODIFICATION (RM) AND CLASSICAL CONDITIONING OF THE RABBIT'S NICTITATING MEMBRANE RESPONSE (NMR) TO ELECTRICAL STIMULATION OF BRAINSTEM STRUCTURES AS AN UNCONDITIONED STIMULUS (UCS). A. J. Nowak and I. Gormezano, Dept. of Psychology, Univ. of Iowa, Iowa City, Iowa 52242.

An impressive body of research has accumulated indicating that the cerebellum may contain the neural circuitry necessary for NMR conditioning. Nevertheless, the possibility that the brainstem may also contain the circuitry sufficient for NMR conditioning has not been eliminated from consideration. The present study employed electrical brain stimulation (EBS) of the pars oralis of the spinal trigeminal nucleus (TRIG), accessory abducens nucleus, abducens nucleus and reticular formation to determine their role in elicitation, RM (i.e., alteration of UCRs by a preceding neutral stimulus), and classical conditioning of the NMR. The major findings were that robust and significant levels of RM and CR acquisition occurred with EBS of TRIG but there were no significant effects of EBS upon RM or CR acquisition at each of the other three sites. These results indicate a convergence of CS and UCS pathways at TRIG and are in agreement with the well-documented findings that CS-UCS interactions occur at sensory nuclei. However, whether the convergence at TRIG reflects neural plasticity at this brainstem structure or at sites efferent to TRIG (e.g., inferior olive, cerebellum, red nucleus) remains to be determined. (Supported by NSF Grant BNS-8419772 to I.G.)

4.12

LOSS OF CONDITIONED RESPONSES FOLLOWING CEREBELLAR CORTICAL LESIONS IS NOT A PERFORMANCE DEFICIT.
C.H. Yeo and M.J. Hardiman. Dept. Anatomy, University College London, London WC1E 7JG, U.K.

Lesions of cerebellar cortical lobule HVI (Yeo et al Behav. Brain Re. 13:261,1984) or cerebellar deep nuclei (McCormick and Thompson, Science 223:296,1984) abolish classically conditioned nictitating membrane responses (NMR) in rabbits. Welsh and Harvey have recently reported deficits in the unconditioned NMR to airpuff. Loss of conditioned responses may be due to a general performance deficit.

Here we made aspiration lesions of cerebellar cortex. NMR conditioning to light and white noise conditioned stimuli (CS) was abolished and there was no sustained reacquisition within 6000 trials over 30 sessions though some responses were emitted erratically in some sessions. Unpaired presentation of the CS did not reveal any extra long latency responses. In contrast to nuclear lesions, these cortical lesions elevated NMR amplitudes and reduced response thresholds to periorbital stimulation, consistent with a loss of cortical inhibition upon the deep nuclei.

We may conclude that loss of conditioned responses is not due to a simple performance deficit. The cerebellar cortex may be crucial for the formation of conditioned responses, their expression, or both.
(SPON:EBBS)

TRANSPLANTATION FOR MOVEMENT DISORDERS

5.1

THE EFFECT OF INTRAVENTRICULAR DA INFUSION ON ROTATION AND STRIATAL BIOCHEMISTRY IN THE RAT. L.C. Kao, P.M. Carvey, J. Kroin*, T.J. Zhang* R. Penn and H.L. Klawans*. Neuropharmacology Research Laboratories. Rush University, Chicago,IL 60612.

It has been assumed that autologous adrenal medulla transplants will secrete significant quantities of DA, and therefore have therapeutic benefit in the treatment of Parkinson's Disease (PD). To examine this hypothesis we evaluated the effect of intraventricular (VENT) or intrastriatal (STR) DA infusion for 10 days in animals that had unilateral 6-OHDA lesions of the medial forebrain bundle (MFB) and compared them with animals with MFB lesions only (UNI).

18 animals with stable contralateral rotation rates for 1 month (apomorphine 0.2 mg/kg) were assigned to 1 of the 3 treatment groups. Cannulae were placed in the striatum or the lateral ventricle ipsilateral to the lesion and connected to 14 day minipumps delivering 10 mcg DA/hr. In contrast to UNI and VENT animals, STR animals exhibited spontaneous contralateral rotations which gradually diminished over the 4 days following implantation. After 7 days UNI and VENT animals did not exhibit significant reductions in apomorphine-induced rotation rate while STR animals exhibited a 58% reduction. After 10 days all animals were sacrificed and the brains frozen in liquid freon within 90 secs of sacrifice. HPLC analysis of 1 mm striatal punches revealed that DA on the ipsilateral side was only able to diffuse a distance of approximately 1 mm in either STR or VENT animals and in neither group was contralateral DA content elevated in any striatal punch. These results strongly suggest that striatal tissue acts as a barrier to DA penetration and that mechanisms other than medullary DA release must be responsible for the clinical efficacy observed in patients following medullary transplants.

5.2

THE EFFECT OF INTRAVENTRICULAR ADRENAL MEDULLA TRANSPLANTS INTO INTACT RATS. P.M. Carvey, J. Kroin*, L.C. Kao, R. Singh*,T.J. Zhang*, R. Penn and H.L. Klawans*. Neuropharmacology Research Labs. Rush U. Chicago,IL 60612

Rat-pup adrenal medulla was transplanted into the lateral ventricle of adult rats in an effort to examine the mechanisms responsible for the reported efficacy of autologous adrenal medulla transplants in Parkinson's Disease patients. Stereotypic behavior (SB) induced by apomorphine (0.6 mg/kg) was decreased in medulla transplant- ed animals (n=6) relative to sham-operated controls (n=6) 3 months following implantation. DA and DA activity ([HVA]/[DA]) were increased in the striatum bilaterally (ipsilateral > contralateral) although both sides were elevated relative to shams. DA-2 receptor numbers were decreased in the striatum (ipsilateral > contralateral) although both sides were reduced relative to shams. This profile of decreased SB and DA-2 receptor number with increased DA activity is consistent with an increase in striatal DA tone subsequent to medulla transplantation. In a second study alterations in [14]C-2 deoxyglucose incorporation were evaluated in intraventricular medulla transplanted animals (n=4) and compared to animals in which sciatic nerve was transplanted into the ventricle (n=4). After 2 months, 3 of 4 medulla transplanted animals exhibited a disproportionate increase in glucose metabolism in the striatum ipsilateral to the transplant (primarily in the dorso-lateral quadrant). Other asymmetries were also observed in the lateral habenula and substantia nigra whereas sciatic implanted animals exhibited no asymmetries. This data also suggests that adrenal medulla transplants increase striatal DA tone. The lack effect in sciatic implanted animals argues against the probability that the effects observed following adrenal medulla transplant are a nonspecific injury potential.

5.3

AUTOLOGOUS ADRENAL MEDULLA TRANSPLANTS REDUCE A DA NEURON SPECIFIC CSF ANTIBODY. H.L. Klawans*, P.M. Carvey, R.D. Penn, C.G. Goetz*, C.M. Tanner*, A. Dahlstrom (1) and A. McRae (1). Rush U. Chicago,IL 60612; (1) U. Goteborg, Sweden 40033.

It has been previously observed that the CSF from 2 Parkinson's Disease (PD) patients contains an antibody (ab) which reacts with dopamine (DA) neuronal populations in the CNS. We have examined the ventricular CSF of PD patients undergoing autologous adrenal medulla transplantation for the presence of this ab. Rat brains were perfusion fixed with 5% glutaraldehyde, coronally sectioned, and incubated overnight with the CSF from patients. Prior to transplantation all 7 transplant patients exhibited immunocytochemical reactivity against rat substantia nigra (SN) neurons. This reactivity gradually decreased in the months following surgery and, in 4/7 patients, disappeared. In contrast, ventricular CSF collected from 7 non-parkinsonian patients did not exhibit this reactivity. In additional studies, 6/7 lumbar CSF samples collected from non-transplanted PD patients exhibited this reactivity whereas lumbar CSF from non-neurologic controls (n=20) did not. In addition, 50 Alzheimer's and multi-infarct dementia as well as 2 multiple sclerosis CSF samples have been examined and were found immunonegative in the rat SN.

Taken together this data suggests that PD patient CSF contains an ab which appears to react with SN neurons and may therefore participate in the patho-physiology of this disease.

5.4

IMPLANT INDUCED CATECHOLAMINERGIC SPROUTING IN MPTP PARKINSONIAN MONKEYS: BEHAVIORAL AND HISTOCHEMICAL STUDIES.
K.S.BANKIEWICZ,R.J.PLUNKETT*,D.M.JACOBOWITZ,I.J.KOPIN, A.CUMMINS*,E.H.OLDFIELD*, NIH,NINCDS/NIMH,Bethesda,MD,20892

We used MPTP hemiparkinsonian (HPD) monkeys as recipients of the brain implants. Tissue was implanted into preformed cavities in the medial caudate nucleus using an open microsurgical technique. We implanted fetal dopaminergic tissue (n=4), fetal non-dopaminergic tissue (n=6), adrenal medullary allografts (n=4) and autografts (n=3), non-dopaminergic adult allografts (n=2). Cavities were left unfilled in six monkeys. Two monkeys were implanted stereotaxicly with an adrenal medulla cell suspension, seven HPD monkeys were left unoperated. Three months after the implantation animals fell into three groups:(i) non-operated and needle implanted monkeys did not change their apomorphine-induced turning over seven months,(ii) 30-60% recovery was observed in animals in which cavities were left unimplanted, non-dopaminergic and adrenal allo and autografted animals,(iii) 70-95% recovery was observed in the fetal tissue-implanted monkeys. Only animals implanted with fetal dopaminergic tissue regained the use of the parkinsonian arm. When examined histochemically we found no tyrosine hydroxylase (TH) activity in the group (i), some TH-positive fibers in group (ii), abundant TH fiber sprouting in the caudate nucleus in the fetal tissue implanted animals [group (iii)]. The sprouted fibers appeared to emanate from the ventral asepect of the striatum in proximity to the n.accumbens. We believe that the sprouted fibers arise from the A-10 cells. These obsrvation indicate that neural sprouting may account for recovery of patients and animals with Parkinson's syndrome after surgical implants.

5.5

MAGNETIC RESONANCE IMAGING OF BLOOD BRAIN BARRIER DISRUPTURE AFTER CATECHOLAMINERGIC IMPLANTS TO THE CAUDATE NUCLEUS OF HEMIPARKINSONIAN MONKEYS. R.S. Miletich,K. Bankiewicz,R.J. Plunkett*,J. Alger*,A. Brunetti*,J. Frank*, G. Di Chiro,E.H. Oldfield*. NINCDS,NIH,Bethesda,MD 20892.

Human trials with adrenal implants for Parkinson's Disease are ongoing with some early favorable results. Several mechanisms have been proposed to explain the post-operative improvement, including blood brain barrier disrupture (BBBD). We investigated the presence of BBBD at the implant site of rhesus monkeys using Magnetic Resonance Imaging with the contrast agent Gadolinium-DTPA,0.1mmol/kg. Animals studied included adrenal autografts, fetal mesencephalic grafts, cavitation without implant and unoperated hemiparkinsonian monkeys. BBBD was manifested as a thin rim of contrast enhancement surrounding the graft bed. BBBD was present within the first 3 months after cavitation or implantation only at the operative site. In one adrenal autograft monkey studied 1 and 3 months after surgery the degree of contrast enhancement decreased over time despite a stable clinical state. After 3 months, no contrast enhancement was seen in any of the animals. The behavioral improvement sometimes seen within a few days of implantation may be partly explained by BBBD. However, these results suggest that BBBD is not the primary mechanism responsible for the long-term clinical improvement seen after certain catecholaminergic implants to the caudate nucleus of hemiparkinsonian monkeys.

5.6

GRAFTING OF HUMAN SYMPATHETIC GANGLIA INTO THE BRAIN OF MPTP-TREATED MONKEYS. P. Pasik, J.F. Martínez*, M.D. Yahr, T. Pasik, G.Holstein and H. Schanzer*. Depts. Neurol., Anat. & Surg., Mount Sinai Sch. Med., CUNY, N.Y., N.Y., 10029.

Sympathetic ganglia were evaluated as a source of dopamine-producing neurons for brain transplants in parkinsonian monkeys. It was expected that the dopaminergic interneurons, contrary to the long-axoned noradreneregic cells, might not be readily damaged by the excision of the ganglia. Two adolescent cynomolgus monkeys were rendered parkinsonian by MPTP treatment. Paravertebral sympathetic ganglia were removed from a patient for the treatment of severe Reynaud's syndrome. After a pathologist verified the nature of the specimen, the remainder was dissected free of connective tissue and divided into small fragments which were deposited stereotaxically into 2-3 loci of the right putamen, after producing a bed by aspiration of putaminal tissue and injecting 10 µl of 0.01% nerve growth factor. One of the monkeys was sacrificed 5.6 months later by aldehyde perfusion, and brain sections were processed for tyrosine-hydroxylase (TH) immunocytochemistry using the PAP technique.

Animals developed parkinsonism after MPTP treatment: hypokinesia, kyphotic posture, rigidity and tremor, and required feeding by nasogastric tube. They recovered spontaneously in about six weeks, and after a second course of MPTP, the full syndrome reappeared. One week following the transplant, they showed improvement and became progressively better, remaining with minimal hypokinesia by 3-4 weeks, and appearing normal by 6-7 weeks. Light microscopy showed: (1) A space at the end of the needle track, partly filled with cells, some of which had neuronal characteristics, lightly stained globular somata, and broad processes. Occasionally, a deeply immunoreactive neuron could be recognized, exhibiting bipolar dendritic branching and an axon penetrating into the host tissue. (2) The neostriatum contained few labeled fibers and, contrary to the n. accumbens septi, appeared very pale. (3) The substantia nigra had few TH+ neurons, but the ventral tegmental area, hypothalamus and locus ceruleus contained numerous labeled cells.

Conclusions: (1) Cross species (human-monkey) grafts of sympathetic ganglia into brain can survive for long periods. (2) Some of its neurons retain the capacity to produce TH. (3) Most probably these neurons are dopaminergic. (4) The behavioral improvement may or may not be attributed to the function of the implant.

Aided by NIH Grants # NS-22953, NS-11631 and the S. & H. Leung Fund.

5.7

IMMUNOHISTOCHEMISTRY OF ADRENAL AUTOGRAFTED AND CAVITATED HEMIPARKINSONIAN MONKEYS. R.J Plunkett*, K. S. Bankiewicz, D.M. Jacobowitz, I.J. Kopin, E.H. Oldfield*, A.C. Cummins*(SPON: A.M. DeLuca) SNB, NINCDS, NIH, Bethesda, Md. 20892 Four hemiparkinsonian Macaque monkeys received adrenal autografts (AM) via an open microsurgical delayed implantation technique; four others had a surgical cavity (CAV) created in the head of the caudate but no tissue implant. In each group, the response to 0.2 mg/kg apomorphine decreased over the ensuing months to less than 50% of the pre-implantation level. Neither group regained use of the MPTP-affected extremity. The AM monkeys were sacrificed 10 days to 8 months after implantation; only the 10 day animal had a significant number of surviving chromaffin cells visualized by tyrosine hydroxylase (TH) immunohistochemistry. The predominant cell at the implant site was the macrophage; there was also a prominent glial response surrounding the site when antibody to gliofibrillary acidic protein was utilized. In 2 of the AM monkeys, and in 3 of the 4 CAV monkeys, there was significant TH reactivity on fibers in the MPTP-lesioned caudate. These TH-positive fibers appeared to be sprouted from the mesolimbic dopaminergic system, in the region of nucleus accumbens. We believe the sprouted fibers are responsible for the behavioral improvement seen.

5.8

ELECTROPHYSIOLOGICAL ASSESSMENT OF REACTION TIME AND MOVEMENT TIME IN PARKINSON DISEASE PATIENTS UNDERGOING ADRENAL-CAUDATE TRANSPLANTATION. R.L. Watts, A.S. Mandir*, B. Hirschfield*, R.A.E Bakay, J.C. Hemphill III*, E.B. Montgomery, Jr. Emory Univ. Sch. of Med., Atlanta, GA 30322 and Washington Univ. Sch. of Med., St. Louis, MO. 63110.

Transplantation of autologous adrenal medullary tissue into the caudate nucleus is a new therapeutic approach for Parkinson disease (PD) patients. Prolonged Reaction Time (RT; a measure of pre-movement neural processing) and Movement Time (MT; the physiological correlate of bradykinesia) are characteristic of PD and are quantitative correlates of functional disability. We are employing a visually instructed RT/MT wrist flexion paradigm in patients both prior to transplantation and at 3, 6, and 12 months post-operatively. We have studied 4 patients with stage IV-V PD when off drugs (ages 48,56,57 & 62), as well as age-matched control subjects for comparison. We measure RT from a "GO" signal both to movement onset (RT/MO) and to onset of agonist EMG (RT/EMG), and MT is measured from movement onset to achievement of a final target. For control subjects, RT/MO is 252±17 (x±SEM msec), RT/EMG is 171±23, and MT is 239±26. At time of RT/MT testing, all patients are examined clinically using a Parkinson disability rating scale. In each patient, pre-op RT/MO (#1-413±26, #2-475±29, #3-434±27, #4-380±32) and RT/EMG (#1-285±47, #2-375±37, #4-280±41) were significantly prolonged from normal controls. Pre-op MT for three of the four patients (#1-418±28, #2-460±40, #3-517±31, #4-220±38) was also significantly prolonged from the control population. At 3 and 6 months post-op, preliminary results from Pt #1 reveal improvement in all movement parameters (3 mo. RT/MO 323±35, RT/EMG 194±46, and MT 348±15; 6 mo. RT/MO 210±16, RT/EMG 120±22, and MT 290±28). Results at 3 and 6 months post-op will be presented for all patients.

5.9

131-I-META-IODOBENZYLGUANIDINE SCINTIGRAMS OF ADRENOMEDULLARY AUTOTRANSPLANTS TO THE CAUDATE NUCLEUS OF PARKINSONIANS. A. Graef*, R.E. Franco-Bourland*, A. Márquez*, I. Madrazo, R. Drucker-Colín, C. Torres*, F. Alvarez*, and E. Manzano* (SPON: V. Alemán). Especialidades "La Raza" IMSS 02990 DF, Inst Nal Nutrición SZ 14000 DF, UNAM 45000 DF MEXICO

The autotransplantation of adrenomedullary tissue to the caudate nucleus has been reported by Madrazo et al. (N Engl J Med 316:831,1987; Surg Forum 38:510,1987) to ame - liorate Parkinson's disease symptoms in humans. We now report on the location and viability of the graft after surgery using 131-I-meta-iodobenzylguanidine (131-I-MIBG). This radiopharmaceutical is used to locate pheochromocytoms. It is selectively taken up by storage granules of chromaffin cells by an active, ouabain sensitive mechanism (J Nucl Med 25:P124,1984). Three autotransplanted parkinsonians(P) one patient who underwent neurosurgery of the right frontal lobe(FL) and one suspected of having a pheochromocytoma(PH) received iv 2.5 mCi (P,FL) or 1.25 mCi (PH) 131-I-MIBG. 131-I thyroid uptake was blocked with oral Isodine (5ml,3/d) before (3d) and after (7d) receiving the tracer. The 48h and 72h scintigrams were obtained with a scintillation camera (360 keV, 15% window, high energy parallel collimator) and a Scintiview data processor. Only the brain images of the autotransplanted parkinsonians showed the specific uptake of the label to the caudate nucleus, thus demonstrating the viability of the adrenomedullary graft.

5.10

BIOCHEMICAL ANALYSES OF LUMBAR AND VENTRICULAR CSF FROM PARKINSONIANS BEFORE AND AFTER ADRENOMEDULLARY AUTOTRANSPLANTATION TO THE CAUDATE NUCLEUS. R.E. Franco-Bourland*, I. Madrazo, R. Drucker-Colín, C. Torres*, F. Alvarez*, P. Carvey*, D. O'Connor*, R. Sierra & J. Tamayo (SPON: J. Villarreal). Inst. Nal. Nutrición SZ 14000 DF, "La Raza" IMSS 02990 DF, UNAM 45000 DF, MEXICO; Rush-Presbyterian Chicago IL 60612, Vet Adm Med Ctr San Diego CA 92161 USA.

Madrazo et al. (N Engl J Med 316:831,1987; Surg Forum 38:510,1987) have reported on the amelioration of Parkinson's disease symptoms after adrenomedullary autografting to the caudate nucleus in humans. In order to acquire an insight into the biochemical mechanisms underlying the beneficial effects of this procedure we have undertaken a collaborative study to assess in 6 operated parkinsonians their pre- & post-transplantation levels of HVA, 5-HIAA, MHPG (by HPLC-ED, PC), chromogranin A (by RIA, DO) & cAMP (by RIA, RS & JT) in lumbar (L) and ventricular (V) CSF over a period of 3-4 months. Pre- & post-surgery,L & V levels of HVA & 5-HIAA were not significantly different; L levels ranged between 20 & 120 ng/ml, & between 20 & 60 ng/ml, respectively; V levels ranged between 120 & 300 ng/ml & between 60 & 100 ng/ml, respectively. Pre- and post-surgery, L & V levels of MHPG were indistinguishable & fluctuated between 5 & 20 ng/ml. L & V levels of chromogranin A also remained unchanged after surgery. L levels varied between 20 & 80 ng/ml and V levels between 0 & 20 ng/ml. Pre and post cAMP,L & V levels were similar(20-60 pm/ml).

5.11

HUMAN FETAL SUBSTANTIA NIGRA AND ADRENAL MEDULLARY TISSUE HOMOGRAFTS TO THE CAUDATE NUCLEUS OF PARKINSONIANS.
I. Madrazo, R. Drucker-Colín, R.E. Franco-Bourland*, F. Ostrosky*, M.C. AGuilera*, and C. Torres*. Especialidades La Raza, IMSS, 02990 DF, UNAM, 45000 DF, INNSZ, 14000 DF, México.

We recently made a preliminary report (N Engl J Med : 318:51,1988), of two cases of human fetal transplants to relieve Parkinson's disease (PD). One case is a 50-year-old man with a 9 year history of PD, who received a substantia nigra fetal homograft to the caudate nucleus. At the time of surgery he had a UPRS score of 59 points and was on a dose of Sinemet of 1000 mg. Three months after surgery he had a score of 45 points. Now, 6 months after surgery he has a score of 5 points, and is receiving 375 mg Sinemet to control the remaining tremor and an oral dose of cyclosporine of 1 mg/kg bw/day.

The second case is a 35-year-old woman with a 5 year history of PD, who had a preoperative UPRS score of 71 points, while under treatment with 750 mg Sinemet. She received a fetal adrenomedullary homograft to the caudate nucleus. Three months after surgery her score was 35 points and now she has a score of 13 points, and is receiving a dose of 375 mg Sinemet, and 1 mg/kg bw/day cyclosporine.

The excellent postoperative tolerance to the procedure in these patients, and the extensive neurobiological data appear to support human fetal grafting as the best procedure to treat PD, but further experience is needed to confirm this contention.

5.12

CATECHOLAMINES AND METABOLITES IN TISSUES AND IN CSF OF PATIENTS WITH PARKINSON'S DISEASE AFTER AUTOLOGOUS TRANSPLANTATION OF ADRENAL MEDULLA TO CAUDATE NUCLEUS.
G. M. Tyce, J. E. Ahlskog*, S. W. Carmichael, S. L. Chritton*, S. L. Stoddard, J. A. van Heerden*, and P. J. Kelly*. Dpts. of Physiology, Neurology, Anatomy, Surgery, and Neurosurgery, Mayo Clinic, Rochester, MN 55905.

Measurements were made of the concentrations of norepinephrine (NE), epinephrine (E), and dopamine (DA) in host and in transplanted tissues in seven patients who were undergoing autologous transplantation of adrenal medulla to caudate nucleus as treatment for Parksinson's disease. In addition, free and sulfated NE, E, and DA, 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA) were measured in lumbar CSF taken before and at two 6-weekly intervals after the surgery. Quantitation of analytes was by HPLC with electrochemical detection. The concentrations of DA in small samples of caudate (removed to create a donor bed) and in samples of adrenal medulla were similar (~4 µg/g). NE and E were barely detectable in caudate but were ~502 and 3,492 µg/g, respectively, in adrenal medulla. In all CSF samples the concentrations of MHPG and free and sulfated NE and E were in the normal range. Free DA was never detected (<40 pg/ml) in CSF. After the transplant sulfated DA increased modestly in CSF, but HVA was unchanged. In summary, analyses of catecholamines in lumbar CSF suggested that the adrenal graft did not produce NE and E after the transplantation, and that production of DA sulfate was modestly increased.

5.13

IMPLANTS OF HUMAN VENTRAL MESENCEPHALIC NEURONS AND PERFUSED ADRENAL MEDULLARY TISSUE INTO THE CAUDATE NUCLEUS OF PARKINSON'S PATIENTS PRELIMINARY RESULTS.
Juan J. Lopez-Lozano[1,2], G. Bravo[3*], J. Abascal[4*] and CPH Neural Transplantation Group*. Dept. of Exp. Surgery[1], Neurology[2], Neurosurgery[3] and Surgery[4]. Clinica Puerta de Hierro. University Autonoma. Madrid. Spain.

The encouraging results obtained by Madrazo et al., Jiao et al, Molina et al. and Lopez-Lozano et al., after transplantation of autologous adrenal medulla (AM) into the caudate nucleus of patients with Parkinson's disease seem to indicate this technique as an alternative treatment of the disorder. Since the clinical improvement reported has not been completely satisfactory, because of the high morbidity and mortality, we attempt new sources of donor tissue. This report examines and compare the effect of transplanting perfused neonatal adrenal medullary or fetal ventral mesencephalic (VM) tissue into right caudate nucleus of Parkinson's patients. VM was dissected from human fetuses of different ages of gestation. In the case of AM tissue, the adrenal gland was cannulated and perfused (JJLL,'88) to improve the dissection procedure. Fragments of either ventral mesencephalon or adrenal medulla were selected to be implanted into the right caudate nucleus, in contact with the lateral ventricle wall, of immunosuppressed Parkinson's patients. To analyze the viability and purity of the implanted tissue, the remaining VM or AM tissue was dispersed by an enzymatic-mechanical procedure. The recovered cell suspension were cultured or implanted into 6-OHDA lesioned rats and subsequently studied by catecholamine-containing histofluorescence and TH-ICC, showing that they contained catecholamine and expressed TH. All patients have been evaluated pre- and post-operative with video, Columbia rating scale, NWDS, Unified Parkinsonism rating scale. Catecholamines and their metabolites have been measured in CSF by HPLC before and after transplantation. This report will show the clinical and neurochemical data obtained after transplantation and will evaluate and discuss the clinical usefulness of cited source donors vs autotransplanting adrenal medullary tissue.
Supported by FIS 87/687, CACYT grants to JJLL. CPH-NT group: A. El Barkani[3],

EPILEPSY I

6.1

ABNORMALITIES IN TEMPORAL LOBE EVENT-RELATED POTENTIALS PREDICT HIPPOCAMPAL CELL LOSS IN TEMPORAL LOBE EPILEPSY.
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Relationships between task-related Event-Related Potentials (ERPs) recorded intracranially from sites in the temporal lobes of epileptic patients and the side of seizure onset have been noted by a number of investigators (*e.g.*, Squires *et al.*, 1983; McCarthy *et al.*, 1987; Meador *et al.*, 1987). Here we demonstrate that abnormalities in task-related ERPs predict the severity of hippocampal cell loss in the affected temporal lobe.

Twenty-four patients in whom both temporal lobe ERPs and cell counts on excised temporal lobe tissue were available were studied. Cell counts (neurons per mm^3 in CA1, CA2, CA3, CA4, and dentate gyrus) and ratings of ERP normality were made blind with respect to each other. Correlations (Pearson *r*) between ERP ratings and cell counts in the four CA fields and dentate gyrus ranged from .57 – .67 and were all statistically significant ($P < .01$). Corresponding rank-order correlations were lower by about .05 and were again all significant ($P < .01$). Cell counts in the CA fields and dentate gyrus were themselves highly correlated ($.5 \leq r \leq .7$); mean cell count across fields was correlated .72 with ERP ratings ($P < .01$).

These results demonstrate that task-related ERPs recorded from the medial temporal lobe are as highly correlated with cell counts in the hippocampal CA fields and dentate gyrus as the cell counts are among themselves. Such ERPs may therefore be of value in estimating the severity of hippocampal pathology before temporal lobe excisions. These results also implicate the hippocampal CA fields and dentate gyrus as generators of, or part of the circuit which generates, task-related ERPs in the medial temporal lobe.

6.2

INCREASED AMYGDALA ACTIVATION OF SUBICULAR NEURONS IN HUMAN TEMPORAL LOBE EPILEPSY.
M. Isokawa-Akesson, C.L. Wilson and T.L. Babb. Brain Research Institute and Dept. Neurology, UCLA, Los Angeles, CA 90024-1761.

In our previous study (Isokawa-Akesson et al., 1987, NS Abstr. 13:365), interictal recordings showed a decreased neurotransmission in hippocampal-subicular pathways of human temporal lobe epilepsy based on extracellular single unit recording and single pulse stimulation. The present study was designed, using the same experimental procedure, to investigate whether another afferent to the subiculum, i.e. the amygdalo-subicular path, is altered in its neurotransmission in correlation with the decreased hippocampal-subicular path in temporal lobe epilepsy. Single pulse electrical stimulation was administered to the amygdala (AMYG) and single unit recordings were obtained from the presubiculum (PRESUB). In controls (in the temporal lobe contralateral to the seizure origin), 3 out of 14 (21.4%) PRESUB neurons responded to AMYG stimulation and the responses were only inhibitory with an average latency and duration of 75 msec and 273 msec, respectively. By contrast, in the epileptic, AMYG stimulation evoked PRESUB responses in 6 out of 8 neurons (response probability: 63.6%), and the responses were characterized by excitation (3/6cells), inhibition (2/6cells), or preceding excitation followed by inhibition (1/6cells). The average latency of excitation was 32 msec and the average latency of inhibition was 52 msec. Mean inhibition lasted 165 msec. Interestingly, this response pattern in PRESUB by epileptic AMYG stimulation could be elicited in the normal PRESUB 56% of the time by normal hippocampal (HIPP) stimulation. (When epileptic HIPP was stimulated, neither excitation nor inhibition was evoked in any of the 7 neurons recorded from the adjacent PRESUB.) Our results suggest that AMYG activation of the PRESUB is enhanced in epileptic human hippocampal formation during interictal periods.
Supported by NIH Grant NS 02808.

6.3

SOMATOSTATIN AND β-ENDORPHIN LEVELS IN INTERICTAL AND POSTICTAL CSF OF EPILEPTIC PATIENTS. A. Pitkänen* and P.J. Riekkinen. Dept. of Neurology, Univ. of Kuopio, P.O.Box 6, SF-70 211 Kuopio, Finland.

Neuropeptides are proposed to play a role in the regulation of interictal behavior and seizure threshold in experimental models of epilepsy. In this study we, firstly, compared the levels of somatostatin (SLI) and β-endorphin (BEP) by radioimmunoassay in interictal CSF of unmedicated (n=18) and medicated (n=24) epileptic patients with the levels of peptides in controls (n=20). Patients with chronic medicated epilepsy had SLI level 80 % (p=0.003, Mann-Whitney U-test) that of the controls and 76 % (p=0.011) that of the unmedicated patients. BEP in the interictal CSF did not differ in unmedicated, medicated and control patients. Secondly, to study the peptidergic systems in postictal stage of human epilepsy we collected CSF from nine patients within two hours after generalized tonic-clonic convulsion (GM), and the second sample from the same patients was obtained interictally after 1-4 days without any kind of clinically observable seizures. BEP was elevated postictally (p=0.044, Wilcoxon's test) compared with the interictal level. SLI was similar in both samples. The present study suggests that BEP is released into extracellular space during GM and may be involved in the depression of seizures. Instead, during the period when epilepsy becomes chronic the somatostatinergic cells are affected.

6.4

ELECTROGRAPHIC RECORDINGS DURING AUDIOGENIC SEIZURES IN FREELY MOVING RATS. N. Ludvig* and S.L. Moshé, Dept. of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461

The EEG changes during audiogenic seizures (AS) have not been well described in freely moving rats because of the technical difficulties introduced by the movement artifacts. With the use of a special recording apparatus equipped with a field effect transistor and a liquid commutator we were able to eliminate most of the movement artifacts and could study the EEG of the AS in the genetically AS-susceptible GEPR-9 rats. Recordings were made from the inferior colliculus (IC) which is considered to be a crucial structure in the pathogenesis of AS. We found that 7.6 ± 1.7 (mean ± S.E.M.) sec after the acoustic stimulus (104 dB), repetitive spike and wave discharges occurred lasting 20-50 sec. The epileptiform discharges were initially associated with wild running seizures and later on with clonic-tonic convulsions. One - two minutes after the end of the convulsions interictal spikes appeared. During this period the rats were immobile with fixed gaze. The data indicate that AS are associated with paroxysmal electrographic discharges recorded from the IC.

6.5

PRINCIPAL COMPONENT ANALYSIS OF LAMINAR CURRENTS PRODUCED BY PENICILLIN SPIKES IN RAT CORTEX. D.S.Barth., S.Di* and C. Baumgartner*(1) Dept. of Neurology and Psychology, Univ. of California, Los Angeles, CA 90024. (1).Neurological Univ. Clinic, Vienna, Austria.

Recent studies using laminar microelectrode recordings have suggested a systematic sequence of neuronal activation in distinct cortical lamina during both normal evoked responses and epileptic spiking. In the present study, we mapped laminar field potentials from the temporal cortex of 5 rats during the direct cortical response (DCR) produced by electrical stimulation and during electrically evoked interictal spikes (EIIS) of the penicillin focus. Current source-density (CSD) distributions were computed from the field potentials to highlight laminar changes in transmembrane currents during the time course of the evoked responses. Finally, the spatial and temporal pattern of CSD was studied with principal component analysis (PCA).

PCA indicated that cellular currents overlapping in space and time during both the DCR and the EIIS are produced by a highly stereotyped interaction between neurons in the upper and the deepest cortical lamina. The sequence, polarity, and location of neuronal activation was not altered by penicillin. The main effect of penicillin was to triple the duration and amplitude of membrane currents during the EIIS as compared to the DCR. These data suggest that penicillin spikes use the same neuronal circuitry as the DCR, but with pathologically enhanced synaptic currents.

6.6

CHRONIC EFFECTS OF SYSTEMIC APPLICATIONS OF KAINIC ACID ON mRNA LEVELS OF NEUROPEPTIDES IN RAT BRAIN. D.K. Meyer*, C. Olenik* and G. Sperk* (SPON: D.J. Dooley). Dept. of Pharmacol., University of Freiburg, D-7800 Freiburg (FRG); Dept. of Pharmacol., University of Innsbruck, A-6020 Innsbruck (Austria).

Intraperitoneal injection of kainic acid causes in rats acute limbic motor seizures and subsequently long-lasting changes in levels of the neuropeptides somatostatin and cholecystokinin in substantia nigra, striatum, frontal cortex and hippocampus. To find out whether these changes are concomitant with altered gene transcription, concentrations of mRNA's coding for the respective prepropeptides were measured with a filter hybridisation technique.
In all areas investigated, concentrations of mRNA's coding for both prepropeptides were enhanced 2 days after kainic acid. This suggested an increased synthesis of both peptides subsequent to their presumed enhanced release during the acute seizures. With the exception of the frontal cortex, levels had returned to control values 8 days later. In frontal cortex, the increases in mRNA concentrations for both prepropeptides persisted up to 30 days.
These changes in mRNA together with the previously observed increases in peptide levels indicate that somatostatin- and cholecystokinin-neurons in frontal cortex have a persistently increased activity, which may be related to the enhanced seizure susceptibility of these rats.

6.7

DIFFERENTIAL RESPONSES OF SOMATOSTATIN, NPY, CCK-8 AND VIP IN KAINIC ACID-INDUCED EPILEPSY IN THE RAT. G. Sperk*, J. Marksteiner* and D.K.Meyer* (SPON: O. Hornykiewicz). Dept. of Pharmacology, Univ. of Innsbruck, Innsbruck, Austria.

Somatostatin, neuropeptide Y (NPY), cholecystokinin octapeptide (CCK-8) and vasoactive intestinal polypeptide (VIP) were measured in various brain areas after limbic seizures induced by kainic acid (10 mg/kg, i.p.) in the rat. Reversible decreases (20-60%) in immunoreactivities of the peptides were found 3 h after injection of the toxin suggesting significant release during the preceding acute seizures. In contrast to this, marked increases of somatostatin, NPY and CCK-8 occurred in the frontal cortex (somatostatin by 60%; NPY, 200%; CCK-8, 35%) and of NPY and CCK-8 in the hippocampus (150% and 50%, respectively) after 10-60 d. The time course of maximal elevation of these peptides was slow but long lasting and was accompanied by an increase of glutamate decarboxylase (Vmax, 22%) in the frontal cortex. This was contrasted in the cortex by an only transient increase of VIP (50%) already 3 d after kainic acid which was again normalized after 10 d. Whereas the reversible increase in VIP (partly contained in local cholinergic neurons) may relate to the acute seizures, the late and non-reversible increases of somatostatin, NPY, CCK-8 and of glutamate decarboxylase may reflect activation of a family of neuropeptide/GABA neurons as a response to the long lasting decreased seizure threshold in the animals.

6.8

SEIZURE ACTIVITY OF TWO PEPTIDES DERIVED FROM DIAZEPAM BINDING INHIBITOR (DBI) AFTER INTRAHIPPOCAMPAL INJECTION IN RATS. C.Ferrarese[1], M.A.Stasi*, R.Samanin* and A.Vezzani. [1]Clinica Neurologica, Università di Milano, Ospedale S.Gerardo, Monza and Istituto "Mario Negri", Milano, Italy 20100

Fragments (FR) 42-50 and 43-50 are products of tryptic digestion of DBI (104 aminoacids), an endogenous peptide that modulates GABAergic neurotransmission. The two peptides were unilaterally injected into the dorsal hippocampus (granule cells of dentate gyrus) of freely-moving adult rats. The EEG pattern was continuously recorded from bilateral hippocampal and cortical electrodes. 100 nmol FR 42-50 induced in all the rats seizures and EEG alterations quantitated as follows: total No seiz.11±2.5 with a latency of 11± 3.5 min, a total time in seiz. of 947±322 sec and a total time of EEG alterations (seiz.+spikes) of 1338±289 sec (n=7). 150 nmol FR 43-50 induced a qualitatively similar but less intense EEG pattern. During EEG seizures the rats had the typical "frozen appearance" and "wet dog shakes" often occurred. Tonic-clonic seizures were not observed. Protection from seizures induced by FR 42-50 occurred with 90 mg/kg PK 11195, a selective antagonist of the peripheral-type of the benzodiazepine receptors. Blockade of the N-methyl-D-aspartate-type receptors in the hippocampus did not suppress seizures. The two fragments induced only marginal, possibly non-specific, neuronal degeneration. The possible role of DBI-related peptides in the pathophysiology of epilepsy will be discussed.

6.9

MODULATION BY SMS 201-995, A SYNTHETIC ANALOG OF SOMATOSTATIN (ST), OF LIMBIC SEIZURES INDUCED BY INTRAHIPPOCAMPAL INJECTION OF QUINOLINIC AND KAINIC ACIDS IN RATS.
A. Vezzani, M.A. Stasi*, R. Serafini*, M.G. De Simoni*, F. Fodritto* and R. Samanin* Istituto "Mario Negri", Milano, Italy

One hundred twenty and 60 nmol quinolinic acid (QUIN) or 0.19 nmol kainic acid (KA) were unilaterally injected in the dorsal hippocampus of freely-moving adult rats. Fifteen min later, 5 μg SMS 201-995 (SMS), a ST analog highly resistant to enzymatic degradation, were delivered at the same site. The EEG pattern was continuously recorded from bilateral hippocampal and cortical electrodes. SMS displayed neither anticonvulsant nor proconvulsant activity when EEG seizures were induced by 120 and 60 nmol QUIN injected in stratum radiatum (SR) CA1. SMS significantly protected from seizures induced by 0.19 nmol KA injected in SR CA1 (PBS+KA: No seiz.11±1 (8), total time in seiz. 1085±174 sec; SMS+KA: No seiz. 2.6±0.8 (8), total time in seiz. 199±60 sec) and a delay in the onset of seizures was also observed (16±3.9 min vs 66±24 min) ($p < 0.05$ for all measures, Student's t-test). SMS was ineffective on seizures when KA was injected within the hilus of the dentate gyrus. KA was equipotent as a convulsant after injection in the SR CA1 and in the hilus.

The effects of QUIN and KA on the endogenous hippocampal ST, measured by in vivo voltammetry, will be shown.

The role of the cholinergic system in the modulatory action of ST on seizures will be discussed.

6.10

A_1 ADENOSINE RECEPTOR ACTIVATION BLOCKS SEIZURES INDUCED BY BICUCULLINE METHIODIDE IN THE RAT PREPIRIFORM CORTEX.
P.H. Franklin, Ge Zhang*, E.D. Tripp* and T.F. Murray. College of Pharmacy, Oregon State University, Corvallis, OR 97331.

It is now clear that adenosine receptors play a primary role in the mediation of CNS inhibitory function. In accordance adenosine or metabolically stable adenosine analogs express anticonvulsant activity in a number of experimental models of epilepsy. Neither the anatomical nor the functional basis for the anticonvulsant effects of adenosine (ADO), however, have been well established in the CNS. We have previously reported (Franklin et al., European J. Pharmacol., in press, 1988) that seizures induced by microinjection of bicuculline methiodide (BMI) into the prepiriform cortex (PC) are potently (ED_{50}= 32.2±0.3 pmol) and completely blocked by prior injection of the ADO agonist 2-Chloroadenosine (2-ClA). To characterize the ADO receptor involved in this response, dose response relationships for a series of ADO agonists were evaluated. The rank order of potencies for these compounds is as follows: N-Ethylcarboxamidoadenosine>Cyclohexyladenosine> Cyclopentyladenosine>R(-)(2-Phenylisopropyl)-adenosine> 2-ClA>>S(+)(2-Phenylisopropyl)-adenosine>>2-Phenylamino-adenosine. These results suggest that potency at adenosine A_1 receptors is the basis for protection by these compounds against seizures induced by BMI in the PC. (supported by NS-23227 to T.F.M.)

6.11

D-1 BUT NOT D-2 DOPAMINE RECEPTOR STIMULATION REDUCES THE THRESHOLD FOR PILOCARPINE-INDUCED EPILEPTIC ACTIVITY IN RATS.
P. Barone*, S.A. Parashos, V. Palma*, C. Marin*, G. Campanella* and T.N. Chase. Experimental Therapeutics Branch, NIH, Bethesda, MD 20892; Dept. of Neurology, University of Naples, Italy; Ist. Sci. Sanatrix. Venafro, Italy.

Experimental evidence indicates a role for GABAergic striatonigral projections in inhibiting the propagation of epileptic activity. D-1 dopamine receptors, found on such terminals in nigra pars reticulata probably modulate GABA release. The present study evaluated the effects of dopamine receptor stimulation on pilocarpine-induced limbic seizures. Pilocarpine produces limbic stereotypies, but not convulsions or seizure-related brain damage at a dose of 200 mg/kg ip. Administration of the D-1 agonist SKF38393 5 min prior to this dose of pilocarpine induced generalized convulsive activity as revealed by behavioral and electroencephalographic alterations and histopathological findings (ED_{50}=1 mg/kg, ip). Similar effects were produced by a high dose of pilocarpine (400 mg/kg, ip). The D-1 antagonist SCH23390 prevented both pilocarpine (400 mg/kg) and SKF 38393 plus pilocarpine (200 mg/kg) induced seizures in a dose dependent manner. Response to pilocarpine was unaffected by the D-2 agonist LY171555 (0.1-10 mg/kg, ip). Conceivably pharmacologic manipulation of dopaminergic transmission could provide an alternative approach to the treatment of secondarily generalized epilepsy.

6.12

KINDLING ANTAGONISM: THE EFFECTS OF COMBINED NEONATAL AND ADULT 6-HYDROXYDOPAMINE TREATMENT.
J. Burchfiel and C. Applegate. Dept. Neuroscience The Children's Hospital, Boston, MA 02115.

Recent evidence suggests a role for norepinephrine (NE) in the development of site suppression and dominance observed following concurrent, alternate stimulation of the septal nucleus (S) and entorhinal cortex (EC; kindling antagonism). Neonatal treatment with 6OHDA produces a forebrain depletion but a hindbrain hyperinnervation by NE at adulthood, and the development of kindling antagonism remains intact. To what degree is brainstem innervation by NE necessary for the development of antagonism?

Rats treated with 6OHDA as neonates were subsequently administered a low dose (75ug; N=8) or a high dose (200ug; N=9) of 6OHDA or vehicle (N=8) as adults. Rats were implanted with stimulating electrodes into the S and EC and were stimulated in the antagonism paradigm. High dose treatment, but not low dose or vehicle treatment interfered with the development of kindling antagonism. This suggests that some brainstem innervation by NE is necessary for the development of antagonism. The quantitative relationship between regional biochemical treatment effects and antagonism seizure profiles are currently being assessed.

6.13

SENSORY KINDLING IN GERBILS IS A PROTEIN DEPENDENT PROCESS REQUIRING PREVIOUS SEIZURE.
Paul L. Prather* & W.B. Iturrian, Pharmacology, Univ. of GA, Athens, GA 30602.

We show "sensory"kindling" (KIN) in gerbils with planned periodic reexposure to handling and report pharmacological correlates of KIN development and expression. In naive animals over 90 days old, only 40% seizure on initial test. Four seizure tests with a 3 day inter-test interval and a 5th test a week later produces kindling with 95% seizures. Seizure incidence and severity were recorded at all tests and drugs were given before tests 2, 3, and 4 only. Diazepam (1mg/kg) before testing was anticonvulsant reducing seizure incidence about 75%. Phenytoin (50mg/kg) was only half as effective. Both drugs retard full expression of KIN since test 5 (drug free) seizure incidence was reduced 20-30% relative to saline control. Cycloheximide (CXM) (25mg/kg) inhibited KIN development 40% and expression by 32%. Clonidine (0.075 mg/kg) was unique in that although seizure incidence during development was reduced 40%, KIN was fully expressed at test 5. Yohimbine (1mg/kg) and CGS 8216 (4mg/kg) had no effect on either KIN development or expression. These results indicate that CXM sensitive proteins induced by testing facilitate, while alpha$_2$ mechanisms inhibit KIN development. Full KIN expression depends on a prior CXM sensitive convulsive experience suggesting a protein dependent memory-like process. Endogenous BDZ's do not appear to be involved. Finally, development and expression of KIN are controlled by different endogenous systems.

7.1

SELECTIVE VULNERABILITY OF MESENCEPHALIC NEUROMELANIN-PIGMENTED NEURONS IN PARKINSON'S DISEASE. E. C. Hirsch*, A. M. Graybiel, Y. A. Agid*. (SPON: F. O. Schmitt) Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

It is well established that mesencephalic dopaminergic neurons are affected in Parkinson's disease (PD), but it is not yet clear whether a subpopulation of these neurons is more vulnerable than the others. The number of catecholaminergic (CA) neurons (immunostained with antiserum against tyrosine hydroxylase), neuromelanin-pigmented neurons and non-CA neurons in mesencephalic dopamine-containing cell groups was estimated in control (n=3) and parkinsonian (n=4) midbrains. Only a subpopulation of the catecholaminergic neurons contained pigmented neuromelanin in controls. These cells were most numerous in the substantia nigra pars compacta, less common in cell group A8 and least common in the central gray substance. In PD the cell loss among the catecholaminergic neurons was directly correlated (r=0.97) with the percent of pigmented neurons normally in these cell groups. The non-pigmented-CA neurons were relatively spared compared to those containing visible neuromelanin pigment. The preferential vulnerability of neuromelanin-pigmented neurons in PD could be related to the capacity of neuromelanin to bind toxic compounds such as MPP+. The topography of cell loss may also be important. *Supported by the Seaver Institute, the Edith C. Blum Foundation, the Tourette Syndrome Association, the McKnight Foundation, the Whitaker Foundation, the Fondation Simone et Cino del Ducca, and INSERM.*

7.2

LOCALIZATION OF TYROSINE HYDROXYLASE mRNA IN TYROSINE HYDROXYLASE IMMUNOSTAINED CELLS IN HUMAN MESENCEPHALON BY IN-SITU HYBRIDIZATION. F.JAVOY-AGID*,S.DUMAS* (1),E.HIRSCH*, J.C.BISCONTE*(2),Y.AGID*,J.MALLET*(1),(SPONS:E.L.GUSTAFSON) Inserm U 289 Hopital Salpêtriere,75013 Paris,(1) Neurobiologie Cellulaire et Moleculaire CNRS,Gif-sur-Yvette 91190, (2) Biocom S.A. Çourtaboeuf 91942, FRANCE.

In Parkinson's disease,dopamine cells in the substantia nigra are more dramatically affected than those in ventral tegmental area suggesting biochemical distinctions between the two cell groups. Detection of specific messenger RNA (mRNA) by in situ hybridization histochemistry was investigated to study the level of tyrosine hydroxylase (TH) gene expression in the two populations of dopamine cells identified by their content in TH. 35-S-labelled cDNA probe complementary to TH mRNA was used on paraformaldehyde picric acid fixed tissue sections. In situ hybridization was followed by TH immunohistochemistry on the same section. Radiolabelled hybrids were detected by autoradiography with nuclear emulsion.

TH mRNA was detected in TH immunoreactive neurons. Differences in the levels of TH mRNA were observed among the dopamine cells. The number of cells expressing TH mRNA decreased dramatically in patients with Parkinson's disease.

7.3

DIVERSITY OF ULTRASTRUCTURAL CHANGES OBSERVED IN THE NEUROPILE OF THE CAUDATE NUCLEI OF PARKINSONIAN PATIENTS. JP.Machado-Salas,D.Martínez*J.Aceves A.Cornejo*O.Ibarra*J.Valadez*J.Kuri*J.Guzmán*and A. Bazaldúa*Neuromorfología,Neurocs,ENEP Iztacala UNAM,Neurociencias,CIEA,IPN,México,D.F.,Neurocir Neurología Cto Med Occid IMSS Guad,Jal.,and Neurocir,Hosp Espec. Puebla, Puebla. MEXICO

Recent attempts to cure Parkinson's disease by autologous implant of adrenal medulla into the Caudate nucleus, have shown rather diverse and incongruous clinical evolution.Our multidisciplinary and interinstitutional research group has approached this question.Here, we report our preliminary E/M findings made in the neuropile of caudate nuclei obtained during surgery, while lodging the implant in Parkinsonians.Each patient showed a different degree of neuropile degeneration, but the presence of cytoplasmic neuronal vacuoles, spinulae (at axo-spinous synapses),elongated nuclei in degenerated cells, axonal degeneration and diverse amount of dense-core vesicles were the most oustanding differences from one case to another. Our findings provide the first ultrastructural data that allow to postulate the existence of several populations of PK patients, who shall respond differently to medical and surgical therapies.

*Res.Proj.partly supported by PADEPUNAM y ALPHA Ls

7.4

AUTOPSY FINDINGS IN A PARKINSON'S DISEASE PATIENT TREATED WITH ADRENAL MEDULLARY TO CAUDATE NUCLEUS TRANSPLANT F.C. Dohan* J.T. Robertson* C. Feler* J. Schweitzer* C. Hall * J.H. Robertson* (SPON:A.R.Wyler)Dept of Neuropathology,Univ. of TN,Memphis, TN 38163

We report a patient with Parkinson's disease transplanted with autologous adrenal medulla to caudate necleus who had multiple post operative complications and died 121 days after surgery. During hospitalization she showed no clinical improvement. At autopsy, microscopic examination revealed the characteristic CNS findings of Parkinson's disease, but examination of the graft showed necrotic tissue, macrophages, blood vessels, fibroblasts, collagen, chronic inflammatory cells, and occasional foreign body giant cells. No chromaffin cells could be identified in numerous sections stained by both H & E as well as the immunoperoxidase method. The results of this study suggest the need for caution in interpreting the mechanism for any long term beneficial result that might be obtained from this procedure.

7.5

CATECHOLAMINE LEVELS ARE REDUCED IN THE ADRENAL MEDULLA OF PARKINSON PATIENTS RECEIVING AUTOLOGOUS TRANSPLANTATION. M.G. Hartman*, N.G.F. Cooper and J.T. Robertson* (SPON: W. L. Byrne). Depts. of Neurosurgery, Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163.

Adrenal medulla harvested at the time of autologous transplantation into caudate nucleus for treatment of intractable Parkinsonism was assayed for catecholamines. Tissue was obtained from five patients age 46 to 64 (mean 56.6) with symptom duration of 5 to 27 years (mean 11.8). All had severe bradykinesia, with varying degrees of rigidity and tremor. Each was maintained on carbidopa/L dopa until surgery. Grossly, the medullary tissue appeared atrophic and was unremarkable on histologic examination. Medulla was disected free from the gland in Euro-Collins solution (4 ^{0}C) and then stored in liquid nitrogen prior to analysis. High performance liquid chromatography with electrochemical detection was used to assay the tissue levels of norepinephrine, epinephrine, and dopamine. Catecholamine levels (ug/mg protein) were markedly decreased in four of five patients. Mean reductions of 75% from post mortum controls were noted in each catecholamine assayed, although one patient had a level approaching that of age-matched controls. Compared to medulla obtained from brain-dead patients at the time of organ procurement, concentrations were significantly reduced. Epinephrine was the predominate catecholamine, with mean level of 84.4 µg. It accounted for 91.5% of the total assay,and norepinephrine 8.2%. Mean norepinephrine concentrations of 7.6 µg were found. Dopamine was present in small amounts, mean 0.21 µg, or 0.3% of the total catecholamines measured. No age, drug, dose, or duration of symptom correlation was identified. Since anesthetic technique, and timing of surgery were identical in each case, and as no post mortum interval was involved, these levels are felt to be highly representative of the *in vivo* status of medullary tissue. This data suggests that the catecholamine depleted adrenal medulla from Parkinson's patients may be unsuitable for autologous transplantation. Supported in part by NIH:NEI EY02708.

7.6

TRANSCORTICAL INTRAVENTRICULAR ADRENAL MEDULLARY GRAFTING IN THE TREATMENT OF HEMI-PARKINSON MONKEYS. R. A. E. Bakay, K. Sweeney,* H. Colbassani,* R. L. Watts, P. M. Iuvone, L. Byrd. VA Medical Center, Emory University, Atlanta, Georgia 30322.

In order to investigate the most commonly used adrenal medullary (chromaffin) grafting technique. Nine M mulatta (2-4 years old) were used in this study. After baseline studies were obtained, hemiparkinsonism was induced by titrated intracarotid injections of 0.4-0.8 mg/kg MPTP. Followup studies performed for at least 4 months following MPTP intoxication. Once an adequate data base was achieved, chromaffin tissue was grafted to the head of the caudate.

Apomorphine (0.15 mg/kg)-induced rotation varied up to 35% in control subjects but did not improve over 6 mos. or longer. A marked decrease in rotational behavior was observed 1 mo. after grafting and persisted for at least 9 mos. Amphetamine (0.5 mg/kg)-induced rotation was significantly less in transplanted subjects as compared to controls. Biochemical and electrophysiological studies were also performed. Symptomatic hemiparkinson animals anatomical studies demonstrated a >80% depletion of substantia nigra TH immuno-reactive cells. Chromaffin volume was reduced to 30-20% of original implant size and surviving chromaffin cells were less than 10% of the original graft. Chromaffin cells were identified by TH, DBH, PTNM immunocytochemical staining and two separate types of chromaffin histological techniques.

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7.7

REJECTION OF FETAL SUBSTANTIA NIGRA ALLOGRAFTS IN MONKEYS WITH MPTP-INDUCED PARKINSON'S SYNDROME. C.R. Freed, J.B. Richards, C.J. Hutt*, E.H. Kriek, and M.L. Reite, Depts. of Med., Pharm. and Psych., Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

A severe Parkinsonian syndrome was established in a male Bonnet monkey (m. Radiata) with the neurotoxin MPTP-HCl. Two months after lesioning, the animal was transplanted unilaterally into right caudate, putamen and globus pallidus with substantia nigra from an 8 weeks' gestation fetus. By 11 weeks after transplant, the animal could sustain a posture and the left hand became the preferred hand. Seven months after transplant, a second graft was placed into left caudate and putamen to improve motor function on the right side. Within six days, the animal lost function of the previously improved left arm and leg. Pathologic examination showed the continued presence of tyrosine hydroxylase positive cells and fibers in all transplant tracks on the right. A focus of lymphocytes was seen in one transplant track. The newly transplanted tracks on the left showed only tissue debris. Transplant rejection was then studied in a group of eight Patas monkeys which were lesioned with MPTP and then transplanted with fetal substantia nigra from 7 to 9 week monkey fetus. Three months after transplant, the animals were challenged with a second fetal transplant to look for graft rejection. These results indicate that rejection may occur following a second transplant of fetal tissue.

7.8

CEREBRAL METABOLIC EFFECTS OF DOPAMINERGIC IMPLANTS IN CAUDATE NUCLEI OF HEMIPARKINSONIAN MONKEYS. L.J.Porrino[1,2], E.Palombo[2]*, K.S.Bankiewicz[1], J.Viola[2,3], J.Jehle[2]*, and I.J.Kopin[1]. [1]NINCDS; [2]NIMH; [3]HHMI, Bethesda, MD 20892.

A recent approach to the treatment of Parkinson's Disease has been the use of dopamine-producing tissue implanted into the brains of patients with the disease. We have used the quantitative autoradiographic 2-[^{14}C]deoxyglucose method to assess alterations in functional activity that accompany implants of dopamine-producing adrenal and fetal tissue into the caudate nuclei of MPTP-induced hemiparkinsonian monkeys. After implant of adrenal medullary tissue, recovery of voluntary movement of the affected limbs was observed, but did not persist. The pattern of local cerebral glucose utilization (LCGU) seen in monkey with adrenal implants was identical to that seen in untreated hemiparkinsonian monkeys. In contrast, following fetal mesencephalic tissue implants, recovery of motor function was observed, along with reversal of the LCGU changes associated with hemiparkinsonism; i.e. the increase in the external globus pallidus, the decrease in the subthalamus, and the increases in the striatum on the lesioned side appear to be normalized. Fetal mesencephalic implants not only normalize motor performance, but also restore symmetry in cerebral metabolic rates not seen after adrenal implants.

7.9

HEMIPARKINSONISM IN MONKEYS AFTER UNILATERAL CAUDATE NUCLEUS INFUSION OF MPTP : BEHAVIOR, HISTOLOGY AND CHEMISTRY. H. Imai, T. Nakamura*, N. Miyashita*, K. Nishi*, and H. Narabayashi*. Dept. of Neurology, Juntendo Univ. Sch. of Med., Tokyo 113, Japan.

In an attempt to clarify the role of the dopamine nerve terminals and cell body in MPTP toxicity and to construct a model for pure hemiparkinsonism, we administered 1-4 mg of MPTP HCl directly into the unilateral caudate nucleus of crab-eating monkeys via an Alzet 200-µL osmotic minipump for 14 days. Within a week after the start, the monkeys exhibited a flexed posture and hypokinesia of the contralateral limbs, and spontaneous circling toward the MPTP-treated side. After treatment with apomorphine the circling motion was reversed. These disturbances continued to increase for 3 months and then reached a plateau. No parkinsonian tremor at rest was observed in any of the monkeys. The number of tyrosine hydroxylase (TH) immunoreactive cell bodies in the MPTP-treated side of the substantia nigra was markedly reduced along the entire rostrocaudal extent relative to the untreated side. Neuronal loss in the MPTP-treated side of the ventral tegmental area was slight and dorsal raphe and locus ceruleus neurons were completely spared. TH activity in the MPTP-treated side of the caudate mucleus and putamen was also markedly reduced. In conclusion, MPP^+ uptake at the dopamine nerve terminals alone and retrograde axonal transport to the cell body seemed to be sufficient to destroy nigral dopamine cells in the monkey.

7.10

DOSE RELATED CONTRALATERAL DOPAMINE DEPLETION AFTER INTRA-CAROTID MPTP ADMINISTRATION IN CYNOMOLGUS MONKEYS: VALIDATION OF THE UNILATERAL MPTP MONKEY MODEL M. Guttman, H.C. Fibiger, A. Jakubovic, D.B. Calne, University of British Columbia, Vancouver Canada, V6T 1W5

N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been employed to produce a primate model for Parkinson's disease. In an attempt to create a unilateral lesion, MPTP has been administered directly into the internal carotid artery to produce hemiparkinsonism (Bankiewicz et al. 1986). Four animals had infusion of normal saline and two groups of four monkeys had either 1.25 mg or 2.5 mg of MPTP-HCl infused into the left carotid. Six to ten weeks later, all MPTP-lesioned animals displayed right sided parkinsonism. Two of the higher dose animals displayed signs of flexed trunk posture and reduced activity. HPLC analyses of caudate nucleus and putamen showed that in all MPTP-lesioned animals there was greater than 97% reduction of dopamine (DA) on the left. In the 2.5 mg MPTP group, the right side also showed reduced DA; in the caudate by 61.4%, and in the putamen by 37.8%. Contralateral DA reduction and abnormalities of DA utilization (HVA/DA ratios) were not present in the 1.25 mg MPTP-treated monkeys. These findings demonstrate that unilateral carotid injection of MPTP can damage contralateral DA systems; caution should therefore be exercised in the interpretation of data with this model. With the lower dose, the lesion appears to be purely unilateral, enabling the use of the contralateral side as an internal control.

7.11

IN VIVO BRAIN IMAGING OF THE BASAL GANGLIA (BG) OF NORMAL AND RECOVERED MPTP-TREATED MONKEYS BY POSITRON EMISSION TOMOGRAPHY (PET) USING 6-FLUORO-L-DIHYDROXYPHENYLALANINE (6FDOPA). D.J. Doudet, R.M. Cohen*, R.D. Finn*, H. Miyake*, T.G. Aigner, T. Bruecke*, R.Q. Wan*, C.A. McLellan*, B. Francis*, R. Adams*, J.J. Chen* and C.C. Chiueh. Clinical Brain Imaging/LCM; Lab. of Neuropsychology, NIMH and Radiopharmacy, NMD, NIH, Bethesda, MD 20892.

The sensivity of PET measurements of 6FDOPA accumulation to detect clinical and preclinical damages to the dopaminergic (DA) system of the BG was assessed in 1-Methyl-4-Phenyl-1,2,3,6- Tetrahydropyridine (MPTP)-treated rhesus monkeys. Four animals were given MPTP (2 to 4.8 mg/kg i.v.) 20 to 24 months before the PET studies. They acutely displayed akinesia and rigidity but spontaneously recovered and were evaluated normal neurologically by investigators blind to their previous treatment. Two monkeys received .8 and 1.5 mg/kg in the right internal carotid artery and, after recovery of the acute effects, remained impaired in their left body side. Five normal rhesus matched for age and sex were used as controls. For the PET study, the anesthetized monkeys were first injected with O-15 labeled water to verify that the BG were visualized in at least one slice and that differences in blood flow did not account for variations in 6FDOPA accumulation. The injection of 2 to 5 mCi of highly purified 6FDOPA demonstrated a substantial loss of 6FDOPA accumulation in the BG of the MPTP-treated animals and in the lesioned side of the hemiparkinsonian monkeys compared to controls. The ratio between BG and a non-rich DA area (occipital/parietal cortex) reached 2.5 to 3 in normals compared to 1.4 to 1.8 in the DA depleted animals and dropped to 1 in the affected side of the hemi-lesioned monkeys.

Thus, 6FDOPA, a PET tracer which preferentially labeled DA neurons appears to be a useful tool in the detection of clinical and preclinical changes in the DA system.

7.12

GM1 GANGLIOSIDE EFFECTS ON THE MPTP-DAMAGED DOPAMINE SYSTEM IN MICE AND CATS. J.S. Schneider and A. Yuwiler, Dept. of Neurology, UCLA School of Medicine and Neurobiochem. Lab., Brentwood VA Hosp., Los Angeles, CA 90024-1769.

Exogenously administered GM1 ganglioside has been suggested to act as a neurotrophic factor in central aminergic and cholinergic systems. The present experiments were performed to see if GM1 ganglioside might have beneficial effects on the MPTP-damaged dopamine (DA) system in mice and cats. Young mature (8-10 wks) C57 black mice which received MPTP and GM1 had significant increases (dependent upon length of GM1 exposure) in striatal DA content as compared to mice which received only MPTP. This DA increase was accompanied by a significant increase in the striatal DAergic terminal field. GM1 had no protective effects against MPTP toxicity, was effective when its onset was delayed in relation to MPTP administration, and its effects appear to be permanent. Interestingly, GM1 did not cause significant striatal DA increases when administered to aged (8-10 mos) MPTP-treated mice. While cats administered MPTP all showed massive striatal DA depletions, animals which received GM1 had significantly increased DA levels as compared to cats which only received MPTP. GM1 appeared to stimulate both terminal and axon collateral sprouting within the denervated striatum but seemed to have no protective effect on nigral cell degeneration. These results suggest that GM1 can stimulate recovery in the DA system in the striatum of young mature animals but that the beneficial effects of GM1 treatment may decline with age.

8.1

BOTH BEHAVIORAL AND NEURONAL PERFORMANCE ARE IMPROVED BY INCREASED ATTENTION. H. Spitzer*, R. Desimone, and T. Moran (SPON. N. Allon). Lab. Neuropsychology, NIMH, Bethesda, MD 20892; Dept. Biomedical Engineering, Technion, Haifa, Israel; and Lab. Clinical Studies, DICBR, NIAAA, Bethesda, MD 20892.

To test whether increasing the amount of attention devoted to a stimulus influences neuronal activity, we studied the responses of neurons in area V4 of two rhesus monkeys to stimuli presented within the context of a discrimination task performed at two different levels of difficulty. The task was a modified version of matching-to-sample. The stimuli were bars varying in size, orientation, and color. Some cells were tested with orientation as the discriminative feature, and others were tested with color. In the nonmatching trials of the easy condition, the sample and test stimuli differed by 90° in orientation or about 77 nm in wavelength, whereas in the difficult condition they differed by 22.5° or 19 nm, respectively. Behavioral evidence indicated that the monkeys' discriminative abilities improved when the task was made more difficult. Correspondingly, neuronal responses to stimuli became larger and more selective in the difficult condition. It was demonstrated in a control experiment that larger responses and narrower bandwidths during the difficult condition occurred only for attended stimuli within the receptive field.

8.2

TASK-SPECIFIC SIGNALS IN AREA V4 OF MONKEY VISUAL CORTEX. J.H.R. Maunsell, G. Sclar* and T.A. Nealey*. Dept. Physiology, Univ. of Rochester, Rochester NY 14642.

In macaque monkeys trained to perform an orientation match-to-sample task, many neurons in area V4 show response modulation that depends critically on which orientation the animal has been cued to look for (Haenny *et al.* **Exp Brain Res.** 1988). Such neurons may be very active during trials in which the animal is cued to look for one orientation, yet unresponsive when identical visual stimuli appear during trials in which the animal is looking for other orientations. Thus these cells seem to encode information about the orientation the animal is looking for, rather than the orientation falling on the retinas.

We have recorded from V4 in a monkey trained to perform an orientation match-to-sample task in which the sample was either presented visually (by showing the animal the correct orientation at the start of each trial) or tactilely (by having the animal feel the orientation of a bar he can not see). Of 36 neurons tested under both conditions, eight (22%) showed statistically significant changes in activity related to the sample orientation in both cases. Because these neurons convey an identifiable signal that can be dissociated from any particular sensory input or any immediate motor response, their signal must be considered an abstract representation of the cued orientation.

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8.3

RESPONSES OF VISUAL CORTICAL NEURONS DURING A FOCAL ATTENTIVE TASK. B.C. Motter (SPON: S.Mitchell) V.A. Medical Center and Depts. Physiology and Neurosurgery, SUNY Health Science Center, Syracuse, NY 13210.

Reponses of neurons in V1, V2, & V4 to flashed stimuli were recorded during the performance of a task requiring visual attention to events at selected spatial locations. Monkeys were trained to discriminate the orientation of a briefly (200ms) flashed bar stimulus appearing 2 to 10 degrees from a central fixation point. The target stimulus was presented alone (25%) or in a circular array (75%) of identical but randomly oriented stimuli. At the beginning of a trial each position of the array was marked by a small dot cue. The target stimulus position was then explicitly cued by removal of all other cues prior to the presentation of the stimulus array. The arrays were positioned so that either the target or another array stimulus was located in the neuron's receptive field. The composition of stimuli and arrays were based on quantitative assessments of the field position and size, and the preferred orientation, color and stimulus size for each neuron studied. Response differences between stimuli presented at attended versus non-attended locations were found in about 50% of V2 & V4 neurons and in about 20% of V1 neurons studied. Such differences were usually limited to responses evoked by stimuli with orientations near the tuning point of the neuron. Stimulus response latencies for V4 neurons at attended locations were often increased.

8.4

BEHAVIORAL ROLE OF STIMULUS SELECTIVE NEURONAL ACTIVITIES IN THE SUPERIOR TEMPORAL SULCUS OF MACAQUE MONKEY. A.MIKAMI and K.NAKAMURA Dept. Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484 JAPAN

In the superior temporal sulcus (STS) of macaque monkey, neurons showed selective response to the complex visual feature including human and/or monkey faces (Bruce et al 1981, Perret et al 1982, Mikami 1987). In order to test behavioral role of the stimulus selective neurons in STS, neuronal activities were recorded during the sequential discrimination task with delay. With pressing lever, the first sample picture was presented for 1 sec. After 1-3 sec of delay period, a picture appeared again. This sequence was repeated until a new picture appeared. Monkey was trained to release the lever when a new picture appeared. In about two third of stimulus selective STS neurons, activities were modulated depend on the number of repetitions and/or the reward contingency. A small number of neurons showed stimulus selective activities during the delay period. These data suggest that the STS neurons may be involved in the process to attend and memorize a specific visual pattern.

8.5

ATTENTIONAL MODULATION OF INFEROTEMPORAL NEURON RESPONSES TO VISUAL FEATURES. J.M. Fuster. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

Neurons of inferotemporal (IT) cortex discriminate and temporarily retain colored stimuli (Fuster and Jervey, J. Neurosci., 2:361, 1982). Are such discriminant and mnemonic neural properties dependent on the degree of attention that the animal devotes to the chromatic stimulus attribute? Four monkeys were trained to perform a delayed match-to-sample task with compound stimuli. The six sample-stimuli utilized were coextensive, isoluminous, and briefly presented, one per trial, in a central location; each consisted of a colored disk (red or green) with a gray symbol in the middle, which indicated whether the symbol itself or the background color had to be remembered for later behavioral decision. Thus, the relevance of color and the attention that the animal was assumed to pay to it were determined by the symbol embedded in it. Extracellular activity was recorded and analyzed from 301 IT units. Analysis of their reactions to the stimuli revealed both symbol- and color-differentiating cells. Some of the latter discriminated the two colors equally well--i.e., with the same differential firing rates--whether color was relevant or not. Others did it better if color was relevant than if it was not; their reaction to their preferred color was enhanced if the contextual cue (symbol) indicated that color had to be attended to. In a few cells, the relevance effect was detectable during the retention period, when the sample stimulus was no longer present. No effects of relevance were noted in 92 striate cortex cells. The latency of the color response of IT cells was correlated with susceptibility to attentional modulation. Long-latency (>180 ms) attention-sensitive cells, were especially common in the lower bank of the superior temporal sulcus. The results indicate that the discrimination and memory functions of IT neurons are facilitated by the focusing of attention on the attributes of the visual scene to which they are attuned; the latency data validate the psychophysical inference of serial processing in selective visual attention (Koch and Ullman, Human Neurobiol., 4:219, 1985).

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8.6

DIRECTING ATTENTION TO COMPLEX OBJECTS. Jeremy M Wolfe*, Kyle R Cave*, and Karen P Yu* (SPON: J. Sandell), Dept. of Brain & Cog. Sci., MIT, E10-137, Cambridge, MA 02139

Visual processing is divided between an initial "pre-attentive" stage where simple features can be processed in parallel across the visual field and a later stage where complex processing is accomplished by directing attention to parts of the field. If pre-attentive processes can only analyze simple features (e.g. color, motion, etc.), how is attention guided in a search for an object (e.g. a cat) not defined by a unique feature? Visual search data suggest that without guidance, attention moves at random in a serial search for a target. However, the apparent ease with which real-world objects are found argues against a haphazard search. We believe that attention may be directed to a complex object by combining information from several feature processors. Thus, while a color processor cannot identify a cat, it can divide the field into loci that can and cannot contain a cat. Attention should not be directed to a locus labelled "green" if one is seeking a cat. In our visual search experiments, target items are defined by triple conjunctions of color, size, and form. Though all other items share one feature with the target, search times are independent of the number of items, as they would be if the target was defined by a unique color or size. This "parallel" search for triple conjunctions strongly suggests that the pre-attentive processes can guide attention even when no single feature defines the target.

8.7

VANISHING TARGETS IN SIMULTANAGNOSIA: FAILURE TO SUSTAIN VISUAL ATTENTION OVER AN ARRAY. M. RIZZO AND D. ROBIN. Div. Behavioral Neurology and Cognitive Neuroscience, Univ. of Iowa, College of Medicine, Iowa City 52242

Simultanagnosia (visual disorientation), the erratic disappearance of targets from direct vision, offers an important opportunity to probe human attention. We studied two subjects with simultanagnosia due to CT/MR verified lesions of the superior visual association cortices. Their defect was not explainable by poor acuity, abnormal eye movements, or visual field defects. Both could orient attention to spatial targets in vision, audition, and even to cross modal targets, comparably to 30 controls. Here we report on a separate paradigm which allowed further assessment of the defect. The task required an immediate response to the appearance or extinction, at unpredictable intervals, of any dot in a static CRT display of 1000 random dots. The subject failed to detect 50% of 400 events without predilection for location, had 19% false alarms, and had prolonged reaction times. The results support the hypothesis that simultanagnosia is due to inability to maintain visuospatial attention across an array. The inefficient processing of simultaneous signals in parallel neural channels may lead to failure to detect targets which thus seem to vanish. The finding suggests that operations of directed and sustained visual attention are dissociable at the level of the visual association cortices.

8.8

ABSENCE OF VISUAL RESPONSIVENESS IN INFERIOR TEMPORAL CORTEX IN MACAQUES LESS THAN FOUR MONTHS OF AGE. H.R. Rodman, J.P. Skelly* and C.G. Gross. Dept. of Psychology, Princeton University, Princeton, NJ 08544.

Although inferior temporal (IT) cortex is necessary for visual learning and pattern perception in adult monkeys, several lines of behavioral and anatomical evidence suggest that IT takes on its full adult role only slowly; moreover, this maturation process may be sexually dimorphic (Bachevalier & Mishkin, 2nd Conf. Neurobiol. of Beh., 1984). Previously, we reported that single-unit response properties in IT are adult-like in female macaques by 4-6 months of age (Rodman et al., Neurosci. Abs. 13: 1539). We now present data on IT properties in male macaques 4-7 months old (139-219 days at the time of recording) and in younger macaques of both sexes (41-113 days at the time of recording).

Recordings were made in repeated sessions under immobilization and N_2O anesthesia. Single units were tested with slits of light, dark edges and a set of complex 3-D objects similar to those used for our previous studies. Models and photographs of monkey faces were also used.

Visual responsiveness in IT was virtually absent in each of the five younger monkeys (three males and two females), except for a few responsive units found in one male in the fourth month of age. Control recordings in striate cortex and extrastriate visual area MT showed normal responsiveness and stimulus selectivity.

In one of the two older males, visual properties in IT (including receptive field size and location and stimulus selectivity) were adult-like, similar to what we found in 4-6 month old females. In the other animal, however, most IT units were unresponsive, despite the presence of normal activity in striate cortex.

8.9

CIRCUIT PROPERTIES OF INFERIOR TEMPORAL CORTEX IN THE MACAQUE. **P.M. Gochin, E.K. Miller, C.G. Gross, G.L. Gerstein.** Dept. Psychol., Princeton Univ., Princeton, NJ 08544, Dept. Physiol., Univ. Penn. Sch. Med., Phila., PA 19104.

We have suggested that the visual scene is represented in inferior temporal (IT) cortex by the pattern of activity across a population of IT neurons each selective for some aspect or aspects of the visual stimulus (Gross et al., in Pattern Recognition Mechanisms, 1985). This view of IT cortex is similar to that postulated for the later stages of some contemporary models of pattern recognition. The purpose of this study was to obtain information about interactions among IT neurons that could help to constrain and develop such models.

We recorded IT activity from bundles of three microelectrodes. Multi-unit activity on each electrode was segregated into single unit activity with a spike sorting apparatus enabling us to study the simultaneous activity of up to nine isolated neurons. The monkey fixated a small spot while stimuli were presented extrafoveally.

Cross-correlation analysis demonstrated that some neuron pairs interact with some combination of shared excitatory input and direct synaptic connections, while other pairs show independent patterns of firing. Furthermore, neurons recorded on the same electrode were more likely to have functional connections and similar stimulus selectivity than neurons recorded on adjacent electrodes. These results will be discussed in relation to models of pattern recognition.

8.10

ORGANIZATION OF VISUAL PROJECTIONS TO PARIETAL AND TEMPORAL CORTEX IN THE MACAQUE. I.S. Baizer, L.G. Ungerleider and R. Desimone. Laboratory of Neuropsychology, NIMH, Bethesda, Maryland, 20852.

Visual information is relayed from striate through prestriate to parietal cortex, which has visuospatial functions, and to temporal cortex, which is critical for object recognition. To identify and compare the distributions of cells in prestriate cortex that project to these two destinations, we made multiple injections of two different fluorescent tracers into the lower bank of the intraparietal sulcus (following removal of the upper bank) and into the inferior temporal cortex in 3 rhesus monkeys. Cells projecting to parietal and temporal cortex were located mainly in different prestriate areas: MT, MST, STP, and peripheral V2 projected only to parietal cortex, whereas TEO projected only to temporal cortex. Areas containing cells projecting to both destinations were V4 (including its ventral and two dorsal subregions, AL and PM) and the posterior bank and floor of the superior temporal sulcus (STS) outside MT. In both V4 and STS, labeled cells projecting to parietal and temporal cortex were intermingled, though the projection to parietal cortex was heavier from peripheral than from central V4. The laminar distribution of labeled cells suggests that V4 provides feedforward information to both parietal and temporal cortex, whereas zones within the STS provide both with feedback information. Supported by RO1MH42130.

8.11

RESPONSE PROPERTIES OF NEURONS IN MACAQUE INTRAPARIETAL SULCUS. C.L. Colby, J.-R. Duhamel and M.E. Goldberg Laboratory of Sensorimotor Research, National Eye Institute, N.I.H., Bethesda, MD 20892.

We have used visual, oculomotor and attentional tasks to study neurons in and around the intraparietal sulcus (IPS) in the alert monkey and have found a variety of cell types which fall into two broad classes.

Cells in the first class have relatively simple visual responses. Receptive fields are restricted and stationary stimuli evoke on/off responses. More robust responses are elicited by moving stimuli and some cells exhibit directional selectivity. Cells in this class appear to have no eye movement related activity and no attentional component to their responses.

Cells in a second class have more complex response properties. These respond well to stationary visual stimuli and their visual responses are enhanced when the stimulus is also the target for a subsequent eye movement or an attentional response. Two types of cells also discharged before the saccade in a task where the target is flashed on briefly and the saccade is made in total darkness. One type fired separate bursts at the time of target appearance and again just prior to the saccade. A second type produced a tonic response, beginning at target onset and continuing until initiation of the saccade, as though it were holding the target position. When tested in a learned saccade task, where no target appeared, some but not all cells in each category fired before saccades to a remembered location.

The results suggest that the IPS contains anatomically and physiologically distinct cell populations.

8.12

ORBITAL POSITION EFFECTS ON SACCADE RESPONSES OF AREA LIP NEURONS. R. Andersen, J. Gnadt, L.Fogassi, M. Bracewell, S. Barash, G. Robertson and G. Essick. Dept. Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Area LIP is a subdivision of the posterior parietal cortex buried within the intraparietal sulcus whose cells have saccade-related activity. The effect of eye position on the saccade responses was examined using a memory saccade task designed to separate visual from saccade-related activity. The task required the animal to memorize the location of a briefly flashed target and to make a saccade to that remembered location after a variable period of time. It was found that the direction and amplitude tuning of the motor fields of the cells did not change but the *magnitude* of the saccade response was modulated by eye position.

Standard linear regression techniques were used to test whether a first order linear model with independent variables of horizontal and vertical eye position would describe the change in gain of the saccade response with change in eye position. Two-thirds of 39 neurons tested showed a statistically significant ($p<.05$) effect of eye position; these cells are said to have gain fields. Of the cells with gain fields a plane was the best fit of the data for 52% of the cells and another 38% showed a significant planar component. Only 8% of the cells had no planar component in their gain fields

These data indicate that a planar model describes a large majority of the gain fields of the saccade cells. A similar planar function for the variation of visual response with eye position has been found for area 7a neurons and a network model trained to localize the location of visual stimuli in head centered coordinate space produced these same planar gain fields (Andersen et al., Science, 1985; Zipser and Andersen, Nature, 1988). The present results suggest that the planar gain function could represent a general strategy by the brain to perform coordinate transformations. In this instance the gain function could be used to transform from the retinal coordinates of the visual target to the head centered coordinates of the location of the eyes in the orbits required to foveate the target.

9.1

EFFECTS OF FMRFamide ON ISOLATED HEART MUSCLE CELLS OF THE MEDICINAL LEECH. K.J. Thompson and R.L. Calabrese, Dept Biology, Emory Univ., Atlanta, GA 30322.

The neurogenic/myogenic leech heart responds to the neuropeptide FMRFamide. Bath application of FMRFamide or nerve stimulation can cause tonic contraction, acceleration of the myogenic rate, and induction of myogenic activity in quiescent hearts (Kuhlman et al, 1985; Li and Calabrese, 1987). Intracellular recording and voltage clamp methods were used to examine the electrical responses of dissociated heart muscle cells to bath applied FMRFamide. Muscle cells depolarize in response to FMRFamide at concentrations as low as $1X10^{-12}M$. This depolarization is accompanied by a conductance increase and continues as long as FMRFamide is present. Response amplitude increases in saline containing 10 mM Ba^{++}, and is severely attenuated by 5 mM Mn^{++} or 50 µM Cd^{++}. In voltage clamp, FMRFamide induces a net inward current that appears to underlie the depolarization observed in current clamp. This FMRFamide-gated conductance increase in isolated heart cells may account for some of the modulatory actions of FMRFamide observed in the intact heart. Supported by NIH NS24072-04 to R.L.C.

9.2

VOLTAGE CLAMP ANALYSIS OF A HYPERPOLARIZATION-ACTIVATED INWARD CURRENT IN HEART INTERNEURONS OF THE MEDICINAL LEECH. J.D. Angstadt and R.L. Calabrese. Dept. of Biology, Emory University, Atlanta, GA 30322.

Previous current clamp studies of heart interneurons (HN cells) in the leech suggested that intrinsic ionic conductances may contribute to the recovery from inhibition exhibited during normal bursting activity (Peterson, 1983; Arbas and Calabrese, 1987). In this study, we used the single-electrode voltage clamp technique to characterize a voltage-sensitive inward current (I_h) activated by hyperpolarization of HN cells. I_h is a slow, non-inactivating inward current with an activation threshold near -50 mV. I_h persists in the presence of Mn^{++} or Co^{++} (5 mM) but is blocked by extracellular Cs^+ (2-5 mM). The reversal potential (-21 ± 5 mV) is shifted by changes in extracellular Na^+ or K^+ but not Cl^-, suggesting that I_h is produced by a mixed Na^+/K^+ conductance. The steady state conductance ($g_{h\infty}$) reaches a maximum between -70 and -80 mV. The voltage sensitivity and kinetics of I_h are consistent with a role for this current in recovery from inhibitory inputs and subsequent burst generation. Supported by NRSA postdoctoral fellowship #NS08089-02.

9.3

CONSTRUCTION OF A COMPUTER MODEL OF THE LEECH HE-MOTONEURON: IMPORTANCE OF SYNAPTIC POTENTIALS.
E. DE SCHUTTER* (SPON: European Neuroscience Association) and R.L. CALABRESE, *Dept. of Neurology, Univ. of Antwerp (UIA), B-2610 Antwerp, Belgium, and Dept. of Biology, Emory Univ., Atlanta, GA 30322.

The HE-cell is the motoneuron that controls the leech *(Hirudo medicinalis)* heart muscle. A compartmental model of the cell was constructed, computer simulations of the response to a hyperpolarizing pulse and of IPSPs were used to determine the equivalent cable parameters of the neuron.

In 13 HE-cells (isolated ganglia, normal Ringer) the response to 10 -0.3nA pulses during the bursting period was averaged (single electrode current clamp, sampling rate 3.6 kHz). Two time constants were always present: 2.1±0.7 ms and 35.5±2.1 ms, in some cells a long time constant of 185±214 ms was found. Average input resistance was 118±12 MΩ. Ten IPSPs in a HE(L4)-cell were digitized and averaged. The hyperpolarization phase could be fitted to a single exponential with a time constant of 2.4±0.6 ms, the final phase of the repolarisation slope had a time constant of 71.9±7.1 ms.

The cell was filled with HRP, developed, fixed and mounted for microscopy without dehydratation. Morphometrical analysis was used to construct a 113 compartmental model. All simulations were done with the Nodus software on a Macintosh computer.

The hyperpolarization experiment was simulated with a membrane capacitance (C_M) of 2.0 µF/cm², a membrane resistance (R_M) of 19 kΩcm² and a cytoplasmic resistance (R_i) from 100 to 200 Ωcm. IPSPs were simulated with a simple kinetic scheme (alpha-function). A normal sized IPSP (5 to 10mV at the soma) was obtained if synapses on at least 50% of the 36 dendrites were fired, IPSPs at 75% of the dendrites gave the best results. The hyperpolarizing phase of the IPSP was approximated with an alpha of 0.9 $^1/_{ms}$, but the repolarisation could only be simulated with higher values for R_M: 38 kΩcm². Both the hyperpolarization experiment and IPSPs could be simulated if different values for R_M in soma, main segment and axon (16 kΩcm²) versus dendrites (50 kΩcm²) were used, with C_M 1.85 µF/cm² and a minimum for R_i of 200 Ωcm.

Conclusion: in this motoneuron membrane resistance is higher in dendrites than in soma and main segment. IPSPs are caused by firing of synapses on most of the dendrites.

9.4

THE ONTOGENY OF THE RESPIRATORY PATTERN GENERATING SYSTEM OF THE FETAL LAMB. L.R.C. Cooke* and P.J. Berger (SPON: D. L. Macmillan). Monash University Centre for Early Human Development, Clayton, Vic., 3168, Australia.

The ontogeny of the neural system which produces the ventilatory rhythm was investigated using a chronically-instrumented fetal lamb preparation. Electrodes were placed in the diaphragm (Di), external intercostal (EIC) and external oblique (EOb) muscles of 13 fetal lambs (47-62 days gestation), using maternal general anaesthesia. After recovery (3 days), electromyogram activity was recorded for 2-hour periods at 5-day intervals until 135 days gestation (term =147 days). Early in gestation (50-70 days), the predominant form of activity recorded consisted of approximately coincident periods of continuous discharge of all three muscles, which lasted up to several minutes. In addition, two stereotyped forms of phasic activity were recorded from the Di. Isolated bouts of up to 50 brief (60-100 ms) bursts, lasting up to 1 min, occurred 1-3 times per hour while prolonged (up to 15 min) sequences of repeating clusters of brief (30-60 ms) bursts occurred every 2-3 hours. The EIC and EOb were silent during short bouts of phasic Di activity; occasionally the EIC discharged in synchrony with the Di during prolonged sequences. The left and right sides of the Di fired synchronously in all episodes of phasic activity. Unilateral phrenectomy abolished all activity on the ipsilateral hemidiaphragm, demonstrating its neural origin. High cervical (C1) transection of the spinal cord abolished all phasic Di activity, but not trains of continuous discharge. Thus, early in gestation, phasic Di activity was generated by neural pathways organised in a manner similar to the mature respiratory pattern generating system. Subsequent development involved progressive increases in the incidence of phasic Di activity and the occurrence of synchronous EIC bursting, together with an increase in burst duration and progressive elaboration of the structure of inspiratory muscle bursts into the mature, progressively augmenting form.

9.5

NEURONAL MECHANISMS CONTRIBUTING TO THE ALTERATION OF THE RESPIRATORY RHYTHM AT FLIGHT ONSET IN THE LOCUST. J.M.Ramirez*, K.G.Pearson, Department of Physiology, University of Alberta, Edmonton, Canada, T6G 2H7.

It has been well established that the respiratory rate in locusts increases at flight onset, but the neural processes underlying this increase are not understood. By means of intracellular recordings from respiratory neurons in quiescent and tethered flying locusts we have found that the respiratory rhythm is reset at flight onset by an activation of inspiratory and inhibition of expiratory neurons. This reset also occurs in deafferented animals thus demonstrating that flight and respiration are centrally coupled. Neurons which may contribute to this coupling were identified in the suboesophageal ganglion. These neurons were tonically active in the quiescent locust and strongly inhibited at flight onset. Injection of hyperpolarizing currents into these neurons increased the respiratory rate and release of excitation following depolarizing current injection sometimes led to the initiation of flight. Reduction of activity also led to an activation of inspiratory neurons, suggesting that the increase in the respiratory rate may be caused by disinhibiting inspiratory neurons in the respiratory rhythm generator. Consistent with this was our finding of a pair of inspiratory interneurons in the metathoracic ganglion which were strongly activated during flight and which increased the respiratory rate when injected with depolarizing currents. We conclude that one mechanism contributing to the increase in respiratory rate at flight onset is a simultaneous disinhibition of the flight and respiratory systems by neurons descending from the suboesophageal ganglion.

9.6

FEEDBACK AND FREQUENCY REGULATION IN LOBSTER (HOMARUS AMERICANUS) CARDIAC GANGLION. A. Berlind, Biology Dept., Wesleyan Univ., Middletown, CT 06457.

The crustacean cardiac ganglion (CG) has traditionally been viewed as a two layered system in which pacemaking is a function of the four small cells. The work reported here shows that there is strong electronic feedback from the five motorneurones (MNs) to the small cells by which endogenous burst-organizing potentials (driver potentials, or DPs) and their hyperpolarizing afterpotentials contribute to regulation of bursting frequency. When the MNs of isolated CGs were selectively silenced with TTX, the small cells continued organizing bursts of activity, which recurred at an average frequency 38% higher than full bursting in normal saline. The average burst duration was not altered. DPs were evoked in TTX-treated MNs by electrical stimulation, by ionic alteration of the medium, or by treatment with the peptide proctolin. DPs which occurred synchronously with small cell bursts prolonged and intensified the bursts. DPs evoked in MNs during the interburst triggered small cell bursts even at very short intervals after a spontaneous burst had occurred. All small cell bursts which were associated with MN DPs were followed by interburst intervals of longer than normal duration. The decrease in instantaneous burst frequency (increase in total burst cycle duration) caused by MN DPs was similar in magnitude to the drop in burst frequency observed when the ganglion recovered normal activity after TTX washout.

9.7

EFFECTS OF LONGTERM REMOVAL OF EXTRINSIC SYNAPTIC INFLUENCES ON BURST-GENERATING PROPERTIES IN THE LOBSTER PYLORIC NETWORK. J.Simmers* and M.Moulins. Lab. Neurobiol. Comp., CNRS, 33120 Arcachon, FRANCE.

The pyloric network in the stomatogastric ganglion (STG) of Jasus produces rhythmic motor output in vitro as a result of the oscillatory properties of pyloric neurons. These properties are conditional, however, since suppression of unpatterned central inputs to the STG by cutting the stomatogastric nerve (stn) rapidly causes loss of bursting in pyloric neurons.

In contrast, when such deafferented quiescent STG are maintained in vitro on a longterm basis (max. 10 days), pyloric rhythmicity returns (from day 4-6) and is identical to the basic pattern normally expressed when the stn is intact. This transition is longlasting since reacquisition of rhythmicity has been observed in in vitro preparations from animals in which the stn was cut up to 12 months previously. Moreover, the fundamental change appears to occur in the intrinsic properties of the pyloric neurons themselves; in rhythmic deafferented ganglia, individual pyloric cells continue to oscillate after further experimental isolation from all other elements in the pyloric network. The results therefore suggest that the prolonged absence of extrinsic inputs to pyloric neurons allows the expression of a true endogenous oscillatory capability that is normally maintained in a conditional state by these same synaptic influences.

9.8

MULTISECOND STORAGE OF STIMULUS-EVOKED EXCITABILITY IN THE SPINAL CIRCUITRY FOR POCKET SCRATCH REFLEX IN THE TURTLE. Scott N. Currie and Paul S.G. Stein. Department of Biology, Washington University, St. Louis, MO 63130.

The Ventral-Posterior Pocket (VPP) cutaneous nerve innervates part of the pocket scratch receptive field in the turtle. Electrical stimulation of the VPP nerve at 1-10 Hz elicits fictive pocket scratch motor patterns in spinal, immobilized preparations. A single "maximal" pulse to the VPP nerve that is sufficiently strong to activate all VPP axons evokes no motor output in a rested preparation. The pulse raises the excitability of the pocket scratch central pattern generator (CPG) for several seconds, however. We reveal the duration of raised excitability by presenting single pulses to the VPP nerve at multisecond intervals. Pulses delivered 5 seconds apart evoke strongly summating central activity: the first pulse elicits no motor response; the second pulse elicits one or more cycles of pocket scratch. The VPP pulse also interacts with pocket scratch activity evoked by natural stimulation of sites far outside of the VPP nerve receptive field, but does not interact with rostral scratch activity. Our data are consistent with the following hypothesis: tactile input from the entire pocket scratch receptive field converges upon a common locus in the spinal cord that 1) stores excitability for seconds after a brief tactile stimulus and 2) drives the pocket scratch CPG. Supported by NIH Grants NS07850 to S.N.C. and NS15049 to P.S.G.S.

9.9

IN SITU APPLICATION OF APV, AN NMDA RECEPTOR ANTAGONIST, ONTO THE SPINAL CORD REDUCES THE EXCITABILITY OF SPECIFIC CUTANEOUS REFLEXES IN THE TURTLE. Paul S.G. Stein and Carla P. Schild*. Department of Biology, Washington University, St. Louis, MO 63130.

A brief mechanical tap to the dorsum of the foot in an immobilized, spinal turtle elicits a flexion reflex in hip flexor (VP-HP) motor neurons. 5 spinal segments comprise the turtle hindlimb enlargement: 3 pre-sacral (D8-D10) and 2 sacral (S1-S2) segments. Afferents from the dorsum of the foot enter the spinal cord in the D10 dorsal root; the D8-D9 segments contain VP-HP motor neuron somata. We apply 50-250 μM APV (D(-)-2-amino-5-phosphonovaleric acid), a specific competitive antagonist of the NMDA (N-methyl-D-aspartic acid) receptor, onto segments D8-D10 and portions of adjacent segments D7 and S1. There is either no Mg or 2.2 mM Mg in the bathing solution. APV reduces flexion reflex excitability. APV also reduces excitability of the ventral-posterior pocket scratch but not that of the rostral scratch. Ventral-posterior pocket scratch afferents enter the spinal cord in the D8 dorsal root; rostral scratch afferents enter in the D3-D6 dorsal roots. Thus excitability of a cutaneous reflex is reduced if APV is applied to spinal segments whose dorsal roots contain the tactile afferents for that specific cutaneous reflex. These results support the hypothesis that NMDA receptors play a role in spinal cord processing of tactile input. Supported by NIH NS15049.

9.10

DIRECT EVIDENCE THAT IDENTIFIED INTERNEURONS MEDIATE THE CROSSED INHIBITION IN THE SPINAL NETWORK OF THE MAUTHNER CELL IN GOLDFISH. J.R. Fetcho and D.S. Faber. Dept. Physiol., SUNY at Buffalo, NY 14214.

We previously identified crossing spinal interneurons that were electrotonically coupled to a M-axon, and we proposed that they produced the short-latency, chloride dependent crossed inhibition of spinal interneurons and motoneurons. We now have direct evidence supporting this prediction. In three experiments we simultaneously recorded intracellularly from a M-axon, an interneuron electrotonically coupled to it, and a probable primary motoneuron inhibited by excitation of the M-axon. In one case an HRP injection confirmed that the coupled interneuron was a crossing interneuron. Firing the M-axon resulted in an action potential in the interneuron and an IPSP (inverted by Cl^- loading) in the spinal cell. Firing the coupled interneuron alone produced a short latency postsynaptic potential in the spinal neuron very similar in time course to the IPSP resulting from activation of the M-axon. In two cases this response was as large as the IPSP produced by firing the M-axon; in the third it was smaller. Hyperpolarizing a coupled interneuron while firing the M-axon blocked the Mauthner initiated spike in the interneuron and substantially reduced the amplitude of the IPSP in the inhibited cell. We conclude that 2 or more of these crossing interneurons mediate the short latency crossed inhibition of each postsynaptic cell. (Support:NIH NS 07593(JRF), 15335(DSF))

9.11

CONSTRUCTION OF THE ADULT ECDYSIS MOTOR PATTERN IN MANDUCA SEXTA: ROLE OF THORACIC DESCENDING INTERNEURONS. K.A. Mesce and J.W. Truman. Dept. of Entomology, Univ. of Minnesota, St. Paul, MN 55108, and Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

The ecdysis behavior expressed by the pharate adult hawkmoth, Manduca sexta, is quite distinct from that expressed by larvae and pupae. Construction of this novel adult ecdysis motor pattern is thought to occur, in part, through the conservation and modulation of the larval ecdysis motor program (K.A. Mesce and J.W. Truman, 1988, J. Comp. Physiol. A, in press).

Expression of the adult-specific motor pattern or the un-masking of the larval-like ecdysis pattern is determined by the activity of descending neurons located solely in the fused pterothoracic ganglion. Anatomical studies indicate that there are only about 15 pairs of thoracic neurons which descend to the unfused abdominal ganglia and whose cell bodies originate in the pterothoracic ganglion. An intracellular study is in progress to determine which of these neurons plays a role in the generation of the adult ecdysis pattern. Thus far, a thoracic descending interneuron (TDI-1) has been identified that, when stimulated to fire at 10-15 Hz for 2-3 secs., causes the co-activation of segmentally repeated abdominal muscles, an adult-specific component of the ecdysis motor pattern. It is not yet known whether some of these descending interneurons are re-modeled larval neurons or specific to the adult stage.

9.12

A NETWORK SIMULATION FOR MODELING OF COMPLEX EXCITATORY AND INHIBITORY INTERCONNECTIONS

C.D. Myre, S.F. Sawyer*, W.K. Smith, D.J. Woodward. *Dept. of Cell Biology and Anatomy, UT Southwestern, Dallas, Texas 75235.*

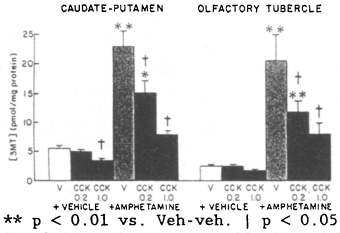
The simulation of a network of one thousand neurons was conducted to examine influences of membrane properties and connectivity patterns on the activity of units. The work was motivated by the extensive local inhibitory feedback system found in the neostriatum. Computation was done in the C language under UNIX on a Motorola 16 MHz MC68020 microprocessor. Neurons in the model occupied positions in a 10 X 10 X 10 cubic array. Each neuron gave inhibitory output connections to all neighboring neurons within a given radius. Neuron characteristics were modeled using state variables representing membrane and threshold values, similar to models reported elsewhere. State variables were tracked using difference equations which accounted for membrane voltage decay, refractory period, and threshold accommodation. Output of the network was recorded as spike events occurring on each neuron at each time increment. One update of a simulation cycle took about 2 seconds of computation in real time, representing about 40,000 synaptic interactions and state variable updates on 1000 neurons. A variety of parameters within the modeling system may be altered to suit the needs of the user. For example, local phasic excitatory input to a zone of 200 neurons was modeled to reveal fine structure of entrainment of neurons near the excited area. These results show that a complex neuronal model can be used in a large network simulation with many interconnections, yet still execute fast enough for realistic modeling experiments to be performed. In the future, modeling experiments will be designed and run, and more realistic neuronal models will be tested.

Support from the Bio. Hum. Found., DA 2338 and AA 3901.

10.1

CENTRAL CCK-B RECEPTORS MODULATE DOPAMINE RELEASE. 1. CCK REVERSAL OF BASAL AND DRUG-STIMULATED RELEASE. W. C. Boyar, P. L. Wood; and C. A. Altar. Res. Dept., Pharma. Div., Ciba-Geigy Corp., Summit, NJ 07901.

The sulfated octapeptide of CCK (CCK-8S; 0.2-1 mg/kg, s.c.) lowered dopamine release (3-MT) and metabolism (DOPAC) in the striatum and frontal cortex in a dose- and time-related manner. 12 Fold higher CCK-8S doses mimicked these actions in the olfactory tubercle. Increases in dopamine release following amphetamine (15 mg/kg i.p., 10') were lowered in a dose-related manner by CCK-8S (s.c, 30'):



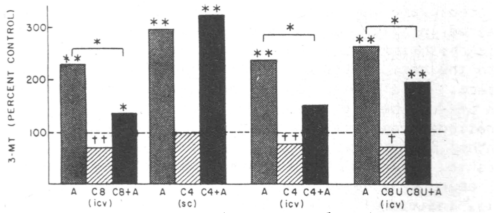
* $p < 0.05$; ** $p < 0.01$ vs. Veh-veh. † $p < 0.05$ below veh.

Haloperidol (0.12 mg/kg, i.p., 25 min) increases in dopamine release and metabolism were also attenuated by CCK-8S. CCK-8S appears to be a suppressor of striatal, limbic, and cortical dopamine release, especially when release is augmented. CCK receptor agonists may be antipsychotic by inhibitng hyperactive dopamine neurons.

10.2

CENTRAL CCK-B RECEPTORS MODULATE DOPAMINE RELEASE. 2. INTRAVENTRICULAR INJECTIONS OF CCK TETRA- AND OCTAPEPTIDES. C. A. Altar and W. C. Boyar. Res. Dept., Pharma. Div., Ciba-Geigy Corp., Summit, NJ 07901.

Butoxycarbonyl CCK tetrapeptide (t-Boc CCK-4) and CCK-8U bind to the brain (CCK-B) but not alimentary (CCK-A) receptor. Basal and d-amphetamine- ("A", 15 mg/kg, 10 min, i.p.) increases in striatal dopamine release were attenuated when 1 or 5 ug of these peptides or 1 ug of CCK-8S was infused into the brain ventricles 12 min before amphetamine but not when t-boc CCK4 (1 mg/kg) was injected s.c:



S.c. CCK-8S lowered basal and stimulated release even with pretreatment of the CCK-A antagonist, L 364,718. These data are the first to demonstrate that CCK-B receptors in brain mediate the suppression of dopamine release by CCK, especially when release is augmented. Thus, lessened peripheral side-effects and treatment of psychotic patients may be obtained with centrally-available CCK-B agonists.

10.3

EXPRESSION AND CHARACTERIZATION IN XENOPUS OOCYTES OF A CHOLECYSTOKININ (CCK) RECEPTOR FROM A HUMAN NEUROBLASTOMA CELL LINE. **TP Segerson*, R Barrett*, J McKelvy, G Mandel*, RH Goodman* (SPON: LP Henderson). Div. of Molecular Med., Dept. of Med., New England Med. Ctr., Boston, MA 02111 and Neurosci. Res. Div., Pharmaceutical Discovery, Abbott Labs., Abbott Park, IL.**

The human neuroblastoma cell line CHP212 expresses peripheral-type CCK receptors (CCK-Rs). Activation of these CCK-Rs causes a 4-5 fold increase in PI hydrolysis. In Xenopus oocytes, an IP_3-dependent increase in intracellular Ca^{++} activates a Ca^{++}-dependent Cl- channel causing transient depolarization. To express the CHP212 CCK-R, Xenopus oocytes were injected with CHP212 RNA, and treated after 72 h with 1 uM CCK while monitoring for changes in either transmembrane voltage or current. RNA injected oocytes depolarized from a mean resting potential of -60 mV to the Cl- equilibrium potential (-25 mV). No response was seen in uninjected oocytes. The size of the CCK-R mRNA was determined by fractionating poly A+ CHP212 RNA on a denaturing agarose gel. Sequential RNA fractions differing by 500 nt screened in oocytes during CCK application. Fractions between 5 and 6 kb elicited a CCK-dependent depolarization. Other fractions demonstrated no depolarization. We conclude that 1) we have expressed CCK-Rs in Xenopus oocytes using mRNA from a human neuroblastoma cell line that expresses peripheral-type CCK-Rs and 2) the size of this CCK-R mRNA is between 5 and 6 kb.

10.4

CHARACTERIZATION OF VASOACTIVE INTESTINAL PEPTIDE BINDING TO RAT BRAIN AND RETINA. J.T. Laitinen and J.M. Saavedra (SPON: F.M.A. Correa). Unit Preclin. Neuropharmacol., Sect. Clin. Pharmacol., Lab. Clin. Sci., NIMH, Bethesda MD 20892.

Vasoactive intestinal peptide (VIP), despite its name, has a wide distribution in endocrine glands and the central nervous system (CNS). Recently, VIP and noradrenaline have been shown to interact synergistically to increase cyclic AMP in rodent nervous tissue in vitro (Schaad et.al. 1987, Nature 328,637; Ho et.al. 1987, BBRC 146,1478). Quantitative autoradiography offered us a tool for a closer look at VIP binding in rat CNS.

Displaceable binding of $^{125}I\text{-}Tyr^{10}$-VIP was seen in several brain areas, including cerebral cortex, dentate gyrus (DG), various thalamic and hypothalamic nuclei. Moreover, binding was detected in the retina and the anterior pituitary. VIP and peptide histidine isoleucine to lesser extent displaced labeled ligand from its binding sites. Neither VIP10-28 nor peptide T (nor its fragment T4-8), the proposed attachment sequence of the human immunodeficiency virus, were able to compete at 1 uM concentration for the binding sites with the ligand. In suprachiasmatic nuclei (SCh), homogeneous anterior-posterior distribution of binding was apparent, when quantified per unit area. Kinetic studies of several brain areas and retina may suggest the presence of two binding sites with K_ds around 0.1-0.5 and 5 nM.

The results further support a functional role for VIP in CNS and possibly also in the retina.

10.5

BIOCHEMICAL PROPERTIES OF BRAIN SOMATOSTATIN RECEPTORS. Kyriaki Thermos, Hai-Tao He*, Neil Margolis and Terry Reisine, Dept. Pharmacology, Univ. Pennsylvania, Phil. PA, 19104

The physical properties of brain and pituitary somatostatin (SRIF) receptors were characterized using photocrosslinking techniques. SRIF receptors in rat corpus striatum and anterior pituitary membranes were covalently bound to the non-reducible SRIF analog, [125I] CGP 23996 using the crosslinking agent n-hydroxysuccinimidyl-4-azidobenzoate and ultraviolet light. In striatal membranes, a protein of 60 kilodaltons was labeled by [125I] CGP 23996. The binding was potently inhibited by SRIF analogs but not by other biologically active peptides. The labeling of the 60 kilodalton protein by [125I] CGP 23996 was diminished by GTPgS, which is consistent with the labeling of a receptor coupled to GTP binding proteins. The migration of the [125I] CGP 23996 labeled protein in native SDS-gels was not affected by the reducing agent DTT indicating that there is a lack of disulfide bridges in the striatal SRIF receptor. The striatal SRIF receptor was solubilized with CHAPS and specifically bound to wheat germ agglutinin suggesting that the receptor is a glycoprotein. [125I] CGP 23996 also labeled a 60 kilodalton protein in anterior pituitary membranes. The characteristics of the binding were consistent with the labeling of a SRIF receptor. Interesting, a comparison of the [125I] CGP 23996 labeled material from striatal and anterior pituitary membranes by 2-dimensional PAGE revealed the presence of several striatal SRIF receptors of varying charge (pI between 6 and 6.5) but only one pituitary receptor. These findings indicate that physical differences may exist between subtypes of SRIF receptors. Supported byNIH grants DK37404 and GM 34781 and the Office of Naval Research (N00014-88-K-0048).

10.6

DEVELOPMENT AND SPECIFICITY OF GONADOTROPIN RELEASING HORMONE (GnRH) RECEPTORS IN RAT BRAIN. **L. Jennes, H. Dine* and B. Dalati*. Department of Anatomy, Wright State University School of Medicine, Dayton, OH 45435.**

The development and structure/function relationships of brain GnRH receptors were studied with "in vitro" autoradiography using the ^{125}I-labeled GnRH agonist D-tert-butyl-Ser^4-Pro^9-ethylamide-GnRH (Buserelin, Hoechst) as ligand. Specific, displaceable binding was first seen on postnatal days 7 and 8 in the hippocampus of young female rats. The distribution of the binding sites in the hippocampus was similar to the adult animal in that the binding was restricted the dorsal and ventral subiculum and to the areas CA_1 through CA_4. In the adult female rat, binding of Buserelin to the hippocampal tissue was inhibited by GnRH as well as by the GnRH agonists D-Ala^6-GnRH and D-Trp^6-Fujino-GnRH and by the antagonist [N-Ac-D-(pyro)Cl-Phe^1,^{2}-D-Trp^3-DLys^6-D-Ala^{10}]-GnRH. In addition, the GnRH fragment Des^{10}-GnRH competed for the brain GnRH binding sites, however with a lower affinity. The binding of Buserelin to the hippocampal GnRH receptors was not inhibited by Salmon-, Lamprey-, and Chicken I-GnRH nor by the GnRH fragments Des^1-GnRH, Des^1-GnRH, 4-10-GnRH and 5-10-GnRH.

In eight days old rats, intraventricular injections of labeled Buserelin resulted in "in vivo" labeling of similar areas of the hippocampus as described for "in vitro" autoradiography. Competition studies with 1 uM unlabeled GnRH show that the "in vivo" labeling is specific and saturable.

The results suggest that specific GnRH binding sites are present in the brain of young female rats and that these brain GnRH binding sites have similar binding characteristics when compared to the pituitary GnRH receptor.

10.7

EFFECTS OF DITHIOTHREITOL (DTT) ON OPIOID RECEPTORS: EVIDENCE FOR A POSSIBLE ROLE FOR DISULFIDE BONDS. T.L. Gioannini*, Y.F.Liu*, H-Y. Park*, J.M. Hiller* and E.J. Simon, NYU Medical Center, New York, NY 10016.

We have reported that purified μ opioid binding protein has a mobility on SDS-PAGE suggesting an apparent M.W. of 65kDa under reducing conditions. In the absence of DTT the apparent M.W. is 53kDa. We attributed this shift to a reduction of disulfide bridges resulting in a more linear conformation of the molecule. This finding has prompted us to study the effects of DTT on both purified denatured μ receptor protein and native opioid receptors. Different concentrations of DTT (from 0.65-325mM) caused SDS-PAGE patterns of purified μ receptor to undergo a stepwise shift in apparent M.W. from 53kDa to 65Da, suggesting the progressive breakage of multiple disulfide bonds. Treatment of cell membranes from various tissues with DTT produced a dose-dependent inhibition of opioid binding. The sensitivity to DTT inhibition varied from type to type in the order $\mu > \delta > > \kappa$. For μ receptors agonist binding was considerably more sensitive than antagonist binding. DTT inhibition is readily reversible and is unaffected by the presence of Na^+ or Mg^{++} ions. Scatchard analysis of saturation data shows that DTT causes a pronounced reduction in K_D with only a slight reduction in Bmax. Receptors solubilized by a modified CHAPS procedure are also inhibited by DTT, but higher concentrations are required than for membrane-bound receptors. These findings, especially the difference in DTT sensitivity between agonists and antagonists, suggest that disulfide bond breakage may play a role in the activation of the receptor induced by opioid agonist binding. (Supported by grant DA-00017 from the National Institute on Drug Abuse).

10.8

ZETA (ζ): A NEW OPIOID RECEPTOR RELATED TO GROWTH. I.S. Zagon, S.R. Goodman* and P.J. McLaughlin. Depts. Anat. and Physiol., Penn. State Univ. College of Medicine, Hershey, PA 17033

Endogenous opioid systems (i.e., opioids and receptors) regulate neural cancer. Utilizing ^{3}H-methionine enkephalin (ME), a ligand prototypic for growth, receptor binding assays were performed with homogenates of S20Y neuroblastoma transplanted into Ajax mice. Specific, high-affinity, and saturable binding to ^{3}H-ME was recorded; K_d = 0.49 nM, binding capacity Bmax = 5.3 fmol/mg protein. Dependency on time, pH, temperature, and tissue concentration was recorded. Na^+ and guanyl nucleotides (GppNHp) markedly reduced binding. Binding assays required a cocktail of protease inhibitors and was sensitive to trypsin, indicating the proteinaceous character of the binding site. An excess of DAGO, DADLE, and/or EKC, to suppress μ, δ, and κ binding, did not alter the profile of binding. Displacement studies showed that ME was the most potent displacer, binding was stereospecific, and ligands prototypic for μ, δ, κ, or ϵ receptors exhibited no distinct pattern of potency. Miscellaneous opioids (e.g., codeine) and non-opioids (e.g., somatostatin) exhibited little ability in displacing ME. These results, in conjunction with functional studies, identify a new opioid receptor, termed zeta (ζ) (G, zoe = life) related to growth. Supported by NIH grants NS-20623 and NS-20500.

10.9

BOMBESIN RECEPTORS ARE PRESENT ON HUMAN GLIOBLASTOMA CELL LINES. T.W. Moody, S. Mahmoud, R. Kris, L. Naldini, D Cirillo and J. Schlessinger. Dept. Biochemistry, George Washington Univ. Med. Ctr., Washington, D.C. and Rorer Biotechnology Inc., King of Prussia, PA.

Bombesin (BN) receptors are present in numerous cells. These include pituitary GH_4C_1 cells, Swiss 3T3 cells where BN stimulates ^{3}H-thymidine uptake, pancreatic acinar cells and SCLC cells where BN stimulates clonal growth. Because BN receptors are present in the mammalian CNS, we investigated if BN receptors were present on certain human neuroblastoma and glioblastoma cell lines.

Continuous human neuroblastoma and glioblastoma cell lines were cultured in serum supplemented medium, harvested and assayed for specific ^{125}I-GRP binding activity. High levels of binding were present on glioblastoma cell lines U-118, U-138 and GM-340 but not neuroblastoma cell line KCNR. On cell line U-118, binding was specific and reversible; ^{125}I-GRP bound with high affinity (Kd = 0.5 nM) to a single class of sites (Bmax = 25,000/cell). Structure-activity studies indicated that the C-terminal of BN or GRP was essential for high affinity binding activity. ^{125}I-GRP was cross linked to a 75 Kdalton protein using 1 mM dissuccinimidylsuberate at 4°C. At 37°C but not 4°C the ^{125}I-GRP receptor complex was internalized. These data indicate that glycoprotein BN receptors are present on human glioblastoma cell lines. Supported by NSF grant BNS 85-00552.

10.10

DISTRIBUTION OF NEUROTENSIN BINDING SITES IN HUMAN BASAL FOREBRAIN. E. Szigethy, R. Quirion and A. Beaudet. Montreal Neurol. Inst., Montreal, PQ, H3A 2B4; Douglas Hosp. Res. Ctr., Verdun, PQ, H4H 1R3.

We have previously demonstrated a selective association of NT receptors with cholinergic neurons in the rat basal forebrain (Neurosci. Lett., 83:47, 1987). In the present study, we sought to document the distribution of ^{125}I-NT-labeled binding sites with respect to that of cholinesterase (AChE) reactivity in human basal ganglia and adjoining forebrain. Slabs of normal human forebrain were cryostat-cut and adjacent sections processed for radioautography (using either conventional film or liquid emulsion dipping techniques) and AChE histochemistry. Dense ^{125}I-NT labeling was observed throughout the caudate and putamen nuclei, where it was found to mirror the diffuse pattern of heavy AChE-reactivity seen in adjacent sections. Moderate ^{125}I-NT labeling was also observed in both the diagonal band nucleus and the nucleus basalis of Meynert (NBM). In both these regions, labeled binding sites were localized predominantly although not exclusively over the large magnocellular neurons identified as AChE-positive in adjacent sections. These data provide an anatomical substrate for NT action on cholinergic neurons in human forebrain. They also suggest that the loss of AChE-positive neurons observed in the NBM of patients with Alzheimer's disease may be correlated with changes in the density of NT binding sites in this area. Supported by MRC.

10.11

NEUROTENSIN: EFFECTS ON THE DEPHOSPHORYLATION OF NEOSTRIATAL PHOSPHOPROTEINS. S.T. Cain, M. Johnson* and C.B. Nemeroff. Depts. of Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.

Neurotensin (NT) is a tridecapeptide which is widely distributed in the mammalian central nervous system. NT displays many of the characteristics of neurotransmitter substances such as synaptosomal localization, calcium-dependent release and specific, saturable high-affinity binding to brain membranes. We have previously reported that NT accelerates the rate of dephosphorylation of a synaptosomal protein with a molecular weight of approximately 51,000 (Cain and Nemeroff, Soc. Neurosci. Abst. 12, 802, 1986). We have now further characterized the effects of NT on the dephosphorylation of this protein.

A lysed synaptosomal preparation containing both synaptosomal membranes and synaptosomal cytosol was phosphorylated for 10 min. in the presence of 500 μM ATP containing [γ^{32}P-ATP]. Identical aliquots of the phosphorylated sample were then incubated for 10 min. in the presence of 7.5 nM NT or vehicle. At various intervals after the addition of NT or vehicle, aliquots were removed and processed for SDS-PAGE. Gels were dried, autoradiographs prepared and the phosphate incorporation into individual proteins quantitated by densitometry. Thirty seconds after the addition of NT to the previously phosphorylated sample, the phosphorylation of the 51,000 MW protein was decreased 29 ± 6.4% (n=3) relative to control values. The rate of subsequent dephosphorylation of the 51 KD protein was not altered by NT. Between 0.5 and 7.5 min., phosphorylation decreased 45 ± 5.31% in the control samples and 52.3 ± 7.31% (n=3 in both groups) in the NT samples. Thus, the stimulation by NT of 51 KD dephosphorylation is of rapid onset and offset. (Supported by NIMH MH-39415 and NARSAD)

10.12

SUBSTANCE P RECEPTOR ANTIBODIES. M. L. SWENBERG*, R. Liu (SPON: M. B. Carpenter). Hypertension & Endocr. Branch, NHLBI, NIH, Bethesda, MD 20892; Dept. of Anatomy, Georgetown Univ. Sch. of Med., Washington, D. C.,20007.

One way to demonstrate isolated receptors is to prepare their antibodies and further clarify the function of the preparations. The scheme for substance P (SP) receptor purification was reported last year (Abstr. Neuros. 87: 118.2, P412.). This presentation provides evidence of their antibody-activities against the receptors.

Purified SP receptors from acrylamide gel were directly emulsified with complete adjuvant and immunized on the back of New Zealand white rabbits. Antisera against both olfactory bulb and intestinal mucosa membrane receptors as well as monoclonal anti substance p antibody, were tested for their activities on : (1) Binding of SP receptors on the Phastgel or western blot and detected by double labeling with radioactive or fluorescent anti-rabbit IgG; (2) Immunocytochemical reactivity on fixed brain slices; (3) Binding inhibition of receptor antibodies on labelled ligand (SP) to fresh tissue slices.

All three antibodies showed similar reactivity but with different titer. Biological effect on guinea pig ileum and rat uteri also showed enhanced contraction (agonistic) for SP.

Fluorescent immunoreactivity can be performed and observed directly on Phastgel(from Pharmacia) or on the nitrocellulose western blot.

Based on this evidence the receptor isolated is homogeneous and antibodies react with specific nerve cells, fibers and terminals of brain tissues which could not be observed in regular autoradiograms.

10.13

NEUROPEPTIDE Y RECEPTORS IN THE RABBIT IRIS ARE RELATED TO SUPPRESSION OF SENSORY NERVE-MEDIATED INFLAMMATION. C. Wahlestedt*, E. Costa, M. Walker*, L. Grundemar *and R. Hakanson*, FIDIA-Georgetown, Univ. of Chicago and Univ. of Lund, Sweden.

Neuropeptide Y (NPY) is a 36 amino acid peptide widely distributed in the central and peripheral nervous systems. Much attention has been given to the coexistence of NPY and catecholamines. The peptide mediates and modulates a wide range of physiological processes.

In the mammalian eye, NPY nerve fibres are found in both the uvea and the retina. The iris is particularly rich in NPY fibres, the vast majority being of sympathetic origin (Sundler et al., Ann. Rev. Cytol. 102:234, 1986). We have examined the rabbit iris with regard to NPY binding and second messenger coupling as well as possible functional roles by means of in vitro and in vivo assays.

By use of 125I-NPY and homogenized iridal tissue, we established the presence of NPY binding sites, the density being high (Bmax 257 ± 19 fmol/mg protein). Scatchard analysis indicated that the peptide bound to two populations of sites (Kd:s 0.09 and 3.2 nM).

NPY inhibited the forskolin-evoked, but not the basal, cyclic AMP formation in pieces of iris. No effect of NPY on basal or adrenergically stimulated accumulation of inositol phosphates could be detected.

NPY did not affect the pupil size upon injection into the aqeuous humour in vivo, nor did the peptide produce any motor effect on isolated iris sphincter and dilator muscles in vitro. The isolated sphincter responded to electrical field stimulation in a complex manner; pharmacological analysis revealed cholinergic, adrenergic and tachykininergic (e.g. substance P-ergic) components. NPY selectively suppressed the tachykininergic component, indicating that it has the capacity to suppress local sensory reflexes. In the rabbit eye, such reflexes are closely related to the inflammatory response to injury (e.g. Wahlestedt et al., Regul. Peptides 16:107, 1986). Preliminary in vivo experiments (infrared irradiation followed by measurement of aqeuous flare response) further support that NPY and thus the sympathetic nervous system has the capacity to attenuate local sensory nerve reflexes, which are known to play a role in intraocular inflammation. (SPON: R. Ekman)

MESSENGER RNA REGULATION I

11.1

EFFECT OF CHRONIC HYPONATREMIA ON TRANSLATION RATE OF VASOPRESSIN (AVP) AND OXYTOCIN (OT) IN RATS. A.G. Robinson, W.A. Evron*, M.M. Roberts*, J.G. Verbalis, L.E. Janocko* and T.G. Sherman. School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261, and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Secretion of AVP and OT varies with osmolality in the rat. Plasma levels of AVP and OT and changes in the content of the posterior pituitary have been described during hyper- and hypo-osmolality, but there are few data on translation. We studied the down-regulation of translation in the neurohypophysis of a rat model of chronic hyponatremia. Sodium was 105-115 mEq/L for up to 14 days. We devised a novel method to measure total translation of AVP and OT by blocking microtubular transport to the posterior pituitary with intraventricular colchicine (7 ug) and measuring accumulation of AVP and OT over 18 h. We measured mRNA with specific riboprobes.

	Days of Hyponatremia			
Translation rate	0	3	7	14
AVP pg/h	1,470	523	0	0
OT pg/h	2,316	1635	211	294
mRNA % control				
AVP mRNA	100	38	16	11
OT mRNA	100	62	40	32

This is the first demonstration of the rapidity with which mRNA and translation of AVP and OT can be shut off. Thus, the neurohypophysis down-regulates promptly with hyponatremia and ongoing stimulated release of AVP and OT is essential to maintain message levels and hormone translation.

11.2

DOWN REGULATION OF VASOPRESSIN AND OXYTOCIN mRNAs: DECAY PROFILE DIFFERENCES BETWEEN HYPONATREMIA AND REHYDRATION. T.G. Sherman, A.G. Robinson, and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109 and Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

The down regulation of hypothalamic magnocellular neuropeptide mRNAs were studied in two independent paradigms: 1) rehydration, the return to baseline decay following six days of salt-loading; and 2) hyponatremia, a down regulation of magnocellular mRNAs during a chronic period of low plasma sodium (see abstract: A.G. Robinson et al.). During a 14-day period of hyponatremia, vasopressin (AVP) mRNA in the paraventricular and supraoptic nuclei decreased to 11% of control values. Analysis of the parallel decay profiles in PVN and SON indicated a close fit to first-order kinetics with $k = 3.6\ hr^{-1}$ and a half-life of 4.6 days. Oxytocin (OT) mRNA was similar to AVP mRNA, whereas dynorphin mRNA decreased much more slowly ($t_{1/2} \approx 7.5$ days). The rate of decay exhibited by AVP during rehydration, on the other hand, did not follow first-order kinetics. Levels of AVP mRNA remained significantly different from controls for as long as 21 days following 6-days of salt-loading. Curve fit analysis of rehydration decay profiles indicated that the data was more consistent with zero-order accumulation/first-order decay kinetics. These results have led to the hypothesis that rehydration decay of AVP mRNA occurs in the presence of control transcription rates, whereas, hyponatremic down-regulation occurs in the absence of significant transcriptional activity. This hypothesis is being tested by examining AVP, OT and dynorphin transcriptional activity occurring during salt-loading, rehydration and hyponatremia.

11.3

VASOPRESSIN mRNA IN THE HYPOTHALAMUS: CIRCADIAN AND OSMOTIC REGULATION OF POLY(A) TAIL LENGTH. B.G. Robinson*, D.M. Frim*, J.M. Gleason*, W.J. Schwartz, and J.A. Majzoub* (SPON: E. Spindel). Neuroendocrine Genetics Lab, Brigham and Women's Hosp, Harvard Med Sch, Boston, MA 02115 and Dept of Neurology, U Mass Med Sch, Worcester, MA 01605.

Vasopressin (AVP) mRNA is localized in the supraoptic (SO), paraventricular (PV), and suprachiasmatic (SC) nuclei of the hypothalamus. We have studied circadian and osmotic regulation of AVP gene expression in these nuclei in male Sprague-Dawley rats. In the circadian experiment, animals entrained to 12 h:12 h LD and DL cycles were sacrificed at various times spanning the 24 h day. RNA was extracted from individual punches of either SO, PV, or SC nuclei, and analyzed by Northern blot. Oligonucleotide-directed RNAase H cleavage was used to determine AVP mRNA poly(A) tail length. In the SC nuclei, location of an endogenous circadian pacemaker in mammals, two distinct AVP mRNA species, with poly(A) tails 250 and 20 nucleotides long, were found predominantly in animals sacrificed in the dark. Only the longer-tailed species was found in the SC nuclei of animals sacrificed in the light. This longer-tailed species was also the sole AVP mRNA found in the SO or PV nuclei at all times. In contrast, an osmotic stimulation experiment (72 h dehydration) resulted in the gradual increase in the size of AVP mRNA poly(A) tails from 250 to 400 nucleotides in the SO and PV nuclei. These data are the first to show circadian and osmotic regulation of mRNA structure in discrete hypothalamic nuclei. Since the poly(A) tail may enhance translational efficiency of mRNA, the circadian and osmotic variation in AVP mRNA poly(A) tail length may provide an additional level of genetic regulation, and may contribute to both the circadian rhythm of AVP peptide levels in cerebrospinal fluid and to the increase in vasopressin synthesis following osmotic stimulation.

11.4

STEROID DEPENDENCY OF VASOPRESSIN mRNA EXPRESSION BY CELLS IN THE BED NUCLEUS OF THE STRIA TERMINALIS BY IN SITU HYBRIDIZATION. M. A. Miller*, J. H. Urban, and D. M. Dorsa (SPON: L. Standish). GRECC, VA Medical Center, Seattle, WA 98108.

Vasopressinergic (AVP) neurons in the bed nucleus of the stria terminalis (BNST) of the rat have been shown by immunohistochemical techniques to be steroid sensitive. To determine whether steroids modulate biosynthetic capacity, we have used in situ hybridization and quantitative autoradiography to measure propressophysin mRNA levels in neurons in the BNST of intact (n=3), castrated (n=6), and castrated male rats treated with testosterone (n=6).

Frozen brain sections (15μm) were hybridized with an oligonucleotide probe and coated with NTB2 emulsion. Sections through the BNST were anatomically matched (4/animal) and read blindly. The number of labeled cells (unilateral) and the average number of grains/cell (based on readings of up to 10 cells/slide) were compared.

Castration significantly decreased ($p<0.01$) the number of labeled cells in the BNST (X ± SEM: 3.2±2.2, castrates; 83.7±20.0, intacts) and reduced ($p<0.01$) the average number of grains/ cell (X ± SEM: 40.4±5.8, castrates; 83.1±10.2, intacts). Testosterone treatment reversed the effects of castration on cell number (90.0±4.6) and grains/cell (108.5±7.0). These results indicate that testosterone and/or its metabolites modulate the expression of mRNA by AVP cells in the BNST of the rat.

11.5

GLUCOCORTICOID (GL) REGULATION OF SOMATOSTATIN (S) GENE EXPRESSION IN NORMAL TISSUES AND IN A S-PRODUCING TUMOR CELL LINE (1027 B2). D.N. Papachristou* and Y.C. Patel* (SPON: J. Randle). Fraser Labs, McGill Univ., Mtl., QUE.

GL have been reported to reduce S secretion and mRNA accumulation in S-producing rat thyroid carcinoma cells whilst adrenalectomy in dogs augments pancreatic S content and induces hypersomatostatinemia. By contrast, GL have been shown to promote S secretion from cultured rat islets. To explain these discrepancies, we examined the effect of dexamethasone (DXM) on S secretion and mRNA accumulation in normal tissues in vivo and in vitro and in 1027 B2 islet cells. S-mRNA was determined by Northern analysis using in vitro synthesized antisense S-mRNA as riboprobe and sense strand S-mRNA as standard. 1027 B2 cells cultured for 24 h with DXM (10^{-10}-10^{-8} M) exhibited a dose dependent increase in S release (from 1.9 to 3.2 ng/ml) accompanied by a 40% increase in S-mRNA accumulation (from 204 ± 26 to 352 ± 32 pg /dish, $p < 0.05$). DXM (10^{-7}-10^{-6} M) reduced S secretion and mRNA by 30% ($p < 0.05$). A similar dose dependent biphasic effect of DXM on S secretion and mRNA was observed in cultured rat islets. Normal rats were injected with saline or DXM (40 ug/day for 7 days and studied on day 7 and 14). DXM increased pancreatic S content (from 0.79 ± 0.15 to 2.6 ± 0.1 pg/ug prot, $p < 0.01$) and mRNA (from 0.57 ± 0.05 to 1.75 ± 0.1 pg/ug, $p < 0.01$) with partial recovery after stopping DXM (1.8 pg/ug S content, and 1.1 pg S-mRNA/ug RNA). Identical changes in S content and mRNA accumulation were observed in cerebral cortex, stomach and jejunum. Conclusions: 1) GL modulate S gene expression in normal and neoplastic S cells. 2) GL effect on S secretion and mRNA accumulation is dose-related and biphasic and may explain their reported stimulatory and inhibitory actions on S gene function.

11.6

MORPHINE DECREASES PROENKEPHALIN mRNA LEVELS. R.E. Harlan and S.L. Chang*. Dept. of Anatomy, Tulane University Medical Center, New Orleans, LA 70112.

Opiate drugs act on the neuronal system mostly through their ability to modify the level of neurotransmitters. We have examined the influence of morphine treatment on the regional expression of the proenkephalin gene, which codes for the principle brain opioid peptide precursor. Six female rats were injected with morphine sulfate (10 mg/kg) or saline vehicle subcutaneously 60-100 min. prior to killing. The rats were injected with pentobarbital prior to decapitation. Brains were removed, dissected and frozen on dry ice. Total cellular RNA prepared from caudate-putamen, hypothalamus and amygdala was subjected to dot-blotting analysis with ^{32}P-cDNA probes complementary to proenkephalin mRNA. Proenkephalin mRNA levels in the caudate-putamen and amygdala of morphine-treated rats were suppressed approximately 45% ($p < 0.001$) and 25% ($p < 0.05$), respectively, relative to those of saline-treated animals. No significant difference was observed in the hypothalamus. Previous studies indicated both caudate-putamen and hypothalamus have high densities of μ-type receptors. The amygdala has few sites of μ-receptors (PNAS, 1980, 6239-6243). Our data suggest that the effect of morphine is not predicted only by the localization of opioid receptor in brain tissue. Our ongoing effort is on the post-receptor mechanism of opiate addiction underlying gene regulation of neuropeptide precursors.

11.7

STEROID REGULATION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) GENE EXPRESSION - EVIDENCE FOR SEXUAL DICHOTOMY. I. Gozes. Dept. of Hormone Research, The Weizmann Institute of Science, Rehovot, 76100, Israel.

VIP gene expression is differentially regulated during ontogeny. Hypothalamic VIP-mRNA reaches a peak at 39 days of age concomitant with rat sexual maturation. Hence, we sought to analyze the influence of sex steroids on rat hypothalamic VIP gene expression. Using densitometric hybridization assays, VIP-mRNA was found to significantly decrease following ovariectomy. This decrease was largely reversed after 3 days of treatment with estradiol dibenzoate (Gozes, Werner, Fawzi, Shani, Fridkin and Koch, J. Molec. Neurosci., in press). In situ hybridization histochemistry suggests that the main changes occur in the suprachiasmatic nucleus as well as in the thalamus (Avidor, Biegon, Baldino, Rostene and Gozes, unpublished results). In contrast to the female rats, no change in VIP-mRNA was observed in the male rats following orchidectomy. Similar sex dependent differences were seen when the peptide levels were measured. These results imply a sexual dimorphism with regard to steroid regulation of hypothalamic VIP-gene expression in the rat, which may reside in sex specific regulatory factors. This sexual dimorphism is VIP specific, indeed, the amounts of somatostatin mRNA in the dorsal portion of the hypothalamic periventricular nucleus were significantly decreased following gonadectomy in both male and female rats (Werner, Koch, Baldino and Gozes, J. Biol. Chem., in press). Finally, our results imply a link between VIP gene expression and the regulation of the oestrous cycle.

11.8

REGULATION OF PREPROCHOLECYSTOKININ mRNA IN THE MEDIAL AMYGDALA BY ESTROGEN: AN IN SITU HYBRIDIZATION STUDY IN THE RAT. R.B. Simerly, B.J. Young* and L.W. Swanson. Howard Hughes Medical Institute and The Salk Institute, La Jolla, CA 92037.

The posterodorsal part of the medial nucleus of the amygdala (MeAp) receives its major sensory input from the accessory olfactory bulb, and projects massively to the encapsulated part of the bed nucleus of the stria terminalis and medial preoptic nucleus (MPN), two sexually dimorphic nuclei thought to play key roles in mediating steroid-sensitive reproductive functions. The MeAp also contains numerous cells that accumulate gonadal steroid hormones and many of these cells project to the MPN (Akesson et al. Soc. Neurosci. Abstr. '87). Recently we reported that levels of CCK-immunoreactivity within cells of the MeAp can be reversibly altered by gonadectomy in male rats (Simerly et al., PNAS, '87), and also appear to vary over the estrous cycle in response to corresponding changes in circulating estrogen in females (Oro et al., Neuroendocrinol., '88). By using a combined retrograde transport/double-immunohistochemical method we have determined that nearly half of the CCK-immunoreactive cells in the MeAp co-contain substance P (SP), and a substantial number of these cells project to the MPN. In order to localize the level at which estrogen regulates CCK expression, as well as to examine the specificity of this regulation, we used ^{35}S-labeled cRNA probes for in situ hybridization histochemistry to evaluate whether preprocholecystokinin (pCCK; see Deschenes, et al., PNAS, '84) and β-preprotachykinin (βPPT, see Krause et al., PNAS, '87) expression are regulated by estrogen at the mRNA level. In male rats, orchidectomy reduced, and treatment with diethylstilbestrol (0.1 mg) increased the number of pCCK mRNA cells within the MeAp. Similarly, the number of pCCK mRNA-containing cells detected within the MeAp was lowest in female animals during metestrus (when blood levels of estrogen are low), and were significantly increased in proestrous animals, which have peak levels of circulating estrogen. Subcutaneous implants of estradiol (0.5 mg) prevented the dramatic decline in pCCK mRNA-containing cells seen following ovariectomy, confirming that changes in pCCK mRNA during the estrous cycle are due, at least in part, to the normally occurring changes in levels of circulating estrogen. In contrast, levels of βPPT mRNA within the MeAp do not appear to be sensitive to acute changes in circulating gonadal steroids in either sex. Although the possibility that post-transcriptional regulation of pCCK mRNA stability may contribute to the observed effects, it appears likely that estrogen stimulates pCCK expression within the MeAp by inducing transcription of the pCCK gene, thereby altering the relative amounts of CCK and SP that are co-expressed within individual cells of the MeAp.

11.9

CLONING AND REGULATION OF RAT HIPPOCAMPAL STEROID RECEPTORS. P. D. Patel, T. G. Sherman, J. P. Herman, and S. J. Watson. Mental Health Research Institute, University of Michigan Medical School, Ann Arbor, MI 48109.

Several lines of evidence implicate the hippocampus in regulation of the hypothalamic-hypophyseal-adrenal axis. The pyramidal cells of the rat hippocampus contain two distinct pools of steroid binding proteins, one showing relative specificity for corticosterone, and the other for the prototypic glucocorticoid, dexamethasone. These are now referred to as the type I and type II corticosterone receptors, respectively. Utilizing the human glucocorticoid (type II) receptor cDNA as a probe in low stringency hybridization screening of a rat hippocampal cDNA library, we have isolated a message encoding the rat hippocampal type I (mineralocorticoid) receptor. The 6.5 kb message has an open reading frame coding for 973 amino acids. The entire open reading frame and 3'-untranslated regions are uniformly high in homology to the human kidney mineralocorticoid receptor (R. Evans), however, the sequence of the 5'-untranslated region diverges abruptly. RNase protection is being used to fully characterize this finding and to determine its tissue specific distribution. Complementary sense RNA probes to unique regions of the rat type I and type II corticosterone receptor messages have been used in protection assays to quantitate their respective mRNAs in a time course after adrenalectomy and after chronic corticosterone treatment. Preliminary results indicate that whereas type II receptor message does vary under this paradigm, type I receptor message is resistent to change, suggesting that control may be exerted at the level of message translatability or in the turnover of a cryptic receptor pool.

11.10

LOCALIZATION AND STEROID REGULATION OF HIPPOCAMPAL GLUCOCORTICOID AND MINERALOCORTICOID RECEPTOR mRNA: A SEMI-QUANTITATIVE *IN SITU* HYBRIDIZATION ANALYSIS. J.P.Herman, P.D.Patel, M.K.-H.Schafer*, S.Burke*, H.Akil and S.J.Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Glucocorticoid (GR) and mineralocorticoid (MR) receptors can be localized to the hippocampal formation of the rat, where they are believed to play a role in feedback inhibition of the hypothalamo-pituitary-adrenocortical axis. In these experiments, we examined localization and regulation of mRNAs coding for these receptor species within discrete hippocampal subfields using semi-quantitative in situ hybridization analysis. Examined were adrenalectomized rats (ADX), ADX rats replaced with daily dexamethasone (DEX) injections (ADX-DEX), sham ADX rats (SHAM), and SHAM rats administered daily DEX injections.

In all groups, GR and MR mRNA could be localized to all hippocampal subfields examined (CA1-2, CA3, CA4 and dentate gyrus (DG)). In SHAM rats, GR mRNA signal was heaviest in subfields CA1-2, with CA3-4 and DG showing approximately equal staining densities. MR mRNA signal was heavy throughout all hippocampal subregions, with no observable tendency toward preferential localization in any given subfield. In ADX rats, GR mRNA underwent a two-fold increase in subfields CA1-2, with no concomitant increases in CA3-4 or DG. The up-regulation of GR mRNA was not observed in the ADX-DEX group, indicating that the adrenalectomy-induced increase in hippocampal GR mRNA was steroid reversible. No change in GR mRNA was observed following chronic DEX treatment. In contrast to GR mRNA, MR mRNA did not respond to adrenalectomy or steroid treatment; no changes in MR mRNA levels were observed among the four groups. These results suggest that hippocampal GR-producing neurons respond to changes in circulating glucocorticoid levels in a region-specific manner, and therefore that neurons localized to subfields CA1-2 play a potentially important role in feedback inhibition of the hypothalamo-pituitary-adrenocortical axis. Evidence for a dynamic response of MR mRNA expression to circulating glucocorticoid levels is presently lacking.

11.11

ESTROGEN INCREASES PROGESTERONE RECEPTOR mRNA LEVELS IN THE HYPOTHALAMUS OF THE RAT. G.J.Romano, P.Chambon*[1] and D.W.Pfaff. The Rockefeller University, New York, NY 10021 and [1]Institute de Chimie Biologique, Strasbourg, France.

Previous studies have shown that estrogen increases progesterone binding in the hypothalamus. To determine whether this results from increased synthesis of new progesterone receptors, we have examined the effects of estrogen on progesterone receptor mRNA levels. Ovariectomized rats were injected s.c. with 10µg estradiol benzoate (EB) and sacrificed 4, 24 or 48 hr later. The 48h group received a second injection of EB after 24h. For in situ hybridization: brain sections were hybridized with a ^{3}H-labelled, single stranded DNA probe complementary to a portion of the human progesterone receptor mRNA. Labeled cells were observed only in regions known to contain progesterone binding sites. As evidenced by the lack of signal in the paraventricular nucleus, the probe did not hybridize to glucocorticoid receptor mRNA. Grain counting analysis revealed that 24h after injection of EB the number of labelled cells increased 3.9-fold in the ventrolateral-ventromedial nucleus and 5.3-fold in the arcuate nucleus. The number of grains/labelled cell in these nuclei also increased by 50%. Despite the second EB injection, the number of labeled cells and grains/cell declined by 48h. Both of these nuclei contain estrogen-inducible progestin binding sites. No effect of EB was observed in the medial amygdaloid nucleus, which contains progesterone binding sites that are not inducible by estrogen.

11.12

DIFFERENCES IN RESPONSE TO VIBRATORY STRESS OF TWO GLUCOCORTICOID REGULATED GENES IN RAT HIPPOCAMPUS. J.N. Masters, N.R. Nichols and C.E. Finch. Andrus Gerontology Center, USC, Los Angeles, CA 90089-0191

To study the molecular mechanisms of glucocorticoid effects on aging, stress and neurodegeneration we have isolated corticosterone-responsive clones from a rat hippocampal cDNA library. Two of these clones were chosen to analyze the effects of stress on their regulation. One of the clones is an unknown neuronal clone (pCR16) while the other has been identified as glycerol phosphate dehydrogenase (GPDH, pCR3) an oligodendrocyte marker. Adrenalectomized (ADX) or intact (INT) animals were placed on a mechanical shaker for two hours followed by isolation of total hippocampal RNA; non-stressed INT animals were used as controls. The abundance of CR16 and GPDH RNA were quantified by RNAse protection experiments using anti-sense cRNA probes. The results are summarized in the table below as pg transcript / ug total RNA. A clone for proteolipid protein (PLP) was used as a non-changing probe.

Clone	ADX	INT	INT + STRESS
CR16	1.4	2.6	3.1
GPDH	0.8	0.9	2.8
PLP	10.9	11.8	12.6

The amount of GPDH RNA is increased 3 fold by stress (INT + STRESS vs INT) but CR16 does not change (1.2 fold). However the amount of CR16 RNA responds to INT levels of CORT (2 fold, ADX vs INT) where GPDH does not. One interpretation of these results is that the high affinity CORT receptor mediates the expression of CR16 while the type II receptor mediates expression of GPDH. Both CR16 and GPDH RNA responses show type II glucocorticoid receptor specificity (RU28362 ≥ CORT > ALDO > DHT = ADX) but this does not preclude an additional type I interaction. We are currently pursuing other experiments to determine what aspects of CORT regulation accounts for the differential responses to stress. Supported by the John D. and Catherine T. MacArthur Foundation and ONR Grant N00014-85-K-0070; MRN is a Brookdale National Fellow.

NEURAL PLASTICITY IN ADULT ANIMALS: LTP I

12.1

INDUCTION, MAINTENANCE AND EXPRESSION OF LONG-TERM POTENTIATION (LTP) ARE DISTINGUISHED BY BLOCKERS AND STIMULATORS OF PROTEIN KINASE C. *D. V. Madison, R. Malinow and R. W. Tsien.* Dept. of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT 06510.

We have previously reported that in the rat hippocampal slice preparation, sphingosine, an inhibitor of PKC at the regulatory domain, blocks LTP when applied before but not after the inducing tetanic stimulation (J. Physiol. 398, 18P). In contrast, H-7, a blocker of PKC at its catalytic site, will reduce potentiated transmission to basal levels, even when applied hours after the tetanus; unpotentiated pathways are unaffected. Upon washout of H-7, LTP is restored. Thus, there must be a signal that persists even in the presence of H-7; the expression of this signal is blocked by H-7.

Synaptic transmission is enhanced by bath application of phorbol diacetate, an activator of PKC, but returns to baseline levels upon washout of the phorbol ester. Thus, PKC activation alone is not sufficient to maintain LTP, even though it mimics the expression of LTP.

To account for these results, we propose a model in which induction of LTP is mediated by sphingosine-sensitive PKC. The model attributes the maintenance of LTP to formation of a sphingosine-insensitive, H-7-sensitive enzyme, such as PKM, the catalytic fragment of PKC. During induction of LTP, PKM levels could be jointly regulated by activation of PKC (as with phorbol esters) and proteolysis of PKC by a Ca-dependent protease.

12.2

SELECTIVE ACTIVATION OF PRE-SYNAPTIC PROTEIN KINASE C (PKC) ENHANCES SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPAL SLICES. R. Malinow, D.V. Madison and R.W. Tsien. *Dept of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven CT 06510.*

Bath application of phorbol ester enhances synaptic transmission in rat hippocampal slices, mimicking tetanus-induced long-term potentiation (LTP) (Malenka, Madison and Nicoll, 1986). We are developing a new technique using selective drug application together with conventional field potential recording to assess the role of pre-synaptic and post-synaptic PKC in LTP. We activate pre-synaptic PKC by focal application of lipophilic agents such as phorbol diacetate or dibutyrate to a selected subset of afferent fibers. When a fluorescent lipophilic agent (Di-I) is applied in the same manner, it can be seen to diffuse within the pre-synaptic axonal membrane to the recording site. The focally applied phorbol ester causes an enhanced synaptic transmission from those fibers (increase to 242 ± 20% of control; n=10). The enhancement decays within about 2 hrs, similar to the effects of bath applied phorbol ester. The action appears to be selectively pre-synaptic since synaptic transmission from independent input fibers to the same post-synaptic cells generally shows no enhancement. The enhancement by focal phorbol ester application is not due to an increased recruitment of axons at the stimulus site, as the pre-synaptic action potential remains unchanged. The relation between this enhancement of synaptic transmission induced by pre-synaptic PKC activation and LTP will be discussed.

12.3

LTP-INDUCED PROTEIN F1 PHOSPHORYLATION IN VITRO: DOES IT REPRESENT AN INCREASE OR DECREASE IN PHOSPHATE INCORPORATION IN VIVO? S. Chan, P. Colley* and A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, IL 60208.

After long-term potentiation (LTP) protein F1 phosphorylation measured in vitro is increased (Brain Res. 399:205). We report here a method to examine the in vivo mechanism for this increased phosphate incorporation in vitro. Rat hippocampal homogenates were boiled for 5 min to deactivate all enzymes. Samples were divided into two aliquots: one was treated with alkaline phosphatase (AP, 37°C, 1 hr, pH 9) to dephosphorylate occupied in vivo phosphorylation sites (AP+), and the other served as a control (AP-). The dephosphorylated protein F1 was then phosphorylated under optimal conditions by exogenous purified PKC (J. Neurosci. 6:3618) in the presence of gamma-^{32}P-ATP. The phosphorylation of protein F1 was quantified by densitometric analysis of audioradiograms. ^{32}P incorporation after AP+ estimated the total amount of protein F1 in the tissue. The value (AP+)-(AP-)/(AP+) * 100 was taken as the % of total sites endogenously phosphorylated.

Initial results indicate that LTP increased the % of endogenously phosphorylated sites (one-way ANOVA test: F=8.96, p<.05, N=4). In addition, there was greater exogenous F1 phosphorylation in controls without AP treatment in 3 out of 4 cases. Such results indicate that the in vivo phosphorylation of F1 is indeed increased after LTP suggesting increased PKC activity, as reported previously (Science 231:587). Thus, no apparent decrease in phosphate incorporation into protein F1 is occurring in vivo, though an inhibition of phosphatase activity cannot be ruled out. An increase in protein F1 substrate is suggested since exogenous protein F1 phosphorylation after AP+ treatment was greater in all cases at 2-4 hrs after LTP. These results suggest that after LTP an increase in kinase activity initially and an increase in protein F1 substrate at later time points (>1 hr) may contribute to the final in vivo increase in F1 phosphorylation. (Supported by MH25281-13 and AFOSR87-0042 to A.R.)

12.4

SYNAPTIC TRANSMISSION BETWEEN INDIVIDUAL CA3 AND CA1 NEURONS IN THE HIPPOCAMPUS. M.J. Friedlander, R.J. Sayer* and S.J. Redman, Experimental Neurology, John Curtin School, A.N.U., Canberra, A.C.T. 2601, AUSTRALIA.

Neurons of the CA3 region of the hippocampal formation provide excitatory input to neurons of the CA1 region. However, the synaptic potentials that underly this convergent input have not been described for connections between individual pairs of neurons. Thus, we employed dual intracellular recording to evaluate the EPSPs in CA1 neurons evoked by impulses in single CA3 neurons. Recordings were made from CA3-CA1 cell pairs in 450-500 µm thick slices of hippocampus prepared in conventional manner from guinea pigs that had been deeply anesthetized with ether. Records from CA1 neurons were averaged in response to single action potentials elicited by brief depolarizing pulses at 2 Hz to a single CA3 neuron. Of 975 cell pairs tested, functional synaptic connections were observed in 65 cases. In most cases (60/65), the unitary CA3 evoked synaptic potential was excitatory onto CA1 pyramidal cells with a mean amplitude of 131.8 µV (range 54-311 µV). The time course of these synaptic potentials is characterized by a mean 10-90% rise time of 3.9 ms (range 0.9-7.4 ms) and mean half width of 14.7 ms (range 5.3-42.4 ms). IPSPs were evoked in 3 cases and EPSPs were evoked in 2 presumptive CA1 interneurons.
Supported by Fogarty 1F06 TW01378 (MJF) & NH&MRC (RJS).

12.5

LONG-TERM POTENTIATION COMPARED FOR UNITARY AND COMPOUND EPSPS IN CA1 NEURONS. R.J. Sayer,* M.J. Friedlander and S.J. Redman, Experimental Neurology, John Curtin School, A.N.U., Canberra, A.C.T. 2601, AUSTRALIA.

To determine whether LTP can be expressed at single cell-to-cell synaptic connections in the hippocampal slice, intracellular recordings of unitary EPSPs were made from CA1 neurons before and after conjunction with tetanic stimuli to the CA1 stratum radiatum. Slices were prepared by standard techniques from guinea pigs deeply anaesthetised with ether. Unitary EPSPs (75-450 μV) were evoked at 2Hz by action potentials elicited in single CA3 neurons by brief intracellular current pulses. Compound EPSPs (2-8 mV) were evoked through the same cathode as the tetani and were interleaved with the unitary EPSPs at 15s intervals. In six experiments in which CA3-CA1 pairs were held for 15-60 min after the tetani and the compound EPSPs showed a persistent post-tetanic increase in amplitude, the unitary EPSPs were unchanged. Trains of depolarizing pulses were delivered to the CA3 neurons coincident with each tetanus to ensure that the CA3 axon was repetitively activated. Implications for LTP mechanisms include a) cooperativity may be required not only for induction but also for expression, b) functional connections may develop for afferents which were previously ineffective.

Supported by NH&MRC (RJS) & Fogarty 1F06 TW01378 (MJF).

12.6

ASSOCIATIVE LONG-TERM POTENTIATION (LTP) OR DEPRESSION (LTD) IS PRODUCED IN HIPPOCAMPUS DEPENDENT UPON THE PHASE OF RHYTHMICALLY ACTIVE INPUTS. P.K. Stanton, J. Jester, S. Chattarji, and T.J. Sejnowski. Dept. Biophysics, Johns Hopkins Univ., Balto., MD 21218.

Hippocampal LTP is an enduring increase in synaptic efficacy following brief high-frequency afferent stimulation. Separate afferents activated together exhibit associative LTP of inputs that alone do not elicit LTP, and inputs silent during postsynaptic burst firing show reduced synaptic efficacy termed LTD. LTP also occurs following short, rhythmic high-frequency bursts (100 Hz) separated at θ rhythm frequencies (5 Hz). We report here that phase of arrival of a weak input within the rhythm of a strong (potentiating) one determines whether associative LTP (inputs in phase) or LTD (out of phase) occurs.

Extra- and intracellular recordings were made in rat hippocampal slices (400 μm thick) in an interface chamber at 34°C. Stimuli were to separate inputs on the Schaffer collateral and subicular sides of CA1 pyramidal cell apical dendrites, or to commissural and mossy fiber inputs to CA3. Strong stimuli consisted of trains of 10 bursts of 5 pulses each (100 Hz burst frequency, 200 msec interburst interval). Weak stimuli (5 Hz trains) were positively correlated (in phase) by superimposing on the middle of each burst, or negatively correlated (out of phase) by symmetric placement between bursts.

When weak and strong inputs were in phase, associative LTP of the weak input epsp and population spike was seen 15-60 min post-tetani in field CA1, if either the Schaffer collateral or subicular side received the weak stimuli (ΔEPSP = +49.8 ± 7.8%, n=20; ΔSpike = +65.4 ± 16.0%, n=14). In contrast, if weak and strong inputs were 180° out of phase, a specific associative LTD of the weak input population spike was observed (-46.5 ± 11.4%, n=10), with no change in epsp slope. In field CA3, commissural fiber synapses also exhibited associative LTP when in phase with strong mossy fiber stimuli, and associative LTD when out of phase. Thus, pyramidal cells show enhanced or reduced efficacy of specific synapses depending upon precise timing between inputs. (Supported by Office of Naval Research Grant #N00014-88-K-0198)

12.7

EVIDENCE FOR THE INVOLVEMENT OF A GTP-BINDING PROTEIN (G-PROTEIN) IN HIPPOCAMPAL LONG-TERM POTENTIATION (LTP). Joanne W. Goh and Peter S. Pennefather, Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 2S2.

Repetitive activation of presynaptic fibres in the hippocampus results in LTP of synaptic transmission. The mechanisms underlying LTP are not entirely clear but there is some indication that activation of protein kinase C (PKC) plays a role (Akers *et al.*, Science 231: 587, 1986; Malenka *et al.*, Nature 321: 175, 1986). The events leading to PKC stimulation during LTP induction, however, are not known. The results presented below suggest that a G-protein is involved in tetanus-induced LTP. It is possible that activation of this G-protein increases phosphoinositide turnover to produce diacylglycerol which, in turn, stimulates PKC.

Experiments were conducted on transversely sectioned rat hippocampal slices. Extracellular population spikes were recorded in the CA_1 cell body layer in response to stimulation of stratum radiatum. Pertussis toxin (PT, 3-4 μg total), an agent that inactivates some G-proteins, or vehicle alone was stereotaxically injected at 2 sites directly above the right hippocampus. Slices were obtained from the rats at 2,3 and 4 days post-injection. It has been shown that PT-uncoupling of G-proteins from their receptors reduces hippocampal neuronal responses to baclofen (Andrade *et al.*, Science 234: 1261, 1986). By monitoring the effect of baclofen on slices from vehicle-injected control and PT-treated hippocampi, it can be established whether PT is indeed inactivating G-proteins. In controls obtained at 3 and 4 days post-injection, LTP could be induced by tetanic stimulation of the input (271 ± 39% SEM of control at 30 min post-tetanus, n = 11) and baclofen (10 μM) produced a marked suppression of the population spike (13 ± 6% of control, n = 11). Experiments performed on the 3rd and 4th days post-PT injection indicated that LTP could not be induced (3rd day: 107 ± 11% of control at 30 min post-tetanus, n = 9; 4th day: 115 ± 11%, n = 6). The blockade of LTP developed in parallel with reduction of the ability of baclophen to inhibit the response (3rd day: 58 ± 4% of control, n = 9; 4th day: 48 ± 9%, n = 6). LTP could be elicited in slices obtained at 2 days post-PT injection (219 ± 47% of control, n = 5) and inhibition of the population spike by baclophen was normal (8 ± 5% of control, n = 5). It, thus, appears that the minimum time required for PT to exert its effect is greater than 2 days. This is not unexpected as the activity of PT is dependent upon a relatively slow uptake process that is followed by intracellular release of the active A protomer (Nogimori *et al.*, Biochem. 25: 1355, 1986).

12.8

TIME-COURSE OF STRESS-INDUCED IMPAIRMENT OF LTP IN RODENT HIPPOCAMPUS. M.R. Foy, T.J. Shors, S. Levine and R.F. Thompson. Dept. Psychology, Loyola Marymount University, Stanford Univ., & USC, Los Angeles, CA 90045

A marked impairment of long-term potentiation (LTP) recorded from the CA1 cell field of hippocampal slices immediately taken from rats exposed to acute stress has recently been reported (Foy et al., Behav. & Neural Bio., 1987,48,138-149). Some variable(s) in the temporal pattern of the PTP (post-tetanic potentiation)/LTP interaction appear to be modulated by dexamethasone (Foy et al., Neurosci. Abstr.,1987,13,976). In the present study, we examined the effects of stress on the pituitary-adrenal axis by measuring the time course of the stress response of LTP impairment.

The procedure was the same as in our previous experiment (Foy et al.,1987), except that following acute stress, rats were either immediately sacrificed (t=0) or returned to their home cages for a period of 1 hr before sacrifice (t=1). In vitro hippocampal slices were then prepared and recordings were made according to standard methods. Control animals (no shock) exhibited LTP following tetanus, whereas both groups of stress animals (t=0) and (t=1) exhibited no LTP following tetanus.

These data indicate that stress can significantly modulate neural plasticity within the rodent hippocampus which may involve long-lasting humoral and/or other effects within the animal following acute stress.

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12.9

PLASTICITY OF RECURRENT EXCITATORY SYNAPSES BETWEEN CA3 HIPPOCAMPAL PYRAMIDAL CELLS. R. Miles* (SPON: T.A. Pedley), Dept of Neurology, Columbia Univ., New York, NY 10032.

CA3 hippocampal pyramidal cells possess axon collaterals which form recurrent excitatory synapses with other CA3 pyramidal cells. Some unitary properties of these synapses have emerged but their plasticity has not been directly examined.

Recurrent synapses were selectively activated in two ways to examine their plasticity. Firstly, CA2-3a pyramidal cells were antidromically activated to evoke recurrent epsps in CA3b cells, while synaptic inhibition was suppressed and divalent cations were elevated. A persistent potentiation of recurrent EPSPs, with constant pre-synaptic activity, was observed 12-20 min after tetanic stimulation. Secondly, recurrent EPSPs were elicited by stimulating one cell and recording from a post-synaptic cell. 4 out of 5 mono-synaptic EPSPs were potentiated 12-20 min after tetanic stimulation of afferent fibres. The mean number of transmission failures at these synapses was reduced from 22% to 9%. The mean number of quanta released, calculated by the variance method, was increased while the quantal amplitude was slightly reduced.

The possibility that modulating transmitters facilitate the plasticity of recurrent synapses is also being investigated. Muscarine (5 uM) and carbamylcholine (5 uM) reduced the amplitude of single recurrent EPSPs but enhanced the post-synaptic depolarization during repetitive stimulation at 20-50 Hz. The probability and magnitude of potentiation of recurrent EPSPs (at 15 min) were enhanced when tetani were delivered in the presence of muscarinic agonists.

In conclusion, plasticity at recurrent excitatory synapses appears to result from a pre-synaptic modification and is facilitated by a transient activation of muscarinic receptors.

12.10

PROTEIN KINASE C IN HIPPOCAMPUS AND SEPTUM FOLLOWING FIMBRIA-FORNIX TRANSECTION. S. SHIMOHAMA, T. SAITOH, AND F.H. GAGE, Dept. of Neurosciences, UCSD, La Jolla, CA 92093, USA.

Protein kinase C (PKC) plays a pivotal role in the regulation of neuronal functions. We have studied the distribution and concentration of PKC in the intact and lesioned septum and hippocampus. Throughout this study, we have employed three anti-(PKC) antisera raised against C-terminal variable region of PK-C α, β-I and γ.

In the intact septum, PKC-α immunoreactive neurons were dense in the lateral septum and PKC-β immunoreactive neurons were present evenly throughout while PKC-γ immunoreactivity was highest in the medial septum. A decrease in the PKC-β and PKC-γ immunoreactive neurons was observed in the medial septum on the side of the lesion and a slight increase in the PKC-β and PKC-γ immunoreactive glia-like cells in the lateral septum.

In the intact hippocampus intense PKC-α immunoreactivity was observed in the subiculum, CA1, CA2 and CA3 regions. The molecular layers of the dentate gyrus (DG) were moderately stained and some PKC-α immunoreactive interneurons were observed in the hilus. Moderate PKC-β and PKC-γ immunoreactivity was observed in the subiculum, CA1, CA2, CA3 and the DG. Some PKC-β and PKC-γ immunoreactive glia-like cells were present in the subiculum and CA1 regions. Following damage there was a large decrease in PKC-α immunoreactivity in the CA1 and CA2 regions. In addition, there was a marked increase in PKC-β and PKC-γ immunoreactive glia-like cells in the subiculum, CA1 and CA2.

These results show that different isoforms of PKC are expressed in neurons and glia, and they may be under distinct regulatory mechanisms.

12.11

CURRENT SOURCE DENSITY (CSD) ANALYSIS OF THE EFFECTS OF PHORBOL ESTERS IN RAT PRIMARY VISUAL CORTEX. N.A. Lambert* and T.J. Teyler. Dept. of Neurobiology, Northeastern Ohio Univ. College of Medicine, Rootstown, OH 44272.

Protein kinase C (PKC) activation has been shown to be involved in the generation of long-term potentiation (LTP) in the hippocampus. Phorbol esters, which activate PKC, produce an effect similar to LTP when applied to hippocampus *in vitro* and blockade of PKC activation by polymyxin B causes an accelerated decay of tetanus-induced LTP. It has recently been demonstrated that activation of PKC induces long-term changes in postsynaptic currents in neocortical neurons (Baranyi, A. *et al.*, *Brain Res. 440*:341, 1988). To determine if PKC activation is involved in LTP induction in the neocortex this study examines the effects of phorbol esters in rat primary visual cortex *in vitro*.

Slices of rat visual cortex were prepared using standard procedures and CSD profiles were obtained from area OclM before and after bath application of phorbol 12,13-diacetate (PDAc). Initial results indicate that PDAc has an effect similar to the potentiation seen in hippocampus. PDAc also made slices susceptable to seizures. This suggests that induction of LTP in rat primary visual cortex also involves activation of PKC. (Supported by grants from NIH, EPA and ONR.)

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION I

13.1

TWO DISTINCT cDNAs FOR RAT PEPTIDYL GLYCINE α-AMIDATING MONOOXYGENASE (PAM) D.A. Stoffers, C.B.-R. Green* and B.A. Eipper, Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD, 21205.

About half of known bioactive peptides require an amidated C-terminus for bioactivity. The copper and ascorbate dependent enzyme responsible for this post-translational modification, PAM, was purified and the cDNA cloned from bovine pituitary (Eipper et al (1987) Mol Endo 1: 777). PAM is found throughout the endocrine and nervous systems. Unexpectedly high levels of PAM activity and mRNA are found in adult rat atrium, where 3.6 and 3.8 kb mRNAs are identified by Northern analysis.

A λZAP cDNA library constructed from rat atrial RNA and screened with bovine PAM cDNA probes yielded two distinct types of rPAM cDNA, rPAM-1 and -2. A 3.8 kb cDNA of the rPAM-1 type was sequenced and exhibits 84% nucleotide identity to the bovine PAM cDNA in the protein coding region. Major features of the protein predicted by bovine PAM cDNA are conserved in rat including signal and propeptide, a lengthy intragranular domain, a putative transmembrane domain, and a short cytoplasmic segment. The positions of all 14 cysteines, the His-rich potential copper binding regions, 8 of 10 pairs of basic amino acids, and 3 potential phosphorylation sites in the cytoplasmic domain are conserved.

Restriction mapping identified cDNAs of the rPAM-2 type which closely resemble the rPAM-1 type cDNA but lack ~200 bp in the protein coding portion.

13.2

EXPRESSION AND POST-TRANSLATIONAL PROCESSING OF TRANSFECTED MUTATED NEUROPEPTIDE Y cDNAs. I.M. Dickerson, J.E. Dixon, and R.E. Mains. Dept. Neuroscience, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

Most neuropeptides are synthesized as a large biologically inactive precursor, which is proteolytically cleaved to a smaller bioactive form. Cleavage occurs primarily at pairs of basic amino acids in the precursor. We have begun to probe the substrate requirements for cleavage by transfecting cDNAs for human proNPY into the mouse corticotrope tumor cell line AtT-20 and isolating stable NPY-producing transformants.

ProNPY is 69 amino acids long and undergoes a single endoproteolytic cleavage at the sequence -Lys^{38}-Arg^{39}- during maturation. Using in vitro mutagenesis, point mutations encoding all possible permutations of this pair of basic residues have been introduced into the wild-type cDNA: -Arg^{38}-Arg^{39}-, Arg^{38}-Lys^{39}-, and -Lys^{38}-Lys^{39}-. We have compared the ability of cell lines transfected with wild-type or mutant proNPY cDNA to synthesize, process and secrete proNPY-derived peptides. ProNPY molecules containing the mutated cleavage sites -Arg^{38}-Arg^{39}- and Arg^{38}-Lys^{39}- are cleaved in AtT-20 cells as is the wild type proNPY which has been previously described (Dickerson et al, (1985) J. Biol. Chem. 262:13646). Like the uncleaved -Lys-Lys- pair in the endogenous β-endorphin, proNPY molecules containing -Lys^{38}-Lys^{39}- are not cleaved.

13.3

DISTRIBUTION OF CARBOXYPEPTIDASE E (EC 3.4.17.10) IN RAT PITUITARY. J. Rossier and E. Barrès*. Lab. de Physiol. Nerveuse du CNRS, 91198 Gif-sur-Yvette Cédex, France.

Carboxypeptidase E, EC 3.4.17.10, also known as enkephalin convertase, carboxypeptidase H and crino carboxypeptidase B, is an important enzyme involved in the biosynthesis of bioactive peptides. Rat anterior, intermediate and neural lobes were homogenized in 1 ml of 0.025 M Tris HCl buffer pH 8 with 5 mg/ml of BSA. After centrifugation, the supernatant was brought to pH 5.6 and centrifuged again. Following a 20 min preincubation with 2 mM $CoCl_2$, supernatant was incubated in a 1.5 ml tube with 0.1 mM of the radioactive substrate (^{3}H)Benzoyl-Phe-Ala-Arg. The 100 µl reaction mixture was stopped with 680 µl of a mixture of 0.25 N HCl/Acetonitrile (4/2.8) and the tube was transferred into a scintillation vial. Econofluor, a water non miscible scintillation fluid, was added. The product, Benzoyl-Phe-Ala, recovered in the organic phase, was counted with no interference from the substrate remaining in the aquous phase. The values expressed in nmol/min/lobe of the product formed were: 193 ± 28, 25.8 ± 1.9 and 10.3 ± 2.0 for anterior, intermediate and neural lobes respectively. After 3.5 days dehydration, these values did not change in anterior and intermediate lobes, but decreased by more than 50% in the neural lobe. In all the extracts 25 µM of GEMSA, a specific inhibitor of carboxypeptidase E, inhibited completely the activity.

13.4

IDENTIFICATION OF PREPROTACHYKININ SPECIFIC PROCESSING ENZYMES. Y.Y.H. Hook, T.J. Krieger*, D. Hegerle*, and H.-U. Affolter*+. Dept. of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD. and +Brain Research Institute, University of Zurich, Switzerland.

The neuropeptides substance P, neurokinin A, and neuropeptide K are derived from a large precursor, that must be specifically cleaved to form the bioactive peptides. In contrast to bovine and human species, rat shows no tissue-specific splicing of the tachykinin gene product. Therefore, the mechanism of tissue-specific production of tachykinin peptides must occur at the level of precursor processing. To identify the endopeptidase(s) that cleave this precursor, authentic ^{35}S-(Met)-ß-preprotachykinin was synthesized by in vitro translation, using a human cDNA derived mRNA (SP6 in vitro transcription system). Processing enzyme activity was found bovine adrenal medulla granules, which have been shown to contain substance P. Sequential cleavage of substrate followed by processing of resultant intermediate products appears to require distinct activities. ^{35}S-(Met)-ß-preprotachykinin cleavage was abolished by pepstatin, whereas further processing of an intermediate product was inhibited by leupeptin, and processing of a second product was inhibited by chymostatin. These data support the hypothesis that multiple types of protease activities may be involved in substance P precursor processing.

13.5

PARTIAL PURIFICATION AND CHARACTERIZATION OF MULTIPLE PREPROTACHYKININ PROCESSING ENZYME ACTIVITIES. T.J.Krieger*, H.-U. Affolter*+, and V.Y.H. Hook. (SPON: G. Mueller). Dept. of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD. and +Brain Research Institute, University of Zurich, Switzerland.

The tachykinins substance P, neurokinin A, and neuropeptide K are synthesized as a larger prohormone which undergoes proteolytic processing to yield the active peptide hormones. A rapid assay for ß-preprotachykinin processing has been utilized in the partial purification of 3 distinct activities from bovine adrenal medulla chromaffin granules. Affinity chromatography on conconavalin A - agarose followed by size exclusion chromatography on Sephacryl S200 yields 3 peaks of activity capable of cleaving human ^{35}S-(Met)-ß-preprotachykinin. The 3 peaks have apparent molecular weights of 205 kD, 58 kD, and 11 kD. The 58 kD activity is the major activity comprising over 60% of the total activity. The 58 kD activity is inhibited by the aspartyl protease inhibitor pepstatin A. The pH optimum for the activity is near 5.0 when the conversion of the prohormone into products is limited. In addition, pH has a profound influence on the extent of proteolytic processing and the identity of the products formed.

13.6

A NOVEL SOMATOSTATIN PEPTIDE CORRESPONDING TO PROSOMATOSTATIN (PRO-S) WITHOUT THE C-TERMINAL S-28 SEQUENCE: CHARACTERIZATION AND TISSUE DISTRIBUTION. S.N. Rabbani*, H. Bennet*, and Y.C. Patel* (SPON: H.H. Zingg). Fraser Laboratories, McGill University, Montreal, Quebec H3A 1A1.

Processing of pro-S to S-28 should theoretically generate a molecule equivalent to pro-S minus S-28 (pro-$S_{[1-64]}$). To identify such a molecule, 1 N acetic acid- pepstatin extracts of rat hypothalamus, antrum, jejunum, and pancreas were fractionated by reverse phase HPLC. The column effluent was monitored by region specific RIAs using antibodies directed against pro-$S_{[1-10]}$, S-$28_{[1-12]}$, and S-14. The concentration of pro-$S_{[1-10]}$ LI (pg/mg) was high in the antrum (56 pg/mg) and hypothalamus (28.5), moderate in the jejunum (12.7) and virtually undetectable in the pancreas (0.3). Eight HPLC peaks of pro-$S_{[1-10]}$ LI were identified and rechromatographed by gel permeation HPLC to reveal the following m.w. forms: 1K, 1.5K, 2.5K, 3.5K, 4.5K, 7K, 8K, and 10K. Only the 10K form was detectable as S-14 LI; it thus corresponded to the full length pro-S molecule. Only the 8K form was detectable as S-$28_{[1-12]}$ LI. The 1K form corresponded to pro-$S_{[1-10]}$. The 7K form was not detectable as S-14 LI or S-$28_{[1-12]}$ LI and thus appeared to represent pro-S without the S-28 sequence. It was relatively abundant in the hypothalamus (50% of pro-$S_{[1-10]}$ LI), present in moderate quantities in the antrum and jejunum (19%, 18% of pro-$S_{[1-10]}$ LI respectively), but undetectable in the pancreas. Conclusions: 1) Pro-$S_{[1-10]}$ LI is heterogeneous. 2) Its concentration is strikingly low in the pancreas compared to other tissues. 3) A novel 7K form corresponding to pro-$S_{[1-64]}$ exists and provides indirect evidence for pro-S to S-28 conversion. Its variable tissue distribution suggests that such conversion is important in the hypothalmaus and gut but not in the pancreas.

13.7

REGULATION OF NEUROPHYSIN PROCESSING IN THE RAT NEURAL LOBE. Robert Newcomb, Daniel Hartline and Jean Nordmann. Békésy Laboratory, Honolulu, HI and CNRS, Strasbourg, France.

An HPLC procedure has been developed for the direct analysis of all major neural lobe peptides (Neurochem. Int. 11, 229). This allows quantitation of the extent conversion of the oxytocin neurophysin (OTNP) to its alternate form, missing the C-terminal glutamate (OTNP'). This conversion occurs on the time scale of secretory granule turnover, making the ratio OTNP'/(OTNP+OTNP') a useful indirect measure of neural lobe secretory granule age. Combination of the HPLC procedure with introduction of 35S-Cys over the supraoptic nucleus provides the in-vivo conversion rate of OTNP to OTNP'. This proceeds with an initial rate constant of 0.1 days-1, and a rate constant for inactivation of this initial rate of 0.14 days-1. With chronic osmotic stress (21 days imbibition of 2% NaCl) the conversion rate shows only a slight increase, suggesting that changes in synthesis with increased secretory activity are comparable for the major secretory granule constituents. Measurements of OTNP'/(OTNP+OTNP') have been made in single neural lobes of animals under varying physiological conditions. Computer simulations incorporating these data show that compartmental models of secretory granule movements are capable of simulating changes in neural lobe (OTNP')/(OTNP+ OTNP'). From these studies, it is concluded that about 60% of secretory granules transported to the neural lobe are not released in the lifetime of a 300 g rat. Changes in affinity for release of aged secretory granules provide an alternative to compartmentation as an explanation for the observed changes in neurophysin content with depletion.

13.8

AN ENDOGENOUS CLONIDINE-DISPLACING SUBSTANCE IS PRESENT IN PERIPHERAL TISSUES OF THE RAT. *M.P.Meeley, M.L.Hensley*, P.M.McCauley*, P.Ernsberger and D.J.Reis* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Clonidine-displacing substance (CDS) is present in extracts of brain and inhibits the binding of clonidine to brain membranes (Meeley et al., *Life Sci* 38: 1119, 1986). Since CDS also contracts smooth muscle of the gastric fundus in rat (Felsen et al., *Eur J Pharmacol* 142: 453, 1987), we sought to determine whether CDS is contained in tissues other than brain. Rats were decapitated and whole blood was collected and serum prepared; the brain and peripheral tissues were dissected, homogenized in boiling water, then centrifuged at 4°C and 100,000xg for 30min. Supernatants and sera were made 7% in trifluoroacetic acid, re-centrifuged and neutralized. Samples were filtered to remove >10,000MW material, then dried and extracted with methanol. The CDS content was measured by radioimmunoassay (RIA) using a polyclonal antiserum to *p*-aminoclonidine (Meeley et al., *Neurosci Lett* 84: 84, 1988). Values are in RIA-Units; ie., the amount of extract giving 50% inhibition *per* gram wet wt. CDS-like radioimmunoactivity was higher in fresh rat brain (8.1±2.5; n=9) than in bovine brain (0.3-0.8). In rat, CDS-like activity was also present in the periphery (n=5-10). Adrenal gland (113±20) and gastric fundus (61±16) contained significantly greater levels of CDS than in any of the other tissues assayed ($p<0.01$, ANOVA with Neuman-Keuls). The heart (7.2±1.7), small intestine (3.1±1.2), kidney (1.6±0.4) and liver (1.3±0.3) also contained measurable amounts. CDS-like radioimmunoactivity in lung and skeletal muscle was near background (0.1-0.6; n=5), whereas CDS levels in serum were substantial (6.2±1.2Units/mL). Dose-dependent CDS-like smooth muscle contractions were elicited by extracts of serum> gastric fundus> small intestine, liver, brain, heart, kidney. These persisted during adrenergic blockade (10µM phentolamine and 1µM propranolol), confirming the presence of bioactive CDS. We conclude that (a) CDS is a unique, biologically-active substance of undetermined origin; (b) two independent assays confirm the presence of CDS in tissues other than brain and in the circulation; and, therefore (c) CDS may play a role as a novel transmitter or modulator, and as a hormone.

13.9

ANGIOTENSIN-(1-7) IS AN ENDOGENOUS PEPTIDE IN THE RAT BRAIN. M.C. Chappell*, K.B. Brosnihan, D.I. Diz, R.A.S. Santos*, M.C. Khosla*, and C.M. Ferrario. Brain and Vascular Research, Cleveland Clinic Fdn, Cleveland, OH 44195.

Recently, we showed that Asp-Arg-Val-Tyr-Ile-His-Pro [Ang-(1-7)] is a potent vasopressin secretatogue (Schiavone et al., PNAS, 1988). Here we compare the distribution of Ang-(1-7) and Ang II in the brain and adenohypophysis (PIT) of 40 male Sprague-Dawley rats (250-350g). Tissue regions were homogenized in ethanol/HCl, cryoprecipitated, and concentrated on a Bond Elut C18 column. Ang peptides were eluted (80% methanol), resolved by HPLC (HFBA solvent system), and quantitated by separate RIAs (Chappell et al., Peptides, 1987). A new RIA (range 1-2000 fmol, sensitivity 5 fmol) was developed which recognized Ang-(1-7) and Ang-(2-7), but not Ang I, Ang II or other N-terminal fragments (<0.05%). ^{125}I-Ang I, -Ang II, and -Ang-(1-7) added prior to extraction yielded recoveries >85%. HPLC revealed that the major immunoreactive Ang peptides detected coeluted with Ang I, Ang II and Ang-(1-7) standards. In PIT and amygdala (AMYG), Ang-(1-7) values were twice those of Ang II [PIT: 1426±85 vs 771±169 pmol/g; AMYG: 689±106 vs 300±75 pmol/g; (n=4 pools of 10 each)]. In contrast, in the hypothalamus(HYPO) and medulla (MED), Ang-(1-7) and Ang II were present in similar amounts (HYPO: 1086±92 vs 1175±75 pmol/g; MED: 683±139 vs 773±125). Thus Ang-(1-7) is present in brain regions and the different ratios of Ang-(1-7) to Ang II suggest that regulation of tissue content may be regionally specific. (Supported by NIH grant HL-6835)

13.10

NEUROPEPTIDE Y-LIKE PEPTIDE IN ADRENAL GLANDS: ISOLATION AND CHARACTERIZATION. H.Y-T. Yang, J. Rubenstein and E.A. Majane, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Neuropeptide Y (NPY) is present in high concentrations in adrenal glands of various species. The analysis of adrenal extract with HPLC-coupled with RIA revealed the presence of an additional NPY-like peptide with a retention time shorter than that of NPY. In rat adrenal glands, NPY-like peptide was detected in older animals but not in younger ones. In bovine adrenal glands, NPY-like peptide was detected in primary culture of chromaffin cells and can be released by 45 mM KCl. In order to explore the possible role of this peptide in adrenal function, NPY-like peptide was purified to homogeneity from bovine adrenal chromaffin granules by BioGel chromatography followed with successive steps of HPLC. The purified material was characterized by sequencing, other chemical analysis and carboxypeptidase digestion. A high degree of homology with NPY was observed and biological activity of this peptide still remains to be studied.

13.11

OXYTOCIN-GLYCINE (OT-G) CIRCULATES IN PLASMA OF HUMANS ADMINISTERED ESTROGEN. J.A. Amico and J. Hempel*. Depts. of Medicine and Biochemistry, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

The OT prohormone contains OT at its N-terminus, OT-neurophysin (Np) at its C-terminus, and glycine (G)-lysine (K)-arginine (R), intervening between OT and Np. Stimuli which release the hormone should also release its associated Np. Estrogen releases into plasma the human OT-Np. The OT immunoreactive (I.R.) material, temporally associated with release of the OT-Np, is not OT but an OT-like peptide (JCEM 60:5, 1985). Co-release of the OT-like peptide with OT-Np suggests that the OT-like peptide originates from the OT prohormone and may be a partially processed form of OT. To test this hypothesis, synthetic OT-G-K-R was cleaved with trypsin and the digest was cleaved with carboxypeptidase-B to form OT-G-K and OT-G, respectively. The identity of each synthetic peptide was verified by compositional analysis and the purified peptides were tested for cross reactivity with the various OT antisera used in this laboratory to distinguish the OT-like peptide from OT. OT-G, but not OT-G-K or OT-G-K-R, showed extensive cross reactivity with the OT antiserum (Ab-1), which is known to detect the OT-like peptide of human plasma. The HPLC separation of plasma from individuals administered estrogen had OT Ab-1 I.R. in fractions eluting with the same retention time as synthetic OT-G. The OT-like peptide of human plasma is a glycine-extended form of OT. Estrogen may alter posttranslational cleavage of the OT prohormone, releasing C-terminal non-amidated forms of OT into human plasma.

13.12

DEMONSTRATION OF THE PRESENCE OF HYPOTHALAMIC HYPOPHYSIOTROPIC FACTORS DISTINCT FROM KNOWN HYPOTHALAMIC HORMONES. A. Arimura*, A. Miyata, N. Minamino, A. Uehara, G. Katsuura, R. Dahl, M. Culler. U.S.-Japan Biomed. Res. Labs., Tulane Univ. Hebert Ctr, Belle Chasse, LA 70037

In order to seek novel hypothalamic hypophysiotropic factors, we began by purifying substances in ovine hypothalamic extracts (OHE) which stimulate adenylate cyclase activity (a nonspecific second messenger involved in the secretion of pituitary hormones) in rat pituitary cell cultures. Boiled and acetone-treated extracts of 3500 ovine hypothalami were loaded on a C18 column, eluted stepwise with 0.1% TFA containing 10, 20, 30, 40, 50, and 60% CH_3CN, and the eluent designated as fr. A, B, C, D, E, or F, respectively. Fr. C and D showed the greatest activity. Fr. C and D were then, respectively, loaded on SP-Sephadex and eluted stepwise with 1 M acetic acid (SP-I), 2 M pyridine (SP-II) and 2 M pyridine acetate (SP-III), respectively. Both SP-II and SP-III derived from C18 fr. C and D showed considerable activity. Both were then subjected to molecular sieving on Sephadex G-50. For each SP fraction a few to several bioactive peaks ranging from approximate mw of 1000 to 5000 were demonstrated. Active peak fractions were pooled and then purified by IEX-HPLC, followed by repeated RP-HPLC until pure materials were obtained. Net charge and hydrophobicity as determined by the retention time on IEX- and RP-HPLC were plotted on the ordinate and abscissa, respectively. Those for synthetic ovine LHRH, CRH, GRH and other neuropeptides were plotted on the same map. About 50% of points for bioactive materials obtained from OHE did not overlap the points for known synthetic neuropeptides. These results indicate the presence of at least several hypothalamic hypophysiotropic peptides which are distinct from known hypophysiotropic hormones. (Supported by NIH DK09094 and a grant from Takeda Chem. Industries.)

CARDIOVASCULAR REGULATION I

14.1

CENTRAL RESPIRATORY GENERATOR (CRG)-RELATED ACTIVITY OF SYMPATHETIC PREMOTONEURONS IN THE RAT ROSTRAL VENTROLATERAL MEDULLA (RVL). J.R. Haselton and P.G. Guyenet. Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908.

The CRG modulation of the lumbar sympathetic nerve discharge (LSND) was examined in 8 rats anesthetized with halothane, vagotomized, paralyzed and artificially ventilated with 100% O_2. At end-expiratory CO_2 levels>6%, phrenic-trigger averaged LSND decreased during early inspiration and exhibited a large peak that occurred at, or immediately after the cessation of the phrenic burst.

In 7 rats prepared as above, recordings of baro-sensitive units were made in the RVL. Forty of 44 neurons exhibited a large CRG modulation at end-expiratory CO_2 levels of 6-10%. Twenty cells displayed a pattern that was strikingly similar to that of LSND. Of these 20, 12 were antidromically activated exclusively from the thoracic spinal cord, 3 from both the spinal cord and pontine central tegmental tract (CTT), 3 only from the CTT and 2 were not driven from any location. The remaining 24 neurons were reticulospinal cells exhibiting one of four CRG-related patterns of activity distinct from that of LSND. The vast majority of the barosensitive cells tested were activated by raising end-expiratory CO_2 to 9-10%.

These results support the notion that RVL sympathetic premotoneurons are involved in cardiorespiratory integration.

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14.2

BETA-ADRENOCEPTOR AND CYCLIC-AMP-MEDIATED EXCITATION OF ROSTRAL VENTROLATERAL MEDULLARY PACEMAKER NEURONS WITH PRESUMED SYMPATHOEXCITATORY FUNCTION. M.-K. Sun and P.G. Guyenet. Dept. of Pharmacology, Univ. of Virginia School of Medicine, Charlottesville, VA 22908.

The rostroventrolateral reticular nucleus (RVL) contains reticulospinal pacemaker cells with presumed tonic sympathoexcitatory and vasomotor function. These cells (N=72) were recorded in 500 μm-thick coronal tissue slices of the rat medulla (Sprague-Dawley 70-110 g). Their mean resting firing rate at 31°C was 8 spikes/s (±1, SEM). Their activity was reversibly increased by forskolin (adenylate cyclase activator, 10 μM; + 57± 12%, SEM) but unchanged by the inactive analog forskolin 1,9-dideoxy, 10 μM N=5). 8-br cAMP (0.5 mM, a membrane-permeable cAMP analog) also increased the activity of these neurons (+ 52 ± 7%, SEM, N=7) while adenosine produced opposite effects (-61 ± 12% at 0.5-1mM, N=8).

RVL pacemaker neurons were also activated by epinephrine (EPI, =+31 ± 7% at 10^{-5}M, N=5) or isoproterenol (ISO, + 52 ± 6% N=5 at 10^{-5}M). The effects of EPI or ISO (both at 10^{-5}M) were antagonized (50-100%) by 10 μM propranolol (N=5). Propranolol alone (10^{-5}M), the alpha-adrenergic agonist phenylephrine (10^{-4}M) or the alpha-antagonist phentolamine (10^{-4}M) were without effect.

It is concluded that the pacemaker activity of RVL cells with putative vasomotor function is regulated by cAMP which is a probable intracellular mediator of the excitatory action of catecholamines on these cells. HL 28785

14.3

AMYGDALA, VAGAL, AND BARORECEPTOR AFFERENT CONVERGENCE ON NUCLEUS TRACTUS SOLITARIUS (NTS) NEURONS IN THE CAT. R.J. Person, Dept. of Physiology and Biophysics, Univ. Okla. Health. Sci. Ctr., Oklahoma City, OK 73190.

Responses of NTS neurons excited from the ipsilateral central amygdala nucleus (AM) were recorded with tungsten microelectrodes in acute cats under chloralose and pancuronium. Stimulating electrodes were also placed on the ipsilateral carotid sinus (CSN) and cervical vagus (V) nerves. AM, V, and CSN responses were evaluated via peristimulus histograms; interactions between inputs were evaluated in conditioning-test (CT) paradigms.

Units received initial excitation from AM at a latency of 8.2 ± 0.7 (SE) msec; 35% had a subsequent 50-200 msec inhibition of spontaneous activity. Of these, many showed a late period of excitation at 175-250 msec. The remainder of the units had a pattern of early excitation with no following inhibitory period or had minimal or no background spontaneous activity.

Twenty-eight % and 16% received convergent CSN or V excitation, respectively, while 10% received convergent excitation from all 3 afferent sources. CT analysis showed that AM input was capable of significantly (>50%) attenuating subsequent CSN and V excitation over a 10-150 msec period.

The AM is apparently capable of modulating carotid sinus baroreceptor input. The inhibition may be interpreted by the as baroreceptor unloading with a subsequent rise in blood pressure. Supported by Ok. Health Research Program, Contract 1702.

14.4

THE ROLE OF ROSTRAL MEDULLA IN THE MEDIATION OF THE CENTRAL ANGIOTENSIN II (ANG-II) PRESSOR RESPONSE. L.R. Portis* and M.J. Brody. Dept. of Pharmacol. and C.V. Center, Univ. of Iowa., Iowa City, IA 52242.

Previous studies in our laboratory have provided functional evidence for mediation of the central ANG II pressor by a periventricular pathway from the antero-ventral third ventricle region to ventromedial peri-aqueductal gray. Our laboratory has also described two functionally and anatomically distinct sites in rostral medulla; rostral ventrolateral (RVLM) and rostral ventro-medial (RVMM) medulla that are both involved in the maintenance of vasomotor tone. Bilateral injection of 4% lidocaine (LIDO) in either RVLM or RVMM produced depressor responses. The present study was designed to determine if either RVLM or RVMM are also involved in the mediation of the ANG-II pressor response. Experiments were performed on Urethane anesthetized rats instrumented for measuring arterial pressure (AP) and heart rate and ventilated artificially. Bilateral injection of LIDO in either RVLM or RVMM produced the expected marked reduction in AP and significantly attenuated the ANG-II pressor response (20 ± 1 vs. 3 ± 1 mmHg) and (20 ± 1 vs. 7 ± 5 mmHg) respectively. The pressor responses and AP both returned to control levels within a 30 minute recovery period. These data suggest that the functional integrity of both RVLM and RVMM are required for mediation of the central ANG-II pressor response. (Supported by HLB-14388.)

14.5

ANGIOTENSIN II AND ANGIOTENSIN (1-7) EXCITE NEURONS IN THE CANINE MEDIAL NUCLEUS TRACTUS SOLITARII (mnTS) IN VITRO. Karen L. Barnes, W. Douglas Knowles, and Carlos M. Ferrario. Brain & Vascular Research and Neurology, Research Institute, Cleveland Clinic Fndn, Cleveland, OH 44195.

Because angiotensin (Ang) II and its heptapeptide fragment Ang (1-7) produce similar dose-related cardiovascular effects when microinjected into the mnTS, we compared the effects of these Ang peptides on mnTS neurons recorded from in vitro slices of the canine dorsomedial medulla. The medulla was removed from halothane anesthetized dogs and blocked in the horizontal plane. 300 μm slices were perifused with artificial cerebrospinal fluid (aCSF, 36°C). Extracellular recordings were obtained from 13 mnTS neurons during microdrop application of Ang II (8 ng/μl), Ang (1-7) (80 ng/μl), or aCSF onto the slice surface. As shown previously [Barnes et al., Hypertension 11: 1988 (in press)], Ang II substantially increased the firing rate of half the neurons tested (6 of 12 cells). Ang (1-7) also excited 4 of 10 neurons tested, eliciting a prolonged (5-10 min) increase in firing rate with a time course similar to the effects of Ang II. In 9 of these neurons the effects of Ang II and Ang (1-7) were compared. Excitatory responses to both Ang II and Ang (1-7) were seen in 3 cells. The other 6 neurons did not respond to either peptide. No inhibitory effects of either Ang peptide on mnTS cells were seen. These studies establish neuronal actions for a member of the Ang peptide family devoid of agonist effects on blood vessels, and suggest a common neuronal substrate for the similar cardiovascular effects of Ang II and Ang (1-7) given into the mnTS. (Supported in part by NHLBI grant HL-6835).

14.6

ALPHA-1 ADRENERGIC DEPRESSION OF SYNAPTIC RESPONSIVENESS IN SOLITARY TRACT NUCLEUS (nTS). P.D. Feldman and R.B. Felder, Dept. of Med., Univ. of Iowa, Iowa City, IA 52242

Our recent work has shown that alpha-2 adrenoceptor activation enhances synaptic excitability in the nTS. To determine the effects of alpha-1 receptor activation extracellular recordings were made from 9 nTS neurons in an in vitro rat brain slice preparation before and during infusion of the alpha-1 agonist methoxamine (MET). A microcomputer generated histograms of postsynaptic spike responses to electrical stimulation of the solitary tract at a fixed stimulus intensity. Agonist was allowed to wash out between drug infusions, and control responses were remeasured before each subsequent infusion of MET. The number of evoked action potentials was not affected by 1 nM or 2.5 nM MET ($P > 0.1$), but fell to 59.9 ± 9.8 % (mean ± SE) of control at 5 nM ($P < 0.05$) and 12.4 ± 0.1 % of control at 25 nM ($p < 0.001$). Infusion of the alpha-1 antagonist prazosin (200 nM) had no effect on synaptic excitability, but completely blocked the decrease of excitability induced by MET ($N = 2$). In contrast, the alpha-2 antagonist yohimbine (200 nM) had no effect on neuronal activity or responses to MET ($N = 2$). These data suggest that activation of alpha-1 adrenoceptors suppresses the responsiveness of nTS neurons to visceral sensory input. We postulate that an opponent process involving alpha-2 and alpha-1 adrenergic receptors may modulate the responsiveness of nTS neurons to autonomic sensory input.

14.7

CORTICOTROPIN-RELEASING FACTOR: ANATOMICAL SUBSTRATES OF AUTONOMIC REGULATION. *R. Giuliano, D.A. Ruggiero, T.A. Milner, M. Anwar and D.J. Reis.* Div. of Neurobiol., Cornell U. Med. Coll., NY, NY 10021

We sought to determine whether areas of nucleus tractus solitarii (NTS) and rostral ventrolateral medulla (RVL) subserving tonic and reflex control of arterial pressure contain corticotropin-releasing factor immunoreactive (CRF-I) neurons and processes and, if so, their source. Antisera against rat/human or ovine CRF were localized in colchicine-treated rats by the peroxidase-antiperoxidase method. Afferents and efferents were identified by injection of wheat germ agglutinated horseradish peroxidase (WGA-HRP) into RVL and NTS. Single sections were processed for HRP histochemistry and immunocytochemical localization of CRF. In NTS CRF-I punctate varicosities filled subnuclei (dorsal, intermediate and ventral) which receive primary sinoaortic baroreceptor afferents and harbor cells projecting to the C1 area of the RVL. Other CRF-I processes were distributed in the gastrointestinal afferent field (parvocellular subnucleus) or lay in close proximity to dorsal motor vagal neurons and surrounded the central canal. CRF-I cells were limited to caudal regions of NTS including an area transitional with area postrema. In RVL relatively few labeled perikarya and processes, distinct from larger CRF-I precerebellar relay cells of the lateral reticular nucleus, were seen. In transport studies WGA-HRP retrogradely labeled neurons in areas containing CRF-I perikarya. After NTS injections dual-labeled neurons were seen primarily in the medial parvocellular division of the paraventricular nucleus of hypothalamus (PVN) and to a lesser extent in RVL. Most HRP-labeled neurons in PVN were adjacent to those with CRF-I. After RVL injections dual-labeled neurons were seen in the dorsal hypothalamic area. Abundant anterograde label from NTS overlapped nuclear groups containing CRF in parabrachial and laterodorsal tegmental nuclei, central nucleus of amygdala, PVN and bed nucleus of the stria terminalis. We conclude that (a) CRF-I processes are distributed viscerotopically in NTS overlapping cardiovascular, gastrointestinal and other primary visceral afferent fields; and (b) CRF-I processes in NTS and RVL may derive from intrinsic and distal sources. CRF in NTS and RVL may contribute to cardiovascular regulation and other autonomic functions. (Supported by NIH HL 18974.)

14.8

THE DISTRIBUTION OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN THE MEDULLARY AND PONTINE TEGMENTUM OF THE CAT. V. John Massari, P.J. Hornby, E.K. Friedman*, R.A. Gillis and P.J. Gatti*, Depts. of Pharmacology; Howard Univ. Med. Sch. & Georgetown Univ. Med. Sch., Washington, D.C. 20059

We have examined the distribution of NPY perikarya and nerve terminals in the brain-stem of the cat. Four cats received IVT injections of colchicine (1000ug/200uL) 24-36 hours before sacrifice. Another cat did not receive colchicine. Sections were processed for immunohistochemistry according to a peroxidase antiperoxidase method. NPY immunoreactive perikarya were found predominantly in the medullary tegmentum. They were found in the medial nucleus of the solitary tract, the lateral tegmental field, in the area of the lateral reticular nucleus, and in the superficial layer of the spinal trigeminal nucleus. Tyrosine hydroxylase immunoreactive neurons with similar morphology and location were also observed in the first 3 areas noted above. Dense plexuses of NPY terminals were seen in the nuclei of the solitary tract, the first two layers of the spinal trigeminal nucleus, and in the Nn. raphe pallidus, obscurus, and magnus. Less dense terminal labelling was seen in the lateral tegmental field, the ventrolateral medulla and the N. locus coeruleus. These data indicate that the distribution of NPY in the medullary and pontine tegmentum of the cat is similar to, but not identical to that of the rat.
Supported by a grant from the A.H.A., Nation's Capital Affiliate.

14.9

RECIPROCAL POTENTIATION OF NEUROPEPTIDE Y AND α_2 AGONISTS IN THE NUCLEUS TRACTUS SOLITARII. C.J. Tseng*, R. Mosqueda-Garcia, D. Robertson* (SPON: R.A. Margolin). Pharmacology Department, Vanderbilt University, Nashville, TN 37232.

In several systems, α_2-adrenoreceptor agonists and neuropeptide Y (NPY) potentiate one another. We reported recently that NPY is a potent depressor agent in the nucleus tractus solitarii (NTS). The purpose of this study is to investigate the possible modulation of α_2 agonist effect by NPY in this site. Microinjection (60 nl) of NPY, anti-NPY antiserum, the α_2 agonist α-methylnorepinephrine (α-MNE), clonidine, and the α_2 antagonists yohimbine and idazoxan were made into the NTS.

Administration of idazoxan (0.2 nmol) prior to the injection of NPY (2.3 pmol) attenuated the potent depressor and bradycardic effect of NPY (from -32±3 to -10±2 mmHg for MBP and from -110±9 to -43±2 bpm for HR, respectively, n=6, p<0.001). There was a similar attenuation of yohimbine's effect. Similarly, prior administration of the anti-NPY antiserum attenuated the depressor effect of the central antihypertensive agents, α-MNE (from -23±1 to -9±1 mmHg after 20 nmol α-MNE, n=9, p<0.001), and clonidine (from -23±2 mmHg, -49±6 bpm to -9±1 mmHg, 12±4 bpm after 5 nmol clonidine, n=7, p<0.001), whereas inactivated antiserum or control normal rabbit serum were not able to attenuate these effects. Even a subdepressor dose of NPY (47 fmol) could potentiate the effect of α-MNE .

These studies demonstrate a reciprocal potentiation of NPY and α_2 agonist in the brainstem, and suggest that NPY and catecholamines interact in central cardiovascular regulation.

14.10

SYMPATHO-INHIBITORY AND EXCITATORY MECHANISMS IN THE MEDIAN FOREBRAIN BUNDLE (MFB) OF RATS. H. Ohta and M.J. Brody. Department of Pharmacology and Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

We reported previously that neuronal interruption by lidocaine or electrolytic lesion in the MFB produced pressor and tachycardic responses in conscious rats. These results indicate that tonic sympathoinhibitory neurons are either located in or pass through the MFB. To investigate whether cell bodies or fibers of passage are activated in MFB to produce hemodynamic responses, the effects of lidocaine and several excitatory amino acids were compared. When kainate (KA; 1-10 ng) and N-methyl-D,L-aspartate (NMDA; 5-200 ng) were injected into the MFB, pressor and tachycardic responses were observed that were similar to those induced by lidocaine. Glutamate (1000 ng) had no effect. The responses induced by lidocaine, KA and NMDA were all blocked by ganglionic blockade but not by adrenal demedullation. The pressor response induced by all three agents was potentiated by sino-aortic deafferentation but the tachycardia was not affected. These data demonstrate that the injection of lidocaine, KA and NMDA into the MFB increases sympathetic outflow to the cardiovascular system. Since the same actions were produced by local anesthetic and excitatory amino acids, it appears that complex sympatho-inhibitory and excitatory mechanisms exist in the MFB. (Supported by HLB-14388.)

14.11

PREGANGLIONIC STIMULATION ALTERS THE PATTERN OF IMMUNOREACTIVITY TO NEUROPEPTIDES IN THE SUPERIOR CERVICAL GANGLION (SCG) OF THE CAT. J. Ciriello, M. Bachoo*, M.M. Caverson and C. Polosa*. Depts. of Physiology, Univ. of Western Ontario, London, Canada and McGill Univ., Montreal, Canada.

The effect of supramaximal stimulation (120 min at 40Hz) of the preganglionic input (cervical sympathetic trunk) and of the postganglionic output of the SCG on the pattern of immunoreactivity (IR) to neurotension (NT), enkephalin (ENK), somatostatin (SS), substance-P (SP) and corticotropin releasing factor (CRF) was studied in the acutely decentralized SCG of the cat. In the presence of the cholinergic antogonists preganglionic stimulation evoked a contraction of the nictitating membrane (NM) that outlasted the stimulus and decayed slowly. With continuous stimulation the NM response was lost after about 1 hr. In the non-stimulated SCG fibers and terminals containing NT, ENK, SS, SP and CRF IR were observed throughout the SCG, but with regional differences in density of labelling for the different peptides. This pattern did not change after postganglionic stimulation. However, after preganglionic stimulation the IR for NT and ENK was drastically reduced. These data suggest that NT and LE are released from preganglionic axon terminals and are involved in mechanisms mediating the non-cholinergic NM response. (Supported by HSFO, QHF and MRC).

14.12

EFFECT OF PROLONGED PREGANGLIONIC STIMULATION ON RADIOIMMUNOASSAYABLE NEUROTENSIN (NT) IN THE SUPERIOR CERVICAL GANGLION (SCG) OF THE CAT. M.M. Caverson, M. Bachoo*, J. Ciriello and C. Polosa. Depts. of Physiology, McGill Univ., Montreal, Canada & Univ. Western Ontario, London, Canada.

During block of cholinergic transmission, stimulation of the cut cervical sympathetic trunk (CST) evokes a slow contraction of the nictitating membrane. Prolonged stimulation produces an irreversible loss of the slow response and a coincident loss of NT-like immunoreactivity in SCG fibers. This finding suggests that stimulation depletes preganglionic axon terminals of NT and that the depletion contributes to the loss of the response. In the present experiments, a radioimmunoassay specific for the C-terminal 5 amino acid sequence of NT was used to estimate the effect of CST stimulation on NT content of the SCG. After stimulation of the R-CST (2h, 40Hz, supramaximal intensity), the content of NT was determined in 0.5M acetic acid extracts of R- and L-SCG tissue (n=3 animals). NT content of the stimulated R-SCG (6.3±1.8 pmol/g tissue wt) was 44.3±2.6% lower than that in the unstimulated L-SCG (11.9±4.1 pmol/g tissue wt). In animals in which the CST was not stimulated, NT content was similar in the R-SCG (6.5±0.3 pmol/g tissue wt;n=3) and L-SCG (6.3±0.6 pmol/g tissue wt;n=2). These findings suggest that NT is released by stimulation of preganglionic axons in the CST and that NT acts as a transmitter at the sympathetic ganglionic synapse in the SCG.

(Supported by MRC of Canada and Quebec Heart Foundation)

SEROTONIN, HISTAMINE AND OTHER BIOGENIC AMINES I

15.1

ISOLATION OF PEPTIDE FRAGMENTS OF SBP THAT CONTAIN 5-HT BINDING SITES: LABELING WITH A NOVEL PHOTOAFFINITY PROBE. M. Adlersberg, H. Tamir*, P.Y.Yu, S.H. Hsiung and K.P. Liu. N.Y. State Psych. Inst. Div. Neurosci. N.Y., NY 10032.

Serotonin binding protein (SBP) is a soluble constituent of synaptic vesicles of central and peripheral (enteric) serotonergic neurons that form *in situ* a complex with serotonin (5-HT). Two forms of the protein that differ in molecular weight (45 kDa and 56 kDa) but share antigenic determinants, have been charecterized. The current study was undertaken in order to define the relationship of the two forms of SBP to each other. The 45 and 56 kDa SBP were purified as previously described (J. Neurochem. 44, 1289,1985; J. Neurochem. 49, 1105, 1987) and were labeled with a new photoaffinity probe, N-(4-azido-2-nitrophenyl)-5-hydroxytryptamine (NAP-5-HT) and its tritiated counterpart, ^{3}H-NAP-5-HT were synthesized by condensation of 5-HT or ^{3}H-5-HT with 3-fluoro-4-nitrophenyl azide. NAP-5-HT inhibited the binding of ^{3}H-5-HT to 45 and 56 kDa SBP (ID_{50}=1nM). ^{3}H-NAP-5-HT was found to bind strongly to both forms of SBP,and when photolysed (visible light; 4° C; 6min.) to bind irreversibly. The structure of NAP-5-HT presents the advantage of having the azido group remote from the hydroxyindole moeity which is essential for binding. ^{3}H-NAP-5-HT-SBP (45 and 56 kDa) were fractionated by SDS/PAGE and their migrating positions were determined by slicing a strip of the gel and counting. The radioactive bands were excised from the gel and overlaid with 5 µg S. aureus V_8 protease or 0.4 µg proteinase K/well in 5% stacking gel and digested for 2.5 hr. The generated peptides were fractionated by 13%-16% SDS/PAGE. The resulting peptides were located and quantitated by staining with Coomassie Blue and densitometry, followed by excising the protein bands and counting. Partial proteolysis with V_8 protease generated very similar pattern of radiolabeled peptides from 45 and 56 kDa SBP (10.5; 12; 14 kDa). Partial proteolysis with proteinase K generated only one radiolabeled peptide from the two forms of SBP that shared the same M.W (15kDa) .The radiolabeled peptides generated from the two forms of SBP were electroblotted to PVDF membranes and excised in order to sequence them by the method of Matsudaira(1987).It is expected that if the 56kDa form of SBP is the precursor of the 45 kDa form,there will be a strong sequence homology between radiolabeled peptides generated from the two proteins. The sequence study will also enable the synthesis of a DNA probe. Supported by NIMH grant 37575.

15.2

ULTRASTRUCTURE OF THE SEROTONIN INNERVATION IN THE RAT NEOSTRIATUM: RADIOAUTOGRAPHIC AND IMMUNOCYTOCHEMICAL CHARACTERIZATION. J.J. Soghomonian, L. Descarries and K.C. Watkins, Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Québec, Canada H3C 3J7.

High resolution radioautography after cerebroventricular administration of tritiated serotonin (5-HT) and PAP immunocytochemistry with an antiserum against 5-HT-glutaraldehyde-protein conjugate (kindly donated by M. Geffard) were used in parallel to investigate the intrinsic and relational features of 5-HT axon terminals (varicosities) in the neostriatum of adult rat. The uptake-labeled varicosities were examined in single thin sections from the paraventricular neostriatum and their immunostained counterparts in serial thin sections from the same area plus a dorsal neostriatal sector. These 5-HT axon terminals were generally small (0.5-0.6 µm in average diameter). Their internal features, best viewed in radioautographs, included small, pleomorphic synaptic vesicles with occasional large granular vesicles and mitochondria. Junctional 5-HT terminals from both sectors synapsed exclusively, and with equal frequency, on dendritic spines or shafts, almost always with asymmetric membrane differentiations. However, only 10-12% of the 5-HT varicosities from either sector exhibited synaptic junctions, as opposed to at least 70% of randomly selected unlabeled or immunonegative varicosities similarly sampled in the surrounding neuropil. Whether synaptic or not, the 5-HT varicosities were directly apposed to a variety of structures comprising mostly other axon terminals, dendritic spines and branches, but rarely neuronal somata. This microenvironment appeared similar in both neostriatal sectors examined. These results indicate that the 5-HT innervation of adult rat neostriatum is predominantly non junctional. They also suggest that, in neostriatum, 5-HT could exert its action upon a multiplicity of cellular targets in addition to the dendritic spines and shafts which are synaptically contacted by this type of terminal. (Supported by MRC grant MT-3544).

15.3

SELECTIVE IN VIVO MEASUREMENT OF SEROTONIN WITH NAFION COATED CARBON FIBRE ELECTRODES (CFE) COMBINED WITH DIFFERENTIAL PULSE VOLTAMMETRY. F. Crespi*, K.F. Martin* and C.A. Marsden* (SPON: S Auerbach). Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK.

CFE were made with single carbon fibres (30 µm o.d.) which were washed in chromic acid, electrically pretreated then coated 4 times with Nafion, a polymer selectively permeable to cations (Crespi. F. et al, Br. J. Pharmac., 92:565P, 1987). These nafion-CFE combined with differential pulse voltammetry (DPV) detect oxidation of dopamine (Peak A at 70 mV) and serotonin (5HT, Peak B at 250 mV) but not that of ascorbate, DOPAC, 5HIAA or uric acid (UA) 10 µM in vitro. In vivo, in the frontal cortex (FC), an oxidation peak at 250 mV with an amplitude of 0.5 nA (corresponding to 5 nM 5HT in vitro) was recorded every 5 min with the Nafion-CFE and was stable up to six hours. 5HT (1 µM) but not 5HIAA or UA (10 µM) increased Peak B when infused into the FC close to the Nafion-CFE (+130%). Infusion of KCl (0.1M) or p-chloro amphetamine (4 µg) also increased Peak B by 170 and 200% respectively without changing 5HIAA or UA measured with a conventional CFE (Crespi, F. et al, Neurosci. Lett., 43:203, 1983). Administration of the MAO inhibitor pargyline (100 mg/Kg i.p.) increased Peak B (+65%) but 5HIAA was decreased by 70%, while the 5HT$_1$ receptor agonist RU 24969 (10 mg/kg s.c.) decreased both Peak B and 5HIAA levels in FC by ~50%. Peak B was also increased by electrical stimulation of the dorsal raphe (10V, 10Hz, 10 min) but only during the stimulation period (+330%) while 5HIAA did not increase until after stimulation reaching a maximum 30 mins later (+75%).
These data indicate that Nafion CFE selectively detect 5HT in vivo.

15.4

SEROTONIN RELEASE AND METABOLISM IN RAT CORTEX IN VIVO. E.Carboni, L.Perezzani and G.Di Chiara. (SPON. G.De Montis). Inst.of Exp.Pharmacology and Toxicology, Univ.of Cagliari, 09100 Cagliari, Italy.

Transcerebral dialysis has been utilized for measuring brain levels of serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA). Dialysis tubes were implanted in the frontal cortex and perfused with Ringer. 5-HT and 5-HIAA were quantified in awake rats by reverse phase HPLC with electrochemical detection 24 hours after surgery. Out-put of 5-HT was calcium-dependent and abolished by tetrodotoxin (1 /uM) added to Ringer 5HT output was increased by the addition of chlorimipramine to the Ringer. The MAO-A inhibitor moclobemide (15 mg/kg s.c.) but not the MAB-B inhibitor RO-19.6327 (5 mg/kg s.c.) stimulated 5-HT output, while lisuride (30 /ug/kg s.c.) and metergoline (2 mg/kg s.c.), respectively reduced and stimulated 5-HT release. It appears that brain dialysis is a suitable method for the study of "in vivo" 5-HT release.

15.5

TRYPTOPHAN AND FOOD INCREASE EXTRACELLULAR SEROTONIN IN THE LATERAL HYPOTHALAMUS AS MEASURED BY MICRODIALYSIS IN RAT. D. H. Schwartz, S. McClane*, L. Hernandez and B. G. Hoebel. Dept. Psychol. Princeton Univ., Princeton, NJ 08544

Administration of l-tryptophan and the ingestion of certain foods are both known to increase brain serotonin synthesis. In order to examine whether this increase in serotonin synthesis leads to an elevation of extracellular serotonin, we used *in vivo* microdialysis. Male, albino Sprague-Dawley rats were stereotaxically implanted with 21 g guide cannulas aimed at the perifornical lateral hypothalamus (PFH). A small, removable microdialysis probe (0.2 mm x 3 mm tip) inserted directly into the guide cannula allowed collection of successive 30 min samples in freely moving animals. Samples were analyzed for serotonin and 5-HIAA using high pressure liquid chromatography with electrochemical detection. After the collection of baseline samples, animals were given one of three experimental manipulations: l-tryptophan (100 mg/kg i.p.), sight and smell of inaccessible diet (mash of Purina Chow plus sweetened condensed milk) or the same diet to eat. The tryptophan injected animals were further divided into two groups: free-feeding and 48-hr food deprived. Tryptophan injection caused a slight increase in extracellular serotonin (186% of saline controls) in *ad lib* fed rats (n=4). Food deprivation enhanced this effect (297% of saline control; n=4). Tryptophan had a similar effect on serotonin's metabolite, 5-HIAA. The sight and smell of an inaccessible, highly palatable diet did not affect extracellular serotonin (n=8). Ingestion of this same diet during a 30 min period caused an increase in extracellular serotonin during the same period. Unlike tryptophan loading, ingestion of the diet had no effect on 5-HIAA. These data suggest that serotonin in the lateral hypothalamus could be involved in the control of feeding behavior.

15.6

RELATIONSHIP BETWEEN DIALYSATE SEROTONIN AND RAPHE UNIT ACTIVITY. L.O. Wilkinson, S.B. Auerbach, and B.L. Jacobs. Program in Neuroscience, Princeton University, Princeton, NJ 08544

Extracellular serotonin (5-HT) was measured in awake, freely moving cats during increases and decreases in dorsal raphe 5-HT unit activity. 5-HT was separated by HPLC and dual amperometric detection was used to verify the authenticity of the 5-HT peak. Activation of dorsal raphe serotonin neurons, (induced either by low intensity electrical stimulation or observed during behavioral arousal) lead to increased dialysate 5-HT (32% and 14% above baseline, respectively) in striatum. Reciprocally, administration of the 5-HT autoreceptor agonist 8-OH-DPAT, which decreased raphe unit activity, produced a dose-dependent decrease in dialysate 5-HT. Though administration of 8-OH-DPAT completely suppressed the activity of 5-HT neurons within 5 mins, the maximal decrease in dialysate 5-HT (40% following 50 ug/kg s.c., and 58% following 250 ug/kg, s.c.) was observed 1 and 3 hours following drug administration. Thus, changes in dialysate 5-HT are less dynamic than those observed in unit activity. These data also suggest that ~60% of measured extracellular 5-HT is released as a consequence of neuronal activity. Finally, we examined dialysate 5-HT from the anterior hypothalamus following fever induced by the pyrogen muramyl dipeptide (50 ug/kg i.p.). Though 5-HT unit activity is unchanged during this manipulation, we observed a decrease in dialysate 5-HT preceeding fever peak. This suggests that 5-HT release may be uncoupled from unit activity in some situations.

15.7

NOREPINEPHRINE-STIMULATED RELEASE OF PINEAL SEROTONIN MAY BE MEDIATED BY ALPHA ADRENERGIC RECEPTORS. B. Benson and W.D. Reynolds*. Dept. of Anatomy, Univ. of Arizona, Tucson, AZ 85724.

The role of alpha and beta receptors was explored in the norepinephrine (NE)-stimulated release of pineal serotonin (5-HT) in vitro. Adult male Harlan SD rats were housed in 12L:12D and sacrificed at mid-light. Pineals were incubated under 95%O_2/5%CO_2 for 4 h at 37° C in vials containing 10^{-4} M ascorbic acid in 100 µl of medium TC-199, in the presence or absence of adrenergic agonists and/or antagonists. Pineal and medium levels of 5-HT were quantitated by HPLC/ECD. NE (10^{-4} M) significantly increased medium levels of 5-HT; this response was not affected by the addition of the beta antagonist propranolol (10^{-4} M). Also, the beta adrenergic agonist isoproterenol (10^{-4} M) was ineffective in the release of 5-HT. On the other hand, the alpha adrenegic receptor agonist phenylephrine (10^{-4} M) markedly stimulated the release of pineal 5-HT into the incubation medium; this stimulatory effect was significantly attenuated by the addition of 10^{-6} M prazosin. The results suggest that NE-stimulated release of 5-HT is mediated by alpha adrenergic receptors. This project was supported by N.I.H. grant HD 19521.

15.8

TRICYCLICS INHIBIT BRAIN MAST CELL SECRETION AND NEURONAL DEPOLARIZATION. M. Lambracht-Hall*, A.D. Konstantinidou*, M. Holdridge* and T.C. Theoharides. (SPON: J.E. Marchand). Dept. of Pharmacology, Tufts University School of Medicine, Boston, MA 02111.

Tricyclics are now widely used for the treatment of major depression because they inhibit the reuptake of serotonin and norepinephrine. We looked at the effects of tricyclics on brain mast cells and neurons in the thalamic-hypothalamic region because they can store and release serotonin (5-HT) and histamine, which have been implicated in mood disorders. We tested imipramine and the tricyclic antihistamine hydroxyzine in a custom-made perfusion system, using fresh rat thalamic slices labelled with ^{3}H-5-HT. The perfusate was assayed for 5-HT, histamine, b-hexosaminidase (a secretory granule enzyme) and LDH. There was no LDH release in any of the concentrations tested. The drugs alone at 0.5x10-^{4}M and 10-^{4}M caused release from mast cells but not neurons. However, over the range of 10^{-5}-10^{-4}M they inhibited subsequent mast cell release induced by the classic mast cell secretagogue, compound 48/80, and neuronal depolarization evoked by high K^+. Tricyclic drugs may, therefore, owe their antidepressant, analgesic, and other psychotropic effects at least in part to their ability to regulate release of certain mediators from brain mast cells and neurons.

15.9

MONOAMINE MODULATION OF HIPPOCAMPAL REACTIVITY TO PERFORANT PATH STIMULATION. G. Richter-Levin*, S.J. Sara and M. Segal*. Center for Neuroscience, The Weizmann Institute of Science, Rehovot 76100 Israel.

Electrical stimulation of the perforant path (PP) in anesthetized rat brain produces a typical EPSP and a superimposed population spike (PS) recorded in the dentate granular layer. Previous studies indicate that norepinephrine (NE) of locus coeruleus (LC) origin and 5-HT of the midbrain raphe can modulate hippocampal reactivity to afferent stimulation. In the present experiments we examined effects of drugs known to interact with the monoamines on hippocampal reactivity to PP stimulation. Fenfluramine (FFA), a 5-HT releaser, enhances PP-evoked PS by 40% without affecting the slope of the population EPSP, indicating that its effect is exerted postsynaptically. FFA is not effective in 5-HT depleted rats and its effects are reestablished when 5-HT levels are restored. FFA does not affect feedback inhibition but may reduce feed-forward inhibition. Idazaxon, an alpha-2 antagonist also enhances PP evoked PS in the dentate gyrus. This is probably mediated by increasing spontaneous activity of LC cells and releasing NE from its hippocampal terminals. The differences between noradrenergic and serotonergic drugs in the sites of action are now being examined.

15.10

SEROTONIN ATTENUATES SLOW IPSP'S IN HIPPOCAMPAL NEURONS. M. Segal* (Spon: M.E. Cohen) Center for Neurosciences, The Weizmann Institute, Rehovot 76100 Israel.

Topical application of 5-HT onto hippocampal slices produces several effects recorded intracellularly in Ca1 pyramidal neurons. These include activation of an outward K current, a blockade of a slow Ca dependent K current underlying the slow afterhyperpolarization, and an occasional late slow depolarization. Afferent stimulation evokes in hippocampal neurons a fast EPSP followed by a fast Cl^- mediated IPSP (fIPSP) followed by a slow K^+ mediated IPSP (sIPSP). The sIPSP and to a smaller extent the fIPSP are reduced by 5-HT while the EPSP is enhanced. The enhanced EPSP is not simply due to a reduced inhibition as its time course is faster and input relations different from those of the IPSP's. These effects are probably mediated by a 5-HT1a receptor as they are sensitive to spiperone. The reduction in sIPSP is seen with a low 5-HT concentration that has no other effects. The reduction in sIPSP is not accompanied by a change in postsynaptic sensitivity to GABA or baclofen. These experiments indicate that 5-HT has a potent presynaptic action to reduce inhibition in hippocampal neurons.

15.11

QUANTITATIVE INHIBITION OF HUMAN AXON REFLEXES BY EPICUTANEOUS APPLICATION OF CAPSAICIN AND LOCAL ANALGETICS. P.Bjerring* and L.Arendt-Nielsen. Dept. of Dermatology, Marselisborg Hospital, Aarhus and Dept of Medical Informatics, Aalborg University, Aalborg, Denmark.

The flare reaction after intracutaneous injection of histamine could be inhibited by epicutaneous application of 1) capsaicin 1% in EtOH once daily and 2) lignocaine 2.5% + prilocaine 2.5% in an emulsion cream (EMLA, ASTRA, Sweden). The cream was applied for 30, 60, 90 and 120 minutes. During the capsaicin treatment the flare area gradually decreased from 12.9 to 1.3 cm^2 after 6 days, when the treatment was stopped. After termination of the treatment the flare area reached the normal range 7 days later.
Analgesia by EMLA cream induced an application time-dependent decrease of the flare area from 9.9 to 2,5 cm^2. As a control dermal infiltration of lignocaine reduced the flare to 0.8 cm^2.
This study indicates, that the axon reflex can be reduced both by capsaicin treatment and by local analgetics. This demeonstrate that the axon reflex may be entirely intracutaneously.

15.12

PHOTOPERIOD-MODULATED MELATONIN BIOSYNTHESIS IN ASEXUAL PLANARIA. J.B. Best, M. Morita* and L. Pei*. Depts. Environmental Health and Anatomy, Colorado State Univ., Ft. Collins, CO 80523.

Previous studies indicate photoperiod modulation of asexual reproduction in planaria analogous to similar modulation of sexual reproduction in higher vertebrates, with evidence that melatonin may mediate such effects. Present studies, of enzymes involved in photoperiod-dependent melatonin biosynthesis in planaria, seek to elucidate these enzymes in asexual Dugesia dorotocephala and ascertain whether they are isoenzymes of the N-acetyltransferase (NAT) and hydroxyindole-O-methyl transferase known to be involved in melatonin biosynthesis in vertebrate pineals. NAT assays utilized tryptamine·HCl and [^{3}H] acetyl-CoA as substrates, and extraction of the [^{3}H] N-acetyl-tryptamine product with toluene:isoamyl alcohol (97:3). HIOMT assays utilized [^{3}H] S-adenosyl-methionine and N-acetylserotonin as experimental substrates, and extraction of the [^{3}H] melatonin product with chloroform. Results of these experiments will be reported.

CATECHOLAMINES I

16.1

IN VIVO TYROSINE HYDROXYLATION IN RAT RETINA: EFFECTS OF PHENYLALANINE INJECTION IN PARACHLOROPHENYLALANINE(PCPA)-PRETREATED RATS. J.D. Fernstrom and M.H. Fernstrom. Departments of Psychiatry and Behavioral Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh PA 15213.

Phenylalanine (PHE), normally hydroxylated to tyrosine (TYR) in liver, may also be converted to TYR in neurons by TYR hydroxylase (TH) and thus be a substrate for TH. However, PHE is also reputed to be an inhibitor of neuronal TH. It is presently not clear which role(s) of PHE occur(s) in vivo. The problem experimentally is that rat liver rapidly converts PHE to TYR after PHE administration. The central nervous system (CNS) levels of both TYR and PHE thus rise, making it difficult to distinguish effects on TH due to increments in PHE from those due to increments in TYR. To circumvent this problem, we have identified a model system in rats in which only serum and CNS PHE levels rise following PHE injection, and applied it to the study of PHE effects on TYR hydroxylation rate in retina. Rats receive PCPA (300 mg/kg ip). Four days later, they receive an injection of PHE or vehicle, followed 1 hr later by NSD-1015 (100 mg/kg ip), and are then killed 30 min later. Four days after PCPA, TYR hydroxylation rate is normal in light-activated retinas, while tryptophan hydroxylation in brain is totally suppressed. In these rats, PHE injection (50-500 mg/kg ip) produces up to 10-fold increments in PHE levels, but neither stimulates nor inhibits TYR hydroxylation rate in light-activated retina. These and other results suggest that PHE is not acutely active in vivo as an inhibitor of TH in retina, even at abnormally high levels.

16.2

PHOSPHORYLATION OF THE N-TERMINAL REGION OF TYROSINE HYDROXYLASE BY A NOVEL PROTEIN KINASE. P.R. Vulliet, F.L. Hall*, J.P. Mitchell, and D.G. Hardie*. Dept. of Vet. Pharm/Tox., Univ. of Calif., Davis, CA 95616 and Dept. of Biochem., Univ. of Dundee, Dundee, Scotland DD1 4HN.

Previous studies have shown that tyrosine hydroxylase isolated from rat pheochromocytoma is phosphorylated in vitro by at least four different protein kinases. Screening for the phosphorylation of TH by a variety of well-characterized protein kinases has demonstrated phosphorylation of the enzyme predominantly at Ser-19 and Ser-40 in the N-terminal region of the native protein. Recently, we identified a novel protein kinase that phosphorylates TH at Ser-8. The site specificity of this kinase enabled the development of an assay for its activity. Originally detected as a contaminant in preparations of highly purified TH, the kinase was found to copurify with TH through ammonium sulfate precipitation and DEAE cellulose chromatography and was only partially resolved from TH by heparin-agarose chromatography. Separation of the kinase from TH was achieved by either phosphocellulose chromatography or sucrose density gradient centrifugation. The kinase eluted during gel filtration as a protein with an apparent molecular weight of 45 kDa. Substrate specificity, activity toward various synthetic peptides, and kinetic properties were investigated. Current investigations are aimed at examining the functional role of this kinase in vivo.

16.3

ISOLATION OF BOVINE cDNA CLONES WITH HOMOLOGY TO HUMAN DOPAMINE BETA-HYDROXYLASE. E.J. Lewis and V. Claflin*. Depts. Biochem. & Med. Genetics, Oregon Health Sciences Univ., Portland, OR 97201.

Dopamine beta-hydroxylase (DBH), the enzyme which catalyzes the conversion of dopamine to norepinephrine, is expressed specifically in noradrenergic and adrenergic tissues. We are interested in understanding the parameters which regulate the expression of the DBH gene.

We have constructed a bovine adrenal medulla cDNA library in lambda gt11 and have screened this library with a 34 base oligonucleotide probe designed to detect DBH sequences. The oligonucleotide contains a mixture of DNA sequences with complementary coding potential for amino acids 471-483 of human DBH (Lamouroux et al, EMBO J. 6: 3931;1987). The ^{32}P-oligonucleotide probe was used to screen 60,000 plaques from the cDNA library. Three positive phage were isolated with cDNA inserts of 0.7, 1.2 and 2.4 kb. Northern blot analysis demonstrated a major band of 2.3 kb when either oligonucleotide or nick-translated cDNA probes were hybridized to bovine adrenal medulla poly A(+) RNA. DNA sequence analysés predict amino acid identity between our cDNA and human DBH in 16/18 residues spanning 432-449 of human DBH. It is likely that these cDNAs code for bovine DBH. (Supported by grants from the NIH and Oregon MRF).

16.4

CHARACTERIZATION AND SEQUENCE ANALYSIS OF BOVINE DOPAMINE B-HYDROXYLASE cDNA. O. Hwang, J. Smith* and T.H. Joh. Cornell Univ. Med. Coll., Burke Rehab. Center, White Plains, NY 10605 and Mass. General Hosp., Boston, MA 02114.

Toward an analysis of the primary structure of dopamine -hydroxylase (DBH), we have independently isolated a 2.3kb cDNA from a bovine adrenal medullary lambda gt11 library using antisera against deglycosylated DBH. To verify the cDNA, we have determined three amino acid sequences of soluble DBH. One of these internal sequences is included in the amino acid sequence deduced from the nucleotide sequence of this cDNA, identifying it as representing DBH. The complete sequence of the coding region representing 351 C-terminal amino acids (41kd) was determined. Comparison with the human DBH sequence (Lamouroux et al, EMBO J. 6:3931, 1987) revealed the following: 1) Both potential glycosylation sites are conserved; 2) 87% homology is found at both nucleotide and amino acid levels in the coding region; 3) The 3′ untranslated regions exhibit low homology (48%); 4) 11 out of 12 cysteine residues are conserved.

Bovine adrenal medulla DBH mRNA is low in abundance (0.018%) and approximately 2.9kb in size. Southern blot analysis revealed that DBH is represented by a single copy gene. (MH24285)

16.5

PRIMARY SEQUENCES FOR BOVINE ADRENAL DOPAMINE β-HYDROXYLASE. D. L. Wong, C. L. Bildstein*, K. Lau* and J.S. Schilling*. Dept. of Psychiatry and Behavioral Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The three subunits of soluble bovine adrenal dopamine β-hydroxylase (DBH), α (71 kD), β (75 kD) and γ (78 kD), were separated on SDS-polyacrylamide gels, transferred to PVDF paper and localized by Coomassie Blue R250 staining [Matsudaira, 1981]. Bands were excised and amino terminal sequences generated by gas phase/gas-liquid phase sequencing. To generate internal sequences, the purified protein was cleaved with cyanogen bromide since only 16 methionine residues were found by amino acid content analysis. Nine peptide fragments were identified on silver stained denaturing gels. Each was immunoreactive with a polyclonal antiserum specific for the DBH protein backbone. Based on relative molecular weights and comparison to the deduced human DBH primary structure (Lamouroux et al., 1988), five of the fragments represented partial digests. The peptides were separated as described above, and the most intensely staining bands sequenced. Amino terminal sequences and partial internal peptide sequences (19-25 residues) for the bovine enzyme showed considerable homology (61-95 percent) to the human enzyme, and represented fragments distributed throughout the polypeptide chain. Synthetic oligonucleotides developed against these amino terminal and carboxy terminal fragments have been used to screen a λgt11 rat adrenal medullary library to bias for the selection of full length rat DBH cDNA clones.

16.6

PRIMARY SEQUENCES FOR BOVINE ADRENAL PHENYLETHANOLAMINE N-METHYLTRANSFERASE. Y.S. Yoo*, K. Lau*, J.S. Schilling* and D.L. Wong (SPON: K.F. Faull) Dept. of Psych. and Behav. Sci., Stanford Univ. Sch. Med., Stanford, CA 94305.

We have previously shown that native bovine adrenal medullary phenylethanolamine N-methyltransferase (PNMT) consists of four charged isozymes. Characterization of these isozymes and preliminary sequencing data suggested that they were very similar, and differences might be primarily attributed to post-translational modifications. This would be consistent with identification of a single cDNA and mRNA for the enzyme by recombinant methodologies (Baetge et al., 1986). Microsequencing of the enzyme should provide further evidence for this hypothesis.

Amino terminal sequencing of purified PNMT showed that the 5' amino acid was blocked. Therefore, tryptic proteolysis and cyanogen bromide cleavage were used to generate peptides. Thirteen tryptic fragments and six cyanogen bromide fragments were obtained. These were separated on 20 percent SDS-polyacrylamide gels or on 20-27 percent gradient SDS-polyacrylamide gels and electroblotted onto polyvinylidene difluoride (PVDF) membranes. Following staining with Coomassie Blue, the bands were excised and were sequenced by automated Edman degradation using a gas/gas-liquid phase microsequencer equipped with on-line detection. Preliminary results show sequence homologies for the peptides ranging from 65-100 percent compared to the deduced sequences. In several cases, non-conservative changes represent single base alterations.

16.7

THE EFFECT OF REMOVAL OF CALCIUM (Ca^{+2}) BY EGTA ON MONOAMINE OXIDASE (MAO) ACTIVITY IN THE RAT BRAIN HOMOGENATE. N.T. Buu and C.Lussier*. Clinical Research Institute of Montreal, Montreal, Que.,Canada.

Mobilisation of intracellular stores of Ca^{+2} and cytosolic Ca^{+2} are necessary for the activation, through phosphorylation, of tyrosine hydroxylase (TH), the rate-limiting step in the synthesis of catecholamines (CA). Because mitochondria is a source of intracellular Ca^{+2} and mitochondrial MAO is the most important enzyme in the metabolism of brain CA, it is of interest to know whether Ca^{+2} availability can influence MAO activity.

MAO-A and MAO-B activities in rat brain homogenates were determined in the presence of 1 mM calcium chloride ($CaCl_2$), 10μM of Ca^{+2}/calmodulin inhibitor N-6-aminohexyl-5-chloro-1-napthalenesulfonamide (W7) or EGTA (2-10 mM).

The results indicated that neither $CaCl_2$ nor W7 affected MAO-A or MAO-B activities. On the other hand EGTA inhibited (from 20 to 60%) both forms of MAO in rat brain homogenates.

Thus the exit of Ca^{+2} from mitochondria which may be required for activating TH to increase CA synthesis may at the same time inhibit mitochondrial MAO suggesting a possible coordination between CA biosynthetic and metabolic pathways. (Supported by MRC of Canada and Canadian Heart Foundation).

16.8

ARE PNMT-CONTAINING CATECHOLAMINERGIC NEURONS ADRENERGIC? Alan F. Sved, Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh PA 15260

The intermediolateral cell column (IML) in the spinal cord is densely innervated by the PNMT-containing neurons of the ventrolateral medulla (C1 cell group). Since PNMT converts norepinephrine (NE) to epinephrine (EPI) and the PNMT-containing neurons in the ventrolateral medulla also contain the other enzymes required for EPI biosynthesis, it has been accepted that EPI is the major catecholamine neurotransmitter produced by these neurons. To examine this hypothesis, a sensitive HPLC-EC assay for EPI has been combined with a microdissection technique to determine the concentration of EPI in the IML. In dissections of rat thoracic spinal cord enriched in IML, as well as total thoracic cord, EPI levels were below the limit of sensitivity of assay, indicating levels less than 0.2 pg/mg tissue. In the region of the IML, EPI levels were less than 0.05% of the NE content. These results suggest that nerve terminals that contain all the enzymes required for EPI synthesis do not necessarily utilize EPI as a neurotransmitter.

(Supported by National Science Foundation grant DCB8711827 and an Established Investigator Award from the American Heart Association.)

16.9

FURTHER OBSERVATIONS ON THE LONG-TERM EFFECTS OF REPEATED COCAINE EXPOSURE ON RAT BRAIN DOPAMINE TURNOVER AND METABOLISM. F. Karoum, R.L. Suddath* and R.J. Wyatt. Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

We have previously reported a preferential long-term reduction in dopamine (DA) turnover in the frontal cortex (FX) and hypothalamus (HY) of rats exposed to 7 days (10 mg 1 kg, twice daily by intraperitoneal injection) cocaine administration (17^{th} annual meeting of neuroscience, 1987, 407.9). DA turnover was assessed by following the changes in central DA and 3,4 - dihydroxyphenylacetic acid (DOPAC) contents. In this communication we report on a replication of our previous work and expand our observations to the effect of a 7 day cocaine exposure on FX and HY DA, DOPAC and homovanillic acid (HVA) concentrations 6 weeks and 3 months after termination of cocaine administration. Ten rats were used at each test point and all analyses were carried out by combined gas chromatography mass spectrometry. Consistent with our previous report, DOPAC concentration in both the HY and the FX remained significantly reduced 6 weeks after termination of cocaine treatment. In the FX, but not the HY, DA content was also reduced. The combined molar concentrations of DOPAC and HVA (HVA + DOPAC) in both the FX and HY were significantly lower than those in the appropriate controls. These results signify a reduction in the total amount of DA released in the FX and HY and suggest a reduction in DA turnover. Interestingly, although DOPAC and HVA concentrations were about normal in the HY one week after treatment, 6 weeks later both DOPAC and HVA were reduced. Three months after termination of cocaine treatment, hypothalamic concentration of DA, DOPAC and HVA returned to baseline levels. In the FX on the other hand, DA and DOPAC concentrations were low but did not reach statistical significance. Rather unexpectedly, HVA concentration in the FX was now significantly low. Combined FX DOPAC and HVA molar concentrations 3 months after cocaine exposure were significantly lower than those of the controls. Apparently, DA turnover continued to be low even 3 months after a 7 day cocaine exposure. The results of the present study support and expand our previous report of a long-term effect of cocaine exposure on HY and FX DA turnover. The previously reported 6 week reduction in FX DA turnover and metabolism following a relatively short period of cocaine exposure is now extended to up to 3 months. The significance of the delayed emergance of a reduction in HVA concentration as compared with that of DOPAC will be discussed.

16.10

ALTERATIONS IN DOPAMINE SYNTHESIS INDUCED BY CHRONIC NEUROLEPTIC ADMINISTRATION: A POSSIBLE BIOCHEMICAL CORRELATE OF DEPOLARIZATION INACTIVATION. A. Y. Deutch and R. H. Roth. Depts. of Pharmacology & Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Electrophysiological studies have revealed that chronic neuroleptic administration results in certain midbrain dopamine (DA) neurons entering a state of depolarization inactivation; biochemical correlates of this condition are lacking. We have examined DA synthesis following chronic haloperidol (HPD) administration. Animals received 20 days of either haloperidol (HPD) or vehicle; on day 21, half of the animals administered chronic HPD received HPD and the other half vehicle injection. Acute HPD (chronic vehicle-HPD challenge) resulted in a marked increase in DA synthesis (as reflected by DOPA accumulation) in the striatum (CP), a somewhat smaller increase in the nuc. accumbens (NAS), and a still smaller increase in the prefrontal cortex (PFC). Corresponding increases in synthesis were seen in the A9 and A10 (but not A8) DA cell body regions. Chronic HPD followed by HPD challenge resulted in a blunted response in the NAS and CP as well as the cell body regions. In animals receiving chronic HPD followed by vehicle on day 21, DA synthesis rates in the CP and NAS (but not cell body regions) were decreased below normal control values. Such decreases in DA synthesis may indicate that the DA neurons innervating these areas are in a state of depolarization inactivation.

16.11

CHRONIC THYROTROPIN-RELEASING HORMONE (TRH) UPREGULATES MAZINDOL (Maz) BINDING AND INCREASES DOPAMINE (DA) METABOLISM IN THE RAT STRIATUM. H. Ikegami*, S.A. Spahn and C. Prasad. Sect. of Endocrinology, Dept. of Medicine, LSU Med. Ctr., New Orleans, LA 70112.

Administration of exogenous TRH, a peptide endogenous to basal ganglia, exhibits a variety of DA-related biologic activities, such as stimulation of DA release. To understand global role of TRH on DA neurotransmission, we have examined effects of chronic TRH on two elements of nigro-striatal DA neuron--DA receptors and DA reuptake sites. Male rats were treated for 10 days with TRH using s/c Alzet-osmotic pumps (0.51±0.02 mg TRH/kg/day). On 11th day rats were killed, striata dissected from brain, and stored frozen. The data presented below show a significant ($p < 0.025$) increase in not only striatal dihydroxyphenylacetic acid (DOPAC) levels (pmol/mg tissue), but also both K_D and Bmax (pmol/mg prot.) of Maz-binding after TRH treatment. In contrast, levels of DA and HVA, or properties of D-1 and D-2 receptors were not affected. While DOPAC elevations may be due to sustained stimulation of DA release by TRH, the mechanism(s) underlying modulation of DA uptake sites remains to be explored.

Treatment	DOPAC	Maz-K_D,nM	Maz-Bmax
Vehicle	20±2(21)	30±6(8)	4.8±0.7(8)
TRH	32±2(7)	51±6(11)	11.3±1.9(11)

16.12

NICOTINE-INDUCED RELEASE OF ENDOGENOUS AND NEWLY-SYNTHESIZED DOPAMINE IN FREELY-MOVING UNANESTHETIZED RATS. T.C. Westfall and L.E. Vickery*. Dept. of Pharmacology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

We have previously observed that nicotine (NIC) causes a dose-dependent release of dopamine (DA) from superfused rat striatal slices. In the present study we examined if such a response could be seen in freely moving unanesthetized rats in vivo. Animals were anesthetized with ketamine/acepromazine and a 21 gauge guide cannula stereotaxically placed in the striatum (A +1.0, L +3.0, V -6.0 with respect to bregma). Five to seven days later, the rats were lightly anesthetized with metofane and a 28 gauge push-pull cannula assembly introduced into the guide cannula to perfuse the striatum with artificial CSF (18 µl/min). Rats were allowed to recover from the anesthesia and CSF effluent collected in 10 min fractions for analysis of DA by HPLC-EC detection. In other experiments newly synthesized DA was measured following perfusion with ^{3}H-tyrosine (40-50 µCi/ml). Drug treatments were carried out 120 min after the start of perfusion to allow the release of ^{3}H-DA to reach a steady-state. Samples were passed over amberlite and alumina columns and analyzed by liquid scintillation spectrometry. Release of endogenous DA (pg/min) was: basal, 9 ± 3; NIC (1 mM) 22.6 ± 6; NIC (1 mg/kg s.c.) 54 ± 4; K^+ (56 mM) 121 ± 23. NIC (1 µM) increased the release of ^{3}H-DA by 117% over control. It is concluded that NIC releases DA from the striatum of freely-moving unanesthetized rats. (Supported by DA02668.)

16.13

INTRACEREBRAL MICRODIALYSIS MONITORING OF STRIATAL DOPAMINE RELEASE AND METABOLISM IN MPTP-INDUCED HEMI- PARKINSONIAN MONKEYS. S.L.Skirboll*, J.Hsaio*, I.Mefford*, R.Miletich, J.Alger*, A.Brunetti*, I.Kopin and K.Bankiewicz (SPON:J.Q.Trojanowski). NINCDS, NIH, Bethesda, MD 20892

In monkeys, unilateral intracarotid infusion of MPTP produces a useful model of hemiparkinsonism. In order to evaluate MPTP-induced neurochemical changes in-vivo, we have employed brain microdialysis to measure extracellular levels of dopamine(DA) and its metabolites (HVA) and (DOPAC) in the putamen. In-vitro recovery rates of these substances averaged 30% at a flow rate of 1 ul/min. 3 mos. after MPTP infusion, the probes were implanted bilaterally into the putamen on a horizontal plane at coordinates calculated from magnetic resonance imaging. Perfusion at 1 ul/min using 0.9% NaCl + 0.1 mM Ascorbate was carried out while the monkey was in a restraining chair under mild ketamine anesthesia. Samples were assayed for DA,HVA,DOPAC, and the 5-HIAA, via HPLC with ECD. Stable baseline values were reached 90 min. after implant of probes. The most striking effect of the MPTP lesion was seen in the 90% reduction in perfusate levels of HVA as compared to the unlesioned side (5.7 vs. 57.5 pimoles/20 ul inj.). A similar reduction was seen with DOPAC though the perfusate levels of it were 230 fold less than that of HVA. Interestingly, DA levels were very low (0.03-0.04 pimoles/inj) equally on both sides. Perfusate levels of 5-HIAA were reduced 32% on the lesioned side. Post-mortem tyrosine-hydroxylase(TH)immunocytochemistry revealed a complete unilateral denervation in the Putamen and total loss of TH(+) cells in the substantia nigra pars compacta. This initial data demonstrates that brain microdialysis can be valuable in describing in-vivo neurochaemical changes in the unilateral Parkinsonian primate model.

16.14

MECHANISM OF MPTP-INDUCED DEPLETION OF NORADRENALINE FROM NERVES IN THE MOUSE ATRIUM. Arthur Hess, Dept. of Anatomy, Robert Wood Johnson Med. Sch., UMDNJ, Piscataway, NJ 08854.

As seen by glyoxylic acid histofluorescence, noradrenergic (NA) nerve fibers extensively innervate the mouse atrium. Two hours after MPTP injection, the fibers are depleted of NA. The atrial nerve fibers, as seen by tyrosine hydroxylase immunohistochemistry, are intact. Inhibition of monoamine oxidase (MAO)-A and B affords no protection from the depletion of NA by MPTP. Desipramine, an NA uptake blocker, provides considerable protection against the depletion of NA by MPTP. Reserpine and amphetamine deplete NA from atrial nerves. Experiments with reserpine--MAO inhibitors--L-dopa sequential administration result in no refluorescence of fibers and indicate that extravesicular storage sites are inadequate for sequestering catecholamines. The refluorescence of many nerve fibers (after MPTP or amphetamine--L-dopa) or almost all fibers (after MPTP or amphetamine--MAO inhibitors--L-dopa) indicate that MPTP or amphetamine causes discharge of NA from the vesicle into extracellular space, with the vesicles themselves left behind in the axon. MPTP depletes NA from atrial nerves by causing release of NA. MPTP (or a metabolite) is taken up selectively by NA axons through NA uptake mechanisms. MPTP then causes discharge of NA from the storage vesicles. Exogenous L-dopa can be sequestered in the vesicles left behind after MPTP-induced depletion of NA. Supported by NIH grant #NS21469.

TRANSMITTERS IN INVERTEBRATES I

17.1

PLASTICITY OF TRANSMITTER EXPRESSION: INDIVIDUALLY IDENTIFIED PEPTIDERGIC NEURONS ALTER THEIR TRANSMITTER PHENOTYPE DURING POST-EMBRYONIC DEVELOPMENT IN THE MOTH. N.J. Tublitz and A.W. Sylwester, Inst. of Neurosci., U. Oregon, Eugene, OR 97403.

It is well known that during embryonic development neurons have the capacity to express a wide variety of transmitters. It is also generally assumed that following differentiation, a mature neuron will synthesize and secrete the same transmitter(s) throughout its lifetime. We present evidence here suggesting that this latter notion might not always be valid and that transmitter phenotype can indeed be altered after completion of embryonic development. The moth, Manduca sexta, contains two cardioregulatory neuropeptides, the cardioacceleratory peptides (CAPs), that had been previously localized in adults to four pairs of midline neurosecretory cells in each ventral ganglion. In contrast larva contain only one pair of medial CAP cells. Results from immunocytochemical studies in larva using the anti-CAP monoclonal antibody revealed, in addition to the 2 midline CAP cells, 8 other neurons that were CAP immunoreactive in larva but not in adults. These cells, whose somata were bilaterally clustered as a group of 4 along the lateral ganglionic margins, have been previously described as synthesizing another neuropeptide, Bursicon (Taghert and Truman, J. exp. Biol., 1982). To understand the physiological significance of the immunocytochemistry results, cell clusters or single cells were dissected from Vth instar larva or day 8 pupa, the stage when the full complement of adult neurons are present, and bioassayed for either CAP or bursicon bioactivities. In larvae, lateral cells contained a small but measureable amount of bursicon combined with high CAP levels. By day 8 of adult development, these proportions were reversed: very high bursicon levels and no detectable CAP activity. By comparison, midline cells contained no bursicon and elevated CAP levels in both larva and developing adults. HPLC analysis of larvae revealed that the cardioactivity present in the midline and lateral cells were due to a single peak that co-eluted with one of the CAPs. By showing that the lateral neurons synthesize primarily one CAP in larva and switch in adults to produce bursicon, these results strongly support the hypothesis that certain neurons can alter their transmitter phenotype during post-embryonic life.

This research is supported by NIH Grant #24613, a NIH Research Career Development Award and a Sloan Fellowship to NJT.

17.2

REGULATION OF TRANSMITTER CHOICE IN AN IDENTIFIED LINEAGE IN *MANDUCA SEXTA*. J.L. Witten & J.W. Truman, Dept. Zoology, U. Washington, Seattle, WA 98195.

We are investigating the interaction of cell lineage and steroid hormones on the regulation of transmitter expression in an identified lineage of neurons ("L") born during postembryonic development of the moth, *Manduca sexta*. The L lineage is heterogeneous in terms of transmitter phenotype, containing neurons immunoreactive to GABA and a peptide similar to molluscan small cardioactive peptide$_B$ (SCP$_B$). We are studying the neurochemical differentiation of these clonally related cells and the influence that lineage position, lineage size and ecdysteroid hormones have on determining transmitter phenotype.

During larval life the L neuroblast generates a discrete cluster of progeny. The differentiation of these neurons is arrested at an early stage of development and their maturation resumes when the appearance of ecdysteroids initiates the onset of metamorphosis. We have followed the neurochemical differentiation of L lineage progeny throughout the postembryonic life stages. GABA and the SCP$_B$-like peptide are not expressed in the partially differentiated cells but first appear halfway through adult development in response to a large rise in ecdysteroid titers. By manipulating ecdysteroid levels during this stage, we are examining steroid regulation of transmitter expression.

To determine the role of cell lineage, we have used immunohistochemical and birth dating methods to map the distribution of these putative transmitters within the L lineage. Preliminary results show that transmitter phenotype is not segregated according to lineage position, which suggests that birth order may not play a critical role in determining transmitter phenotype. Cell lesion techniques are being applied to neurons in the L cluster to investigate the influence of lineage size on transmitter expression.

Supported by grants NS 07936 (JLW) and NS-13079 (JWT).

17.3

THE EGPS - ECLOSION HORMONE AND cGMP REGULATED PHOSPHOPROTEINS IN THE CNS OF *MANDUCA SEXTA*: EFFECTS OF CYCLOHEXIMIDE AND ACTINOMYCIN D. D.B. Morton and J.W. Truman, Department of Zoology, University of Washington, Seattle, WA 98195

Eclosion hormone (EH) is a 62 amino acid neuropeptide that acts on the CNS to trigger the stereotyped ecdysis behavior which occurs at the end of each molt. In *Manduca sexta*, the action of EH is mediated via an increase in cGMP levels followed by the phosphorylation of two 54kDa phosphoproteins, the EGPs (EH and cGMP regulated phosphoproteins)

At 24hrs before ecdysis, injected EH will elevate cGMP levels but will not trigger ecdysis behavior. Two dimensional SDS-PAGE analysis of CNS proteins at this time show that the EGPs are absent or cannot be phosphorylated. The EGPs first appear on fluorograms at the same time that EH will first trigger ecdysis behavior, 8 hrs before ecdysis and the appearance is regulated by the steroid hormone, ecdysone.

The need for protein and RNA synthesis for the development of both EH triggered ecdysis and the appearance of the EGPs has been investigated using the protein and RNA synthesis inhibitors, cycloheximide (CX) and actinomycin D (ACT). Injections of CX (150ug) and ACT (100ug) 4hrs before ecdysis did not prevent either EH triggered ecdysis or the appearance of the EGPs. Injection of CX or ACT at earlier times, however, prevented both EH triggered ecdysis and the appearance of the EGPs on fluorograms. These data are consistent with the hypothesis that *de novo* synthesis of the EGPs is necessary for EH to trigger ecdysis.

Supported by NIH grant NS 13709.

17.4

CLONING OF ADIPOKINETIC HORMONE cDNA M.H. SCHAFFER M.O'SHEA, and B.E. NOYES*. Dept. Psychiatry, Univ. of Texas Health Science Center Dallas,TX 75235.

Adipokinetic hormone (AKH I) is a grasshopper neuropeptide which is of interest as a hormone, neurotransmitter, and member of a structurally related family of neuropeptides which extends throughout the arthropods. cDNA clones of AKH I were selected from a library constructed from polyA enriched RNA derived from 700 S. nitans corpora cardiaca. The library was screened with a 25-base oligonucleotide whose sequence was derived from the peptide sequence and results of a variation of the primer extension method of direct RNA sequencing. The DNA sequence of the recombinants predicts that the AKH I precursor contains one copy of the AKH sequence which is preceded by a signal peptide and followed by the amidation sequence gly-lys-arg. The precursor also contains a second peptide which is abundant in the c.c. named alpha chain by Hekimi and O'Shea (abstract this meeting). Minor variations in sequence found in different cDNAs likely reflect allelic variation. The high abundance of AKH I cDNAs suggests that this library is a favorable source of AKH II cDNAs. The status of this search and that for the AKH I gene will be discussed. A similar cDNA has been identified from another species by Schulz et al. (abstract this meeting).

This work supported by Biological Humanics Foundation.

17.5

MOLECULAR BIOLOGICAL STUDIES ON THE SYNTHESIS OF LOCUST ADIPOKINETIC HORMONES. M.F. Schulz*, E. Roulet*, J. Fischer* and M. O'Shea. Lab. de Neurobiologie, Université de Genève, Geneva, Switzerland. (SPON: G. Kato)

The peptide hormones AKH I and AKH II are synthesized in the neurosecretory cells of the locust corpora cardiaca (CC). Two dimer precursors P1 and P2 are involved in AKH biosynthesis. They produce the monomer AKHs as well as two dimers, the AKH Precursor Related Peptides, APRP 1 and APRP 2 (Hekimi and O'Shea, J. Neurosci. 7:1773-1784). To study the molecular mechanism leading to the P1 and P2 precursors, we have isolated and sequenced candidate clones from a CC cDNA library and analysed in vitro translation products from CC mRNA. Translation of CC mRNA in vitro results in two major proteins of 5 and 6 Kd whose production is blocked in the presence of precursor related oligonucleotides. This indicates that the two in vitro products are related to the P1 and P2 dimers. The sequence of an AKH I and APRP 1 cDNA clone encodes a signal peptide followed by AKH I, a Gly-Lys-Arg processing site, the 28 amino acids of the monomer of APRP 1 α-chain followed by a stop codon. Thus the P1 homodimer is formed from two monomer translation products. Although the α-chain peptide contains an Arg-Lys processing site it is not used during AKH and APRP biosynthesis (see also Hekimi and O'Shea, ibid).

Supported by Ciba-Geigy.

17.6

PRECURSORS OF ADIPOKINETIC HORMONES (AKHs) ARE N-TERMINALLY BLOCKED DIMERS GENERATING DIMER PEPTIDE PRODUCTS IN ADDITION TO THE AKHs. S. Hekimi* and M. O'Shea. Lab. de Neurobiologie, Université de Genève, Geneva, Switzerland.

The neurosecretory cells of the corpus cardiacum (CC) of the locust Schistocerca gregaria produces two peptides called AKH I (10 aa) and AKH II (8 aa). These are released into the blood during prolonged flight and serve to mobilize lipid metabolism. By performing pulse-chase experiments on in vitro cultured CC we have identified two precursors (P1 and P2) of the AKHs. Structural analysis of P1 and P2 by protein sequencing and reduction shows that P1 is a homodimer and P2 is a heterodimer. Both dimers are formed by a single disulphide bond. During processing the P1 and P2 dimers generate the monomer AKHs and dimers we call AKH Precursor Related Peptides or APRPs. Three APRPs have been identified in the CC and complete structures determined. APRP 1 is a homodimer of two 28 residue chains (called α-chain) and is processed from P1. APRP 2 is a heterodimer of the α-chain plus a homologous 28 residue chain (β-chain) and is processed from the heterodimer P2. We have also found and sequenced the β-β homodimer (APRP 3). The APRPs are released peptides and may have functions related to flight as do the co-synthesized AKHs. The APRPs are produced in markedly different proportions at different stages of development.

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17.7

EXPRESSION AND ORGANIZATION OF A NEUROPEPTIDE-ENCODING GENE IN *DROSOPHILA MELANOGASTER*. Paul H. Taghert and Lynne E. Schneider*. Dept. of Anatomy & Neurobiology, Washington University School of Medicine, Saint Louis, MO 63110.

We are interested in the mechanisms that underlie cell-specific neuropeptide gene expression in the nervous system. The *Drosophila* gene that encodes multiple FMRFamide-like neuropeptides (Schneider and Taghert (1988), PNAS **85**, 1993; Nambu et al (1988), Neuron **1**, 55) is expressed in a small subset of CNS neurons. It is transcribed throughout post-embryonic life with a relatively simple pattern as detected by Northern blot analysis; a single band of ~1.7 kb is found in larval, pupal, and adult stages. A smaller band (of ~0.7 kb) is also detected if the probe includes the 3' untranslated region of the cDNA. We are currently examining embryonic stages to detect the initial period of gene transcription. Sequencing of genomic DNA and protection assays indicate that the gene spans ~4 kb and contains a single intron. Exon 1 is 254 bp with a short (27 bp) untranslated region and includes a 41 bp sequence identical to the 5' end of the longest cDNA recovered (Nambu et al, *op. sit.*). The intron is ~2.3 kb in length. Exon 2 is 1351 bp long and contains all 15 FMRF-NH2 encoding sequences. This neuropeptide gene is expressed by a specific complement of CNS neurons (see Schneider, Sun, and Taghert, this volume). In order to identify *cis*-regulatory genomic regions, and as a prelude to germ-line transformation studies, we are currently sequencing the intron and the regions that are 5' to the gene. In addition we are examining comparable regions adjacent to the gene in a related Drosophalid species to find conserved sequences.

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17.8

CELLULAR ANALYSIS OF A NEUROPEPTIDE GENE EXPRESSION IN *DROSOPHILA MELANOGASTER*. L. E. Schneider*, E. Sun#*, and P. H. Taghert (SPON: R. Sinclair). Washington University School of Medicine, St. Louis 63110 and #Monsanto Co., Chesterfield, MO 63198.

The *Drosophila* FMRFamide gene encodes a variety of different peptides that end in Phe-Met-Arg-Phe-amide (Schneider and Taghert (1988), PNAS **85**, 1993 ; Nambu et al (1988), Neuron **1**, 55). In *Drosophila*, antisera raised against synthetic FMRFamide recognize ~30 neurons in the larval and adult nervous systems (White et al (1986) J. Comp. Neurol. **247**, 430). We have examined the expression of the authentic *Drosophila* gene by immunohistochemistry and in situ hybridization. We have raised a rabbit antiserum to the octapeptide FRGDPKQD, a portion of the deduced *Drosophila* FMRFamide precursor. This sequence spans a suspected cleavage site between two adjacent peptides. In larvae, this antiserum specifically labels a single pair of neurons at the ventral mid-line of the subesophogeal ganglion, and a single pair in each thoracic ganglion. The thoracic ganglion neurons correspond to previously identified neuroendocrine cells that are laterally-placed and that project to the Transverse Nerve. This pattern is retained in adults with the addition of a second pair of neurons in each thoracic ganglion and a single cell in the optic lobe areas of the brain. Hence, these immunoreactive neurons comprise only a subset of those recognized by antisera against authentic FMRFamide. To examine the cellular distribution of transcripts from this gene, we used a 41 bp probe (from the 3' end of the translated region) that was tailed with ~15 bases of ^{35}S-dATP. Although this method lacks precise cellular resolution, strong hybridization signals are seen in each CNS region that also contains immunoreactive cells. The regions corresponding to adult-specific cells (optic lobes and in the dorsal region of the third thoracic ganglion) typically contain weaker signals. We are currently studying the establishment of these patterns during embryogenesis and adult development.

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17.9

STRUCTURE AND EXPRESSION OF THE *DROSOPHILA* GLUTAMATE DECARBOXYLASE GENE. F. Rob Jackson, Worcester Fndn. Exptl. Biol., Shrewsbury, MA 01545.

The enzyme glutamate decarboxylase (GAD) catalyzes the synthesis of gamma-aminobutyric acid (GABA), a well-documented inhibitory neurotransmitter of vertebrate and invertebrate nervous systems. We previously reported the isolation of *Drosophila* DNA clones that hybridize to mammalian GAD gene sequences. On the basis of the following experimental results, we now conclude that these clones represent a *Drosophila* GAD gene: (1) The sequence of one *Drosophila* cDNA clone (GAD1) predicts a protein with extensive homology to mammalian GAD (overall sequence identity = 52%). Moreover, a tetrapeptide sequence (NPHK) thought to represent the pyridoxal binding site of mammalian GAD is conserved in the deduced *Drosophila* protein. (2) The GAD1 cDNA hybridizes to RNA transcripts which are more abundant in head than in body poly(A)+ fractions, suggesting that homologous mRNAs are preferentially expressed in nervous tissue. The results of preliminary tissue *in situ* hybridization studies are in agreement with this conclusion. (3) A chromosomal deletion which removes the genetic locus represented by the *Drosophila* clones is associated with reduced GAD enzyme activity.

Northern analyses demonstrate that expression of the *Drosophila* GAD gene commences early in embryogenesis (4-8 h) and continues throughout development. A major 3.1-kb size class of mRNA is detected throughout development, while a minor 2.6-kb transcript is observed only in embryos and pupae. The analysis of multiple cDNA and genomic DNA clones suggests that the 3.1-kb class of mRNA consists of a population of variably spliced transcripts. Such transcripts might encode a family of GAD protein isoforms.

I thank Laurel M. Newby for technical assistance. This research was supported by NIH grant NS25914 and a McKnight Scholars Award.

17.10

SPIDER VENOM TOXINS ACTING ON THREE CLASSES OF SYNAPTIC ION CHANNELS AT THE INSECT NEUROMUSCULAR JUNCTION. M. E. Adams, V. P. Bindokas*, L. Hasegawa* and V. J. Venema*. Div. Toxicology and Physiology, Dept. of Entomology, University of California, Riverside, CA 92521

Spider venoms contain a diversity of paralytic toxins which may serve as useful probes for the characterization of synaptic ion channels. The venom of *Agelenopsis aperta*, a funnel web spider, paralyzes flies (*Musca domestica*) and disrupts neuromuscular transmission by affecting ion channels on pre- and postsynaptic membranes. Multiple acylpolyamine "α-Agatoxins" related in structure to argiotoxins (Adams, M.E. et al, *Biochem. Biophys. Res. Comm.* 148, 678-683, 1987), exert use-dependent block of postsynaptic glutamate-sensitive receptor channels. Such blockade is slowly reversible and is correlated with temporary paralysis produced by the toxins *in vivo*. Two additional classes of presynaptic toxins produce irreversible effects on transmitter release. "μ-Agatoxins" appear to be sodium channel activators, causing spontaneous repetitive activity and massive transmitter release. They synergize the paralysis caused by α-Agatoxins. We have identified six μ-Agatoxins as homologous, cysteine-containing peptides 36-37 amino acids long. A second class of presynaptic toxins ("ω-Agatoxins") irreversibly blocks transmission at nanomolar concentrations without affecting iontophoretic glutamate potentials. Block of neuromuscular transmission by one of these, ω-Aga I, appears to involve presynaptic calcium channels (Bindokas, V.P. and Adams, M.E., this volume).

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17.11

ω-AGA I, A SPIDER VENOM TOXIN FROM *AGELENOPSIS APERTA*, IRREVERSIBLY BLOCKS TRANSMITTER RELEASE AT INSECT AND FROG NEUROMUSCULAR JUNCTIONS. V. P. Bindokas*, and M. E. Adams (SPON: M. A. Baker). Div. Toxicology and Physiology, Dept. of Entomology, University of California, Riverside, CA 92521

In addition to α- and μ-Agatoxins, (Adams, M. E. et al, this volume), venom of the spider *Agelenopsis aperta* contains ω-Agatoxins, a group of irreversible presynaptic blockers. The most abundant of these, ω-Aga I blocks transmission in the fly (*Musca domestica*) longitudinal ventrolateral muscle and in the frog (*Rana pipiens*) cutaneous pectoralis muscle. Studies on the fly neuromuscular junction suggest that ω-Aga I acts on calcium channels involved in transmitter release. Loose patch recordings from individual junctions reveal that the toxin reduces quantal content without apparantly affecting the frequency or amplitude of spontaneous miniature junctional currents. ω-Aga I appears not to affect spike invasion of the nerve terminal. Dose-dependent block of EPSP's by ω-Aga I can be partially reversed by elevating extracellular calcium or by repetitive stimulation. Graded release of transmitter elicited by direct field stimulation of nerve terminals, which is TTX-insensitive but cobalt-sensitive, is blocked by the toxin.

The effects of ω-Aga I are not confined to neuromuscular junctions. For example, the toxin suppresses the calcium component of action potentials recorded in the somata of insect dorsal unpaired median (DUM) neurons. Our data indicate that ω-Aga I may have widespread actions on calcium currents in the nervous system.

Supported by NIH grant NS24472 and USDA grant 86-CRCR-1-2097.

17.12

PEPTIDE CO-TRANSMITTER IN SKELETAL MUSCLE ACTS AT MULTIPLE SITES WITHIN THE EXCITATION-CONTRACTION COUPLING PATHWAY. C.A. Bishop, Dept. of Psychology, Stanford Univ., Stanford, CA 94305.

We have been investigating the site of action of the pentapeptide proctolin in the crayfish tonic flexor muscle, where the peptide serves as a co-transmitter in 3 of 5 excitatory motoneurons and greatly potentiates the tension generated by the conventional conductance-increasing, EPSP-producing neurotransmitter (Bishop et al., *J. Neurosci.* 7: 1769, 1987). Proctolin acts directly on the muscle; it does not affect the EPSP amplitude and therefore probably acts through a separate receptor from that used by the conventional neurotransmitter. The peptide's action is eliminated when extracellular Ca^{2+} is replaced by equimolar Co^{2+}. We have shown previously that proctolin-induced tension enhancement can be elicited in Ca^{2+}-free, Co^{2+}-substituted saline after treatment with the Ca^{2+} ionophore A23187, which causes the release of Ca^{2+} from intracellular stores (Bishop and Wine, *Neuro. Abst.*, 1986). This result suggests that at least part of the peptide tension enhancement may be due to an enhanced release of Ca^{2+} from intracellular stores and is not associated with changes in membrane Ca^{2+} conductance. Recent experiments, however, indicate that proctolin can also potentiate membrane Ca^{2+} conductance in this muscle: Patch-clamp experiments reveal at least one voltage-activated Ca^{2+} channel that is gated by proctolin and voltage-clamp experiments show that an early, transient, voltage-dependent Ca^{2+} current is potentiated by 10^{-8} M proctolin. Together, these results indicate that the proctolin tension enhancement effect in this muscle is probably the result of the peptide's action at multiple sites along the excitation-contraction coupling pathway. (Supported by NIH and Muscular Dystrophy Assn.)

17.13

PEPTIDES MODULATE THE LOBSTER CARDIAC SAC RHYTHM. P.S. Dickinson and E. Marder. Biology, Bowdoin College, Brunswick, ME 04011 and Brandeis Univ., Waltham, MA 02254.

The cardiac sac rhythm is one of four rhythms generated in the lobster stomatogastric nervous system. We have found that red pigment concentrating hormone (RPCH) is present in the stomatogastric (STG), commissural, and oesophageal ganglia of the Panulirus interruptus nervous system; previous work had shown the presence of proctolin in these ganglia. Bath application of either 10^{-6} M proctolin or RPCH activates the cardiac sac rhythm in quiescent preparations; it increases the frequency of the rhythm and the duration of dilator bursts in spontaneously active preparations. When applied to any single ganglion, RPCH alters the cardiac sac rhythm; proctolin is generally effective only on the commissural ganglia. These two peptides may interact in their control of the cardiac sac rhythm, since proctolin, which does not normally induce a cardiac sac rhythm in an isolated STG, will provoke such a rhythm in the presence of sub-threshold levels of RPCH. Moreover, cardiac sac dilator bursts lead to perturbations of the other 3 rhythms (pyloric, gastric, oesophageal) produced by the stomatogastric nervous system. Thus, RPCH and proctolin may modulate these rhythms not only directly, but also indirectly via their effects on the cardiac sac rhythm. Supported by NSF BNS 8706568 (PSD), the Bunting Institute of Radcliffe College and NS 17813 (EM).

18.1

NEUROBEHAVIORAL EVIDENCE FOR KAPPA AGONIST ACTIVITY OF THE MORPHINAN DERIVATIVE 14-ß-METHYL 8-OXACYCLORPHAN. F.B. Jolicoeur[1] R. Rivest[1], D. Menard*[1] S. Lemaire[2] and B. Belleau*[3]. [1]Dept. Psychiatry, Fac. of Med., University of Sherbrooke, Sherbrooke, Quebec, J1H-5N4, [2]Dept. Pharmacology, School of Med., University of Ottawa, Ottawa, K1H 8N5 and [3]Dept. Chemistry, McGill University, Montreal, Quebec, H2A 2K6

The purpose of the present study was to determine if the in vivo neurobehavioral effects of the morphinan 14-ß-8-oxacyclorphan would reflect the kappa agonist activity found in our previous in vitro studies (Lemaire, S. et al., Can. J. Physiol. Pharmac. 64:707, 1986). The effects of intracisternal (IC)administration of various doses (10-80 µg) of 14-ß-8-oxacyclorphan on body termperature, muscle rigidity, nociception of thermal, chemical and mechanical stimuli as well as its ability to induce catalepsy were examined. The effects of intrathecal administration of the same doses of the compound on reactivity of animals to a thermal stimulus were also assessed. Results indicate that the analgesic properties of 14-ß-8-oxacyclorphan resemble those of typical kappa agonists: (IC) administration of the drug failed to affect nociception to a thermal stimulus but markedly reduced the reactivity of animals subjected to chemical or mechanical stimuli. On the other hand, intrathecal administration of the drug significantly attenuated nociception of animals to a thermal stimulus. The in vivo neurobehavioral effects of 14-ß-8-oxacyclorphan appear to be kappa selective since the drug did not decrease body temperature, increase muscular tone or induce catalepsy, three effects generally attributed to µ agonists. Furthermore, the (IC) administration of the compound in a similar dose range was found to significantly antagonize the catalepsy, muscular rigidity, akinesia as well the analgesia in the hot-plate test produced by IC administration of morphine (60µg). In summary, the present results reveal that 14-ß-8-oxacyclorphan displays many features similar to those of well known kappa agonists.

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18.2

Functional U69593 receptors exist in the spinal cord of the 9-16 day old rat.

J.C. Hunter, J.A.M. Smith, C.A. Allerton, G.E. Leighton, P.R. Boden, R.G. Hill and J. Hughes. Parke-Davis Research Unit, Addenbrooke's Hospital Site, Hills Road, Cambridge, CB2 2QB, U.K. (SPON: L.C. Thomas)

The benzeneacetamide U69593 is a highly selective ligand for the kappa opioid receptor. In contrast to intracerebroventricular or intravenous administration, intrathecal application of U69593 and related compounds into adult rats failed to produce analgesia (Leighton, G.E. et al., Br. J. Pharmacol. 93, 553, 1988). Consistent with this observation is the apparent absence of ^{3}H-U69593 binding sites in the spinal cord of the adult rat (Lahti, R.A. et al., Eur. J. Pharmacol. 109, 218, 1985). In this study we have shown the equilibrium binding of ^{3}H-U69593 to homogenates of 9-16 day old rat spinal cord (B_{max} = 60fmol/mg protein, K_D = 2.3nM). The presence and distribution of these sites in 10uM transverse sections of spinal cord was demonstrated using autoradiographical techniques. Superfusion of U69593 onto an in vitro spinal cord preparation resulted in depression of spontaneous and electrically evoked synaptic activity. In these immature rats, intrathecal administration of benzeneacetamides produced dose-dependent analgesia. In conclusion, we have shown that in contrast to the adult, the spinal cord of the immature rat has a high level of U-69593 binding sites, with functional correlates.

18.3

ANALYSIS OF THE DEPRESSION AND LOCOMOTOR EXCITATION INDUCED BY U50, 488, A SELECTIVE KAPPA OPIATE AGONIST. L.B. Estall*, B. de Costa*, K. Rice*, A. Pert (SPON: J. Malick). BPB, NIMH and IN, NIDDK, Bethesda, MD 20892.

In our previous studies, we found that the (S,S) form of U50, 488, a potent kappa agonist, was much more active than the (R,R) form in several behavioral tests following central administration. One of the most profound effects of U50, 488 was on locomotor output. The purpose of this series of studies was to further characterize the effects of U50, 488 on locomotor activity and to define the neurochemical circuits involved. Systemic injections of U50, 488 produced an initial dose-dependent decrease in locomotor output, which was followed by excitation. No tolerance was observed to the locomotor depression following chronic administration while the excitatory effects increased with repetitive injections. Neither MR 2266 nor naloxone antagonized the excitatory effects of U50, 488. 6-OHDA lesions of the nucleus accumbens also failed to alter the locomotor stimulatory effects. The effects of systemically administered U50, 488 on locomotar activity appear to be mediated through different mechanisms than those mediating the effects of centrally administered U50, 488 and do not involve mesolimbic dopamine.

18.4

NEONATAL NALTREXONE ADMINISTRATION: SOMATIC AND BEHAVIORAL EFFECTS ON THE INFANT AND ADULT RAT.

B.Caldarone* & P.Kehoe. Dept. of Psychology, Trinity College, Hartford, Ct. 06106

In an effort to examine the functional changes following neonatal opiate receptor antagonist treatment, naltrexone in doses 1, 25 and 50 mg/kg or saline was injected daily IP from postnatal day 1 until either day 9 or day 21. Pups receiving treatment until day 9 were conditioned on day 12 with exposure to a novel odor in conjunction with morphine (.5 and 2 mg/kg) IP. On day 15 the pups were given an odor preference test. All pups given morphine paired with the odor acquired the preference, whereas prior naltrexone administration had no effect. Additionally, naltrexone treatment resulted in no difference in their daily weight gain. Pups treated with naltrexone until day 21 showed little difference in weight except that males given 50mg/kg weighed somewhat less than saline controls and even significanlty less than those given 1 mg/kg. Female weight gain was unaffected. While females in general showed earlier eye opening, naltrexone in a dose-dependent manner hastened eye opening in males and females. As adults, females defecated less in a novel open field situation than males but both were affected by neonatal naltrexone in that the high dose was associated with an increase in fecal boli number during exploration. These data suggest that while daily naltrexone may accelerate some maturational milestones, certain opiate-mediated functions are either unaffected or altered in complex ways.

18.5

Receptor types involved in opioid-induced antinociception in neonatal rat spinal cord in vitro. **I.F.James, S.B.Ketchum*, M.N.Perkins* and A.Dray. Sandoz Institute for Medical Research, Gower Place, London, England.**

A nociceptive response can be recorded in vitro from the spinal cord of the neonatal rat following stimulation of the tail. Opioids depress this response and we have now characterized the spinal opioid receptor types involved.

Receptor binding, performed in homogenates of spinal cord from 1-2 day old rats, showed high affinity sites for [3H]-DAGO and [3H]-EKC. The DAGO sites showed a mu-receptor profile while the EKC sites that remained after blocking mu-sites with 100nM DAGO, showed a kappa receptor profile. No [3H]-DPDPE binding could be detected. In functional studies where the spinal cord and attached tail were separately superfused, noxious stimulation of the tail with capsaicin or heat produced a ventral root depolarization. Depression of noxious responses was produced by DAGO, DPLPE and U50488 (ED50 = 2, 150 and 130nM respectively) superfused on the spinal cord. The effect of an ED50 dose of DAGO and DPLPE was reversed by a similar concentration of naloxone (2-10nM) but a higher concentration (0.2-1.0 µM) was required to reverse U50488.

These data show that the neonatal rat spinal cord contains functional mu and kappa receptors and while no delta binding is detectable, delta ligands may activate another receptor.

18.6

STEREOSPECIFIC MODULATION OF SOMATIC GROWTH IN PREWEANLING RATS BY OPIOID ANTAGONISTS. S. Pileggi*, P.J. McLaughlin and I.S. Zagon (SPON: T. Pritchard). Dept. Anatomy, Penn. State Univ. Coll. of Med., Hershey, PA 17033.

Somatic and neurobiological growth of rats are regulated by endogenous opioid systems. Perturbation of endogenous opioid-opioid receptor interaction by opioid antagonists has been an important experimental paradigm. To determine if somatic growth is regulated at the level of opioid receptors, a stereospecific response to (-) and (+) isomers of naloxone, an opioid antagonist, was studied. Newborn Sprague-Dawley rats were injected i.p. with various dosages of (-)naloxone. At day 21, rats injected with 20-60 mg/kg (-)naloxone had deficits of 10-23% in body weights from control levels. To study stereospecificity, a dosage of 40 mg/kg (-)naloxone or (+)naloxone, or sterile water was injected daily into rats from birth to day 21. Relative to controls, 21-day old rats injected with 40 mg/kg (+)naloxone did not differ in body or organ weights, whereas rats receiving 40 mg/kg (-)naloxone were 23% lighter in body weight and had 19-52% reductions in weight of liver, thymus, heart, spleen, and skeletal muscle. Opioid agonist challenge experiments indicated that (+)naloxone did not block receptors, while (-)naloxone blocked receptors for at least 6 hr/day. These results suggest that opioid antagonists can perturb the interaction of endogenous opioid systems in a stereospecific manner. Support by NIH grants NS-20623 and NS-20500.

18.7

INTERACTION OF MIDAZOLAM AND MORPHINE IN RATS SPINAL CORD. M. Sabbe*, A. Yanez Gonzalez*, C.W. Stevens and T.L. Yaksh (SPON: J.D. Grabow). Mayo Clinic, Rochester, MN 55905.

GABA-benzodiazepine ionophore complex has been implicated in pain modulatory processing. The present studies sought to characterize the antinociceptive interaction between morphine (MOR) and benzodiazepines given in the intrathecal (IT) space. We used the water soluble agent midazolam (MID). Rats with chronic IT catheters received MOR, MID, or a combination (10 µl). IT MOR (0.5-15 µg) evoked a dose-dependent increase in hot plate (HP)/tail flick (TF) response latency with no effect on motor function. IT MID (20-100 µg) had no effect on HP or TF response, but evoked hindlimb paralysis at 100 µg. Consideration of the effects of IT MOR in the presence of MID (20 µg) revealed no change in the maximum effect produced by any given dose of MOR on the HP, but significantly increased the area under the time effect curve. Naloxone (1 mg/kg, i.p.) antagonized the effects of MOR, MID + MOR, but not MID (100 µg) alone. The benzodiazepine antagonist Ro15-1788 (1 mg/kg, i.p.) had no effect on any dose of MOR, significantly antagonized the motor dysfunction of MID (100 µg), but had little effect on the antinociceptive actions of the MOR + MID antinociceptive effects. These data suggest a probable interaction between intrathecally administered opiate and benzodiazepine agonists. Whether this interaction is pharmacokinetic or pharmacodynamic is not known. (Supported by Janssen (MS) and grant DA-02110 (TLY).)

18.8

THE ROLE OF EFFICACY IN THE MAGNITUDE OF SPINAL ANTINOCICEPTIVE TOLERANCE. C.W. Stevens and T.L. Yaksh, Dept. of Pharmacology, Mayo Clinic, Rochester, MN 55905.

Tolerance is manifest by the decrement of agonist effect with the continued administration of the same dose of agonist or by an increment of dose to produce a previously obtained effect. Using a constant-rate, constant-dose intrathecal (IT) infusion model in the rat (JPET 244: 63, 1986) we have shown that the time course of tolerance development to daily hot plate (HP) testing is not different among µ or δ opioid, α_2 or synergistic combinations of opioid and α_2-agonists. In experiments designed to assess the magnitude of tolerance on HP after 7-day IT infusion we now report that the degree of tolerance induced is related to the efficacy of the infused agent. Morphine (MOR), sufentanil (SUF), DAGO (µ opioid specific), DADLE (δ opioid) and ST-91 (α_2-adrenergic) were each infused in 3 log-spaced concentrations and full dose-response curves obtained by IT bolus of the respective agents. ED_{50} and tolerance ratios (tolerant ED_{50}/saline-infused ED_{50}) were calculated for saline and each infusion dose. At the middle infusion doses, the most efficacious agents, DAGO and SUF, produced minor tolerance ratios (7; 2) whereas the less efficacious MOR, DADLE and ST-91 produce higher ratios (55, 46 and 29, resp.). These results support the view that highly efficacious agents may produce less tolerance as they necessitate a lower fractional occupancy of available receptor pool for a given effect. (Supported by grant DA-02110 and Mayo Foundation.)

18.9

β-FUNALTREXAMINE (β-FNA) VERSUS OPIOID RECEPTORS IN THE SPINAL CORD. E. Mjanger* and T.L. Yaksh (SPON: A.G. Engel). Mayo Clinic, Rochester, MN 55905.

Five opioid receptors are implicated in the mediation of spinal analgesia (µ, μ_1, δ and κ). This study is done to systematically classify the potency of the different receptor agonists in the spinal cord. All injections were given intrathecally in volume of 10 µl saline. Antinociception was measured by hot plate (HP; 52.5°C) and tail flick (TF) tests. Each rat was used only once.

The ED_{50} ± 95% CI (nmol) (n = 12-25 per drug)

Drug	Hot Plate	Tail Flick
DAGO	0.3 (0.1-0.9)	0.6 (0.3-1.2)
β-endorphin	1.1 (0.5-2.4)	1.8 (1.1-2.8)
DSTLE	4.1 (0.8-21.1)	4.4 (0.4-56.1)
DPLPE	13.1 (16.5-29.4)	7.0 (2.4-20.6)
Morphine	20.4 (10.9-38.0)	26.7 (15.2-46.9)
Meptazinol	>100	>100
U50488H	>100	>100

IT β-FNA (1 µg) given 24 hrs prior to test, resulted in a reduction in effect such that as a percent of saline control: morphine (2%) > DAGO (60%) > DPLPE (96%). Given the relative preference of these agonists and the effects of β-FNA, these results suggest a role for distinguishable spinal µ and δ receptors in modulating thermal nociception. (Supported by grant DA-02110, TLY; Odd Fellow, Norway, EM.)

18.10

THE EFFECT OF DOSAGE AND TIMING OF ADMINISTRATION OF NALOXONE ON NEUROLOGIC OUTCOME IN THE RAT VENTRAL COMPRESSION MODEL OF SPINAL CORD INJURY. Edward C. Benzel, M.D., and John Lancon*, B.S. Depart. of Neurosurgery, LSU School of Med., Shreveport, LA 71130

The effects of dosage and timing of administration of Naloxone on rats following spinal cord injury via the ventral compression technique is presented. The ventral compression technique for rat spinal cord injury allows a ventral compression of the spinal cord without the requirement for a prior laminectomy. The first part of this two part study involved the determination of the optimal dose of Naloxone, administered intraperitoneally, at 45 minutes following the creation of the lesion. Of the groups studied (control-10.0 mg/kg), 2.0 mg of Naloxone proved to be superior to both lesser and greater dosages ($P < 0.05$).

The second part of the study involved the administration of a 2.0 mm/kg dose of Naloxone at varying intervals ranging from 10 minutes prior to lesioning to 24 hours following lesioning. A biphasic response was again demonstrated with an optimal time of administration occurring at 45 minutes following the creation of the lesion ($P < 0.05$).

In summary, a significant effect was offered by a mid range dose of Naloxone (2.0 mm/kg), administered at 45 minutes following injury. Experiments not accounting for these biphasic dose and timing of administration responses may lead to erroneous conclusions.

18.11

EFFECTS OF THE ENKEPHALINASE INHIBITOR ANALGESIC SCH 34826 ON EEG ACTIVITY AND SLEEP PATTERNS IN THE RAT. M. Trampus*, A. Conti*, A. Monopoli* and E. Ongini. Research Labs., Essex Italia, I-20060 Comazzo (Milan), Italy.

SCH 34826 is a new orally active enkephalinase inhibitor analgesic (1). We investigated whether the molecule induces sedation by evaluating EEG activity and sleep patterns in the rat. EEG was recorded for 6h. SCH 34826 was tested at 30-300 mg/kg p.o., and compared with morphine (10 mg/kg s.c.) and acetorphan (30 mg/kg p.o.), another enkephalinase inhibitor. Morphine decreased waking and REM sleep, and increased slow wave sleep. There was dissociation between EEG patterns of sleep and behavior. Bursts of paroxysmal activity (spikes) were observed in the EEG. Acetorphan did not modify the sleep patterns. SCH 34826, at 30 and 100 mg/kg, did not alter the sleep-waking cycle. At 300 mg/kg, rats appeared to sleep less than controls and a reduction of REM was observed. This dose is 10-30 times that required for inhibiting the enzyme and at no time did SCH 34826 elicit any of the EEG and behavioral effects observed after morphine. SCH 34826 appears to be devoid of liability for sedation and other EEG changes at doses far above those predicted to be clinically effective. (1) Chipkin et al. (1987), J. Pharm. Exp. Therap., in press, 1988.

18.12

PHARMACOLOGICAL ACTIONS OF A NOVEL MIXED AGONIST-ANTAGONIST OPIATE, NalBzoH. **M.A. Gistrak,* D. Paul,* E.F. Hahn,* and G.W. Pasternak** (SPON: R. Price) The Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Cornell U. Medical College, NY, NY 10021.

NalBzoH, the benzoylhydrazido derivative of naloxone, is an opiate analgesic with mixed agonist-antagonist properties. In binding studies, it potently labels opioid receptors: mu_1>mu_2>kappa>>delta. The sodium effect on ^{3}H-NalBzoH binding is intermediate between that of a pure agonist and a pure antagonist, suggesting a mixed agonist-antagonist. We therefore examined the actions of NalBzoH in tailflick assays alone and against morphine analgesia. NalBzoH antagonizes morphine analgesia (6 mg/kg s.c.) with an ID_{50} of 2 µg/kg (s.c.), a potency 4 times that of naloxone. NalBzoH (1 mg/kg s.c.) also precipitated withdrawl in morphine-dependent mice. These data indicate that NalBzoH is an antagonist at mu receptors. At higher doses, NalBzoH alone is an analgesic, with an ED_{50} of 10-20 mg/kg, s.c. Peak effect occurs 15 to 30 min following administration, with a duration of 90 min. Although it is reversed by naloxone, NalBzoH analgesia was at least 5 times less sensitive than morphine analgesia, implying a non-mu mechanism. Thus, NalBzoH, an analgesic with potent antagonist actions at mu receptors, may be free of many of the side-effects mediated through mu receptors, such as respiratory depression and constipation.

18.13

Chronic naltrexone exposure enhances morphine analgesia but not defeat induced analgesia in mice. M.L. Thompson* and C. A. Paronis* (SPON: L. Shuster), Dept of Pharmacology, Tufts Medical School, Boston, MA 02111

Chronic naltrexone (NTX) exposure via pellet implantation has been shown to increase the number of opiate receptors in certain regions in the brains of mice or rats. These increases have been suggested to be functional in the sense that analgesic response to morphine is enhanced for a period of time following pellet removal. In this expt. we investigated the effects of NTX pellet exposure on the analgesic response observed in mice following defeat in a social encounter.

Male B6AF1 mice, aged 3-4 mo., were implanted sc. with pellets containing roughly 7.5 mg of NTX. A comparison group of mice received cholesterol implants. Pellets were removed after 10 days. 24 hours later mice were injected with 3 mg/kg ip morphine or exposed to social conflict in a resident-intruder paradigm. Analgesia was assessed in the tailflick test to a radiant heat stimulus, (baseline response of 1.5-2.5 sec. and a cutoff of 6 sec.) TF latencies were recorded every 30 min. for 2 hours following morphine, or after bouts of 20 bites in the social conflict paradigm. Latencies were expressed as % maximum possible effect (MPE). Mice tested with morphine showed significantly enhanced analgesia compared to placebo pelleted mice at 30 (75% mpe vs 40%) and 60 min (65% mpe vs 35% mpe) following morphine injection. In contrast, social conflict analgesia was significantly reduced in the NTX pelleted mice. The placebo mice achieved 50% MPE after roughly 20 bites whereas NTX pelleted mice required on average around 40 bites. This data suggests that analgesia produced by morphine or defeat may be mediated by somewhat different subpopulations of receptors with different profiles of response to chronic antagonist exposure.

18.14

THE SELECTIVE μ OPIOID PEPTIDES DAGO AND PLO-17 STIMULATE PROLACTIN SECRETION WHEN ADMINISTERED TO LACTATING FEMALE RATS. M. Baumann, J. Janik and J. Rabii, Dept. of Biological Sciences, Rutgers Univ., Piscataway, NJ 08855.

We have recently reported that morphine does not stimulate prolactin secretion in lactating female rats (Callahan *et al.*, *Brain Res.*, 442:214, 1988). The insensitivity of the prolactin-releasing mechanism to opiates during lactation is dependent upon the suckling stimulus and not related to altered drug metabolism. These data suggested to us that the μ opioid receptor sites mediating opiate-induced prolactin secretion may be down regulated in the lactating female. In this study, the effects of selective μ receptor activation on prolactin release were determined in lactating rats.

Lactating dams were implanted with intracerebroventricular (ICV) cannulae and chronic indwelling jugular cannulae. On the day of the experiment, dams were separated from their pups for 4 hours. DAGO [Tyr-D-Ala-Gly-(Me)Phe-gly-ol] and PLO-17 [Tyr-Pro-(Me)Phe-D-Pro-NH_2] were injected ICV at concentrations of 0.1, 0.5, and 2.5 ug. Serial blood samples were withdrawn immediately before and at selected time periods after opioid treatment.

Both DAGO and PLO-17 elicited dose-related increases in plasma prolactin in lactating females. Moreover, naloxone pretreatment (1.0 mg/kg, iv) completely blocked the effects of both peptides. These results indicate that the μ opioid receptor site is not down regulated during lactation. Thus, the insensitivity of the prolactin-releasing mechanism to morphine stimulation in the lactating female appears to be a phenomenon unique to narcotic analgesic drugs. (Supported by the Busch Memorial Fund and the Anne B. and James H. Leathem Scholarship Fund.)

18.15

FURTHER EVIDENCE FOR MU OPIATE RECEPTOR SUBTYPES: DIFFERENTIAL BLOCKADE BY NALOXONAZINE OF MORPHINE ANALGESIA AND INHIBITION OF GASTROINTESTINAL TRANSIT. D. Paul* and G.W. Pasternak. (SPON: D. Rottenberg). The Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Cntr and Cornell U. Medical College, NY, NY 10021.

Administered systemically, morphine elicits both analgesia and inhibits gastrointestinal (GI) transit through activation of mu opioid receptors. Work from our group has suggested the presence of two distinct subtypes of mu receptors and has correlated mu_1 receptors with supraspinal analgesia. We now report the relative contribution of mu_1 and mu_2 receptor subtypes to the inhibition of GI transit by systemic morphine by examining the effect of the selective mu_1 antagonist naloxonazine (NAZ) on morphine analgesia and inhibition of GI transit in mice. Blockade of mu_1 receptors by NAZ (35 mg/kg) completely antagonizes morphine analgesia at doses of either 2 mg/kg, or 6 mg/kg (s.c.). In contrast, NAZ treatment had no effect on the distance traveled by a charcoal meal through the small intestine. Both morphine analgesia and inhibition of GI transit are mediated through mu receptors, but only analgesia was antagonized by NAZ, supporting the concept of mu receptor subtypes. Based upon its sensitivity towards NAZ, morphine analgesia involves mu_1 receptors, whereas mu_2 sites mediate inhibition of transit following systemic morphine.

18.16

THE ANTAGONIST WIN 44,441-3 DOES NOT BLOCK THE β-ENDORPHIN OR DADLE INDUCED PROLACTIN INCREASE DURING LACTATION. J. Janik, M. Baumann and J. Rabii. Department of Biological Sciences, Rutgers Univ., Piscataway, N.J. 08855.

We have previously reported that lactating female rats are not sensitive to morphine or methadone stimulation of prolactin release. However, this model does show a prolactin secretory response to both β-endorphin and d-ala-d-leu-enkephalin (DADLE) administration which is reversed neither by naloxone nor naloxonazine, a specific antagonist of the μ-1 receptor site. The antagonist WIN 44,441-3 has been shown to effectively block the μ, δ and κ receptor sites (Wood et al., *Pharm. Res.*, 1:46, 1984). The purpose of this study was to attempt to determine the receptor subtype mediating the prolactin increase by examining the effects of this antagonist on the β-endorphin and DADLE induced increases in the lactating female model.

Lactating females between days 6 and 10 post-partum received β-endorphin (0.5, 2.5 or 5 μg, ivt) or DADLE (2.5 or 5 μg., ivt). All doses of β-endorphin and DADLE produced a significant increase in circulating levels of prolactin. However, the magnitude of the response seen in animals pretreated with WIN 44,441-3 was significantly attenuated compared to the saline pretreated controls. Thus, it appears that while the μ receptor may not be involved in the prolactin response to these opiates in the lactating female model, at least part of the response is occurring via the δ and/or κ receptor subtypes. (Supported by the Busch Memorial Fund and the Anne B. and James H. Leathem Scholarship Fund)

18.17

A NOVEL, HIGHLY POTENT AND SELECTIVE DERIVATIVE OF [D-PEN^2,D-PEN^5]ENKEPHALIN: OPIOID ACTIVITY IN VITRO AND IN VIVO. T.H. Kramer*, G. Toth*, V.J. Hruby*, and T.F. Burks. Depts. of Pharmacology and Chemistry, University of Arizona, Tucson, AZ 85724.

Cyclic [D-Pen^2,D-Pen^5]enkephalin (DPDPE) is a conformationally constrained enkephalin analog with demonstrated high affinity and potency at the delta opioid receptor. A newly synthesized derivative, cyclic [D-Pen^2,p-Cl-Phe^4,D-Pen^5]enkephalin (Cl-DPDPE) shows greater potency and selectivity than DPDPE in radioligand binding assays. Cl-DPDPE was tested in the guinea pig ileum (GPI) and mouse vas deferens (MVD) bioassays for in vitro opioid activity. Intracerebroventricular (ICV) injections of Cl-DPDPE were performed in mice, and analgesia was measured using the hotplate test. Cl-DPDPE demonstrated agonist activity in both the GPI (IC50=4.4±0.6 uM) and MVD (IC50=1.9±0.3 nM). Delta receptor selectivity (IC50 GPI/IC50 MVD) of 2304 (±273) indicates improved selectivity in vitro relative to DPDPE. Cl-DPDPE was antagonized by the selective delta antagonist ICI 174,864 in MVD, and the mu antagonist CTP in GPI. ICV Cl-DPDPE caused dose-dependent analgesia in the hotplate test (ED50=1.09 ug±2.1), and was antagonized by naloxone. Cl-DPDPE is a highly delta-selective opioid agonist in vitro, and potently produces opioid analgesia in vivo. We confirm that opioid analgesia is mediated by delta receptors at the supraspinal level (supported by USPHS grants NS19972, DA02163, and DK36289).

18.18

REGULATION OF CYCLIC AMP-DEPENDENT PROTEIN PHOSPHORYLATION BY ACUTE AND CHRONIC MORPHINE TREATMENT IN RAT BRAIN. X. Guitart* and E.J. Nestler (SPON: S. Stine). Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Previous studies have shown that chronic treatment of rats with morphine increases the levels of specific G-proteins, adenylate cyclase, and cyclic AMP-dependent protein kinase in the locus coeruleus (LC), but not in several other brain regions examined. In the present investigation, we have identified a number of phosphoproteins termed "MARPPs" (Morphine and cyclic AMP-Regulated Phospho-Proteins) as additional targets of chronic morphine action.

Rats were treated with morphine for 5 d and used on day 6. Isolated brain regions were then analyzed by endogenous and back phosphorylation assays and by one- and two-dimensional electrophoresis and autoradiography. Among the MARPPs identified were: 1) **MARPP-58**, a 58 kD protein, whose phosphorylation level and total amount were increased by chronic morphine treatment in the LC, but not in other brain regions studied; this protein has been identified as tyrosine hydroxylase. 2) **MARPPs-51, 55, 62, and 165**, proteins whose phosphorylation levels were regulated by chronic morphine in the LC only, despite their presence in other brain regions; their phosphorylation level was also regulated by acute forskolin and morphine in the LC. 3) **MARPPs-14-20** and **145**, proteins whose phosphorylation levels were regulated by chronic morphine in all brain regions studied, as well as by acute forskolin and morphine in the LC; MARPPs-14-20 have been identified as myelin basic proteins.

It is possible that these various phosphoproteins mediate some of the acute and chronic actions of morphine on the nervous system, including the development of tolerance and dependence.

18.19

ACUTE TOXICITY OF METHADONE, MEASURED AS HYPOXIA AND HYPERCAPNIA, IN PREGNANT RATS. R. C. Pleus and S. B. Sparber Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

Two groups of pregnant SD rats were transcutaneously (tc) monitored for CO2 and O2 tension to determine the degree of hypoxia and hypercapnia after treatment with 5 mg methadone/kg, i.p. (M). The acute M group (MA) received saline daily and the chronic M group (MC) received M daily until tc monitoring. Daily treatments for both groups began on gestation day (GD) four. Respiratory depression, leading to significant hypoxia/hypercapnia, is caused by injection of M in early (GD 4 or 5), mid (GD 11 or 12), and late (GD 17-20) pregnancy. Tolerance to this effect is evident 7-14 days after treatment is initiated (Table 1.), although stable (*i.e.* 24 hr) dependence may not yet be achieved (Sparber, *Neuro Toxicol.*, 7:335-348, 1986).

TABLE 1.

		tcpO2		tcpCO2	
GD	Time[2]	MA	MC	MA	MC
EARLY	30	-24.9 ± 7.6		18.28 ± 3.5	
	60	-21.1 ± 5.8		25.6 ± 4.0	
	180	-1.7 ± 6.9		10.6 ± 2.3	
MID	30	-21.9 ± 4.3*	-2.2 ± 2.9	25.0 ± 5.2*	4.7 ± 2.0
	60	-22.8 ± 0.6*	0.1 ± 0.4	25.5 ± 1.4*	1.7 ± 2.6
	180	-7.4 ± 2.8*	7.2 ± 1.0	19.3 ± 7.9	-2.4 ± 1.2
LATE	30	-26.0 ± 2.7*	-0.6 ± 2.6	31.9 ± 3.6*	4.0 ± 2.2
	60	-24.8 ± 2.6*	-1.5 ± 3.8	31.1 ± 6.9*	4.9 ± 2.1
	180	-11.8 ± 4.2*	6.2 ± 6.8	20.9 ± 4.3*	13.3 ± 1.9

1. tc tensions (pre - post injection) expressed as mean ± SE (mmHg). 2. Minutes post injection. * p< .01 between MA and MC using Duncans t test

Hypoxia/hypercapnia may be valid measures of acute toxicity in rats and may be also responsible for some effects in offspring after treating pregnant rats with M before tolerance is attained. Supported in part by USPHS grant DA 01880.

RETINA I

19.1

TRANSDUCTION IN LOCUST PHOTORECEPTORS: FLASH AND WHITE-NOISE STIMULATION. **A.E.C.Pece, A.S.French, M.J.Korenberg*, J.E.Kuster*. Department of Physiology, University of Alberta, Edmonton, Canada T6G 2H7.**

The response of photoreceptors to light is believed to be mediated by a biochemical cascade between rhodopsin excitation and channel opening. Simple cascade models predict a linear relationship between light intensity and depolarization with small stimuli. However, some photoreceptors are nonlinear even when only a few photons are transduced. In locust receptors, nonlinearities have been demonstrated by measuring interactions between flash responses as a function of flash separation, and by comparing the linear transfer functions of the process at different background levels. We have now measured the linear (first order) and second order components of photoreceptor responses by conventional intracellular recordings, while stimulating with a light source modulated by white noise. Kernels were measured at different noise amplitudes. These results are compared with nonlinear effects demonstrated previously, to obtain a more complete characterization of the input-output relationship of these photoreceptors. The nonlinearities demonstrated experimentally will be compared with simulations of some simple nonlinear cascade models of phototransduction.

Supported by Canadian Medical Research Council and Alberta Heritage Foundation for Biomedical Research.

19.2

OUTER SEGMENT CABLE PROPERTIES SET UPPER LIMIT TO cGMP-INDUCED CLAMP CURRENT OF SALAMANDER RODS. D.A. Cameron* & E.N. Pugh* (SPON: L. M. Hurvich) Inst. of Neurological Sciences & Dept. of Psychology, U. of Pennsylvania, Philadelphia, Pa. 19104.

Rods with o.s. in a suction electrode (s.e.) were infused with cGMP from a gigaseal pipette in voltage-clamp mode, and the hv-sensitive current, J, measured. (1) (dJ/dt)max but not Jmax depended on pipette [cGMP] in range 10-20 mM. Means ± 2sem for Jmax & (dJ/dt)max: [cGMP]=10 mM, 1150 ± 160 pA & 350 ± 49 pA/sec (n=12); [cGMP]=20 mM, 1300 ± 160 pA & 556 ± 72 pA/sec (n=29). These data make it unlikely that either Na gradient collapse or [cGMP] distribution in o.s. limited Jmax. (2) When the o.s. was completely drawn into the s.e., the average ratio of s.e. to clamp current was 0.75; for o.s. partially drawn in, this ratio decreased steeply as a function of o.s. length excluded from the s.e. (n=18, [cGMP]=20 mM). (3) Transient analysis showed that whole-cell pipette access resistance did not limit measured Jmax. From analysis of our results with a cable model, we conclude (1) that a longitudinal resistance of 1-3 megohm/um in the o.s. limits Jmax measured by point-clamp, setting a "space constant" of 6-8 um and (2) that a perfectly spaced-clamped rod, with all cGMP-gated conductances instantly gated open, would have a maximum cGMP-induced current of 7-10 nA.

Supported by NIH grant EY-02660.

19.3

LIGHT ADAPTATION DYNAMICS OF THE PHOTORECEPTOR CELLS REVEALED WITH A STIMULATION FEEDBACK CONTROL SYSTEM. K.Djupsund, E.Kouvalainen*, M.Weckström*, M.Kallio* and M.Järvilehto. Dept of Physiology and Dept of Zoology, Univ of Oulu, 90100 Oulu, Finland.

In contrast to conventional open-loop stimulation and recording, a continuous stimulus-response feedback system offers the advantage of monitoring directly the cell sensitivity at a constant or changing level of the response voltage, i.e. the operating point.

The intracellularly recorded voltage response of the photoreceptors of the blowfly (Calliphora erythrocephala) was controlled by changing the light intensity of a LED (green, 555 nm), in order to force the response to an intended time-course. This was performed by comparing the sampled cell response point by point with a reference function. The LED output was calculated with a PID algorhithm by a µPDP-11. The control process ran at 166 Hz.

The changes in sensitivity and the adaptational process, as indicated by the respective changes of the LED intensity, show faster onset for larger amplitude maxima of forced responses. These changes are furthermore accentuated, when the derivative of the forced response step increases. The sensitivity also shows several changes of slope during the ongoing control process. The on- and offset of screening pigment-linked processes temporally fit the findings, but also gain-control mechanisms inherent to the transduction process can be involved.

19.4

INTERACTION BETWEEN CHEMICAL EXCITATION AND PHOTOTRANSDUCTION MUTATIONS IN FLY PHOTORECEPTORS. B. Minke*, C.T. Rubinstein*, S. Barash.*, E. Suss* and Z. Selinger*[+] (Spon: S. Rotshenker). Dept. of Physiology and Biol. Chem.[+] The Hebrew University, Jerusalem, Israel 91010.

Chemicals which affect different steps of the phototransduction cascade were used to localize the site of action of the defective gene products of three mutants; the transient receptor potentials (trp) and retinal degeneration B (rdgB) mutants of Drosophila and the no steady state (nss) mutant of Lucilia. The trp and nss mutants fail to maintain excitation during continuous illumination and become temporarily non-responsive following intense light. In the rdgB mutant the peripheral photoreceptors in each ommatidium degenerate only after exposure to light for several days (24°C). We have found that GTP analogs and F^-, which are known to activate G-proteins and to excite fly photoreceptors, mimicked the effects of light in causing retinal degeneration when applied daily (for 8 days; 24°C) to the eyes in the dark. The metabolic inhibitors CN^- and 2-deoxy-D-glucose did not cause more degeneration than Ringer's solution, while $GDP_\beta S$ reduced the degeneration below the control dark level. GTP analogs, F^- and a combination of inositol trisphosphate + diphosphoglycerate ($InsP_3$+DPG) were applied to the trp and nss photoreceptors. Intracellular recordings and shot-noise analysis revealed that F^- neither excited nor adapted the trp and nss photoreceptors, while $GTP_\gamma S$ markedly reduced the light-response of the mutants without exciting the photoreceptors. $InsP_3$+DPG, in contrast, mimicked the effect of background light on the response of the nss. The results suggest that the defect in trp and nss is at a stage which follows the production of $InsP_3$ and that GTP analogs and F^- activate both G-protein and an unknown step subsequent to $InsP_3$ production. The G-protein operates prior to the rdgB gene-product in the phototransduction cascade.

Supported by NIH grant EY-03529.

19.5

OCTOPAMINE SUPPRESSES INHIBITION IN THE *LIMULUS* LATERAL EYE

George Renninger and Claudia Farrell, Biophysics Group, University of Guelph, Ontario

Octopamine, a putative circadian neurotransmitter in *Limulus*, and other exogenous agents applied to the lateral eye *in vitro* increase the rate at which the eccentric cell (the second-order neuron of each photoreceptor unit) generates impulses at a given level of membrane depolarization during exposure to light [Renninger *et al.* (1988) J Comp Physiol]. The modulation of impulse generation is consistent with a decrease in self-inhibition by the exogenous agents. Activity in efferent nerve fibers from the circadian clock, however, has no significant effect on the impulse generator.

We have now observed directly that self-inhibition is suppressed by octopamine, but not by efferent activity. Positive current steps injected into a eccentric cell *in vitro* in the control condition produced impulses at a steady rate which was about 3 times lower than the initial firing rate. The addition of octopamine (500 μM) suppressed self-inhibition: the same current steps produced a final steady rate which was only about 1.5 times lower than the initial rate. Calculations show that this suppression is consistent with a five-fold decrease in the conductance change underlying self-inhibition. *In vivo*, however, similar current steps produced the same fall in firing rate both before and during natural efferent activity.

Octopamine also suppresses lateral inhibition *in vitro*. Lateral inhibition depressed eccentric firing rates in the control condition. This reduction in firing rate was no longer observed after the addition of octopamine. Furthermore, hyperpolarization of the eccentric cell membrane due to lateral inhibitory interactions was significantly reduced by an octopamine agonist. These results support the notion that the circadian clock reduces lateral inhibition in the compound eye at night [Renninger & Barlow (1979) Soc Neurosci Absts 5:804; Batra & Barlow (1983) Soc Neurosci Absts 8:49].

Suppression of self-inhibition by octopamine agonists, but not by efferent nerve activity, suggests that exogenous agonists can reach receptors on the eccentric cell membrane which are not accessible to the neurotransmitter released from efferent nerve terminals.

19.6

ULTRASTRUCTURE OF RETINAL SYNAPSES IN CULTURE. E.L. Gleason* and M. Wilson. Dept. of Zoology, Univ. of Calif., Davis, CA 95616.

In the simplified conditions of low density culture, synapses between retinal neurons might be more amenable to physiological analysis than is the case in the intact retina. With this in mind, we have followed the time course of synapse formation in culture.

Eight day chick embryo retinal neurons were put into culture (medium consisting of DMEM, 5% FCS and 100units/ml pen-strep), fixed on embryonic equivalent days 12, 14, 16 and 18, then processed for TEM. By day 12, the cells have extended processes and contacted distant cells. These contacts are often characterized by densities at each of the opposing membranes. Vesicles can also be found in processes but are usually not localized to sites of contact. Short synaptic ribbons are seen at the base of some cone cells. Day 14 cultures show a marked increase in the number of vesicles and these vesicles are now localized to sites of contact. After 16 days in culture, conventional synapses are mature. By day 18, synaptic ribbons similar to those found in the innerplexiform layer are observed.

The time course of synaptic development for these dispersed retinal neurons will be compared with that of retinal cells grown in aggregates and neurons in the intact retina.

19.7

PHYSIOLOGICAL AND MORPHOLOGICAL CORRELATIONS OF MUDPUPPY HORIZONTAL CELLS. H.G. Kim* and R.F. Miller (SPON: T.J. Cicero). Dept. of Ophthalmology, Washington U. St. Louis, Mo 63110

The morphology of mudpuppy horizontal cells is unknown even though numerous physiological studies of these cells have been reported, including one observation of both "luminosity" (L-type) and "chromaticity" (C-type) horizontal cell subtypes. We stained physiologically identified horizontal cells of the mudpuppy using intracellular electrodes filled with horseradish peroxidase (HRP: 10%) and/or Lucifer Yellow (LY: 6%) to determine structure/function relationships of these cells. We used cone-matched monochromatic light stimuli to evaluate rod/cone contributions and small slits of light to determine receptive field size.

We found that most mudpuppy horizontal cells receive inputs from rods and cones; staining results show that these cells have one or more long axons, exhibiting diverse morphologies. Receptive fields sizes, determined by displaced slits, varied from 200 μm to 650 μm and could not be correlated with morphological subtype. We found C-type horizontal cells with different morphologies although these cells were rarely encountered. One type responded better to shorter wavelength stimulation, and did not have an axon. Another C-type horizontal cell responded better to longer wavelength stimulation and displayed an axonless morphology different from that of the short wavelength preferring cell. The presence of C-type horizontal cells confirms an earlier observation by Fain and suggests that complex synaptic mechanisms, which underlie C-type responses in teleosts and turtles, may also be present in the mudpuppy, although the receptor basis of this interaction is unknown. Our study extends to the mudpuppy, the concept that the vertebrate retina contains axon-bearing and axonless horizontal cell subtypes.

This work is supported by NEI grant EY03014 and T32 NS07057.

19.8

HORIZONTAL CELLS FROM DARK-ADAPTED GOLDFISH RETINAS RESPOND TO ANNULAR ILLUMINATION AND SHOW INCREASED GAP JUNCTION PARTICLE DENSITIES. W.H. Baldridge*, A.K. Ball*, R. Miller Dept. Anatomy, McMaster University, Hamilton, Ont., L8N 3Z5

The density of goldfish retinal horizontal cell (HC) gap junction (GJ) particles increases with presumed coupling (J. Comp. Neurol. 265:428, 1987). To test the correlation of presumed coupling conditions with HC GJ particle density, we have made intracellular recordings from dark-adapted goldfish HCs and measured the responses to spot and annular illumination to determine coupling state. The retinas were then processed for freeze-fracture electron microscopy and the particle density of HC somas GJs measured. Goldfish were dark-adapted for at least 2 hrs. and intracellular recording of S-potentials made from isolated retinas maintained in a chamber superfused with fish Ringers. HCs showed increasing responses (10-25 mV) to spots ranging from 350 um to 2,750 um. Potentials (20-25 mV) were also recorded in response to annular illumination (3,750 um illuminated surround with a 2,500 um center and 5,250 um illuminated surround with a 2,500 um center). Particle densities of HC soma GJs from these retinas were about 5,000 particles per square micron. The responsiveness of HCs to surround illumination demonstrates that these cells were coupled. The corresponding high HC GJ particle density was nearly identical to previous results where coupling was presumed. We are currently testing the effect of dopamine on the responses to annular illumination and HC soma GJ particle density.

19.9

RECEPTIVE FIELD PROPERTIES OF HORIZONTAL CELLS CORRELATE WITH THE CONNEXON DENSITY WITHIN THEIR GAP JUNCTIONS

R. Weiler, K. Poser*, W. Kolbinger*, G. Kurz-Isler*, W.H. Baldridge*. Dept. Neurobiol. Univ. Oldenburg, 2900 Oldenburg, FRG.

In the fish and turtle retina, dopamine (DA) alters the gap junction coupling resitance of horizontal cells (HC) and consequently their receptive field (RF) properties. DA also influences the connexon density (CD) within the gap junctions and we therefore examined a possible correlation of CD with RF properties. The RF size of HC was measured by intracellular recordings in: a) dark- and b) light-adapted retina; c) DA-incubated and d) DA-depleted retina. DA content was subsequently measured and the CD value was determined in freeze fracture replicas. The largest RF were recorded in DA-depleted retinas where the CD value was significantly higher than in dark-adapted retinas having smaller RF. Light adaptation and incubation in DA resulted in an increasing decline of the overall RF size. These RF properties correlated significantly with a decrease of the CD value. The observed correlation between the RF properties of HC and the CD opens the possibility that DA does not only act through a phosphorylation of a gap junction channel protein. Supported by DFG and NATO.

19.10

SALAMANDER HORIZONTAL CELL RESPONSES IN BICARBONATE- AND IN HEPES-BUFFERED MEDIUM

Jochen Kleinschmidt, Dept. of Ophthalmology, New York University Medical Center, New York, NY.

The dominant extra- and intracellular pH-buffering system in the retina in vivo is likely to be HCO_3/CO_2. Much of previous work on photoreceptor physiology and synaptic transmission in the amphibian retina has been done in HCO_3-free, HEPES-buffered medium. The present study demonstrates dramatic differences in horizontal cell responses recorded in the eyecup superfused with these two media. Changing from HCO_3/CO_2 to HEPES under photopic stimulus conditions (a) depolarizes H cells, (b) increases their response amplitudes, (c) greatly reduces their depolarizing rollback, (d) dramatically alters waveforms of incremental responses during bright background adaptation. Under mesopic and scotopic conditions, rod-to-H cell transmission is greatly enhanced. These changes are completely reversible. Experiments using chloride substitution and the chloride transport inhibitor SITS indicate that chloride plays an important role in shaping H cell responses. These findings suggest that HCO_3 is a physiologically important anion which is involved in chloride regulation in photoreceptors and/or H cells, presumably via Cl/HCO_3 exchange.

Supported by NIH grant EY05213.

19.11

DEVELOPMENT OF NPY REACTIVE NEURONS IN RAT RETINA. D.M. Ferriero and S.M. Sagar. Dept. of Neurology, Univ. of Calif., San Francisco, CA 94143

Neuropeptide Y (NPY) is present in very high concentrations in brain and is colocalized with somatostatin (SLI) in a subpopulation of neurons in rat striatum. SLI has been well documented in retina of many species. Although NPY has been implicated in visual function because of its localization in the suprachiasmatic nucleus, it has been reported only in guinea pig retina. In developing rat retina, SLI concentration is very high prenatally and falls dramatically prior to synapse formation. There is a gradual rise postnatally to adult levels. We have now studied the ontogeny of NPY in rat retina by radioimmunoassay (RIA) and gel permeation chromatography (GPC) from embryonic day (E) 15 to adult. NPY concentration and content gradually increases over pre- and postnatal development with a small surge at eye opening. The different patterns of ontogeny for NPY and SLI in developing retina suggest that phenotypic expression of neuronal subtypes changes over development, and that peptides may have prenatal functions other than "transmission".

19.12

L-GLUTMATIC ACID DECARBOXYLASE mRNA IN MAMMALIAN RETINA: LOCALIZATION BY IN SITU HYBRIDIZATION. P.V. Sarthy and M. Fu*. Department of Ophthalmology, University of Washington School of Medicine, Seattle, WA 98195.

GABAergic neurons in mammalian retinas have been previously localized by ^{3}H-GABA or ^{3}H-muscimol uptake autoradiography, or by immunocytochemistry using L-glutamate decarboxylase (GAD) or GABA-antisera. In this study, we have used a GAD cDNA probe to localize GAD mRNA by in situ hybridization. ^{35}S-labeled DNA and RNA probes were hybridized to cryostat sections of paraformaldehyde-fixed retinas, and localization was carried out using established protocols. In the cat retina, labeled cell bodies were found in the INL and GCL. The majority of labeled cells (~72%) were located in the vitread side of the INL bordering the INL/IPL boundary. The second class of cells (~20%) in the INL had larger somata and were found at the INL/OPL boundary. Double labeling experiments with antisera to parvalbumin, a horizontal cell marker, showed that these perikarya belong to horizontal cells. In the monkey retina, labeled cell bodies were found only in the INL (~83%) and the GCL. In the mouse retina, labeled somata were again observed in the INL and the GCL. No evidence for horizontal cell labeling was, however, found in these retinas. Northern blot analysis showed that these retinas contain a single species of GAD mRNA that is ~4 Kb in size. These results are in general agreement with immunocytochemical data on the localization of GABAergic neurons in the inner retina. Supported by NIH Grants EY03664 and EY01730.

19.13

CORTICOTROPIN RELEASING HORMONE IN FROG RETINA - QUANTITATIVE AND DETAILED ANATOMICAL ANALYSIS. T. Z. Baram*, M. C. Citron and L. Schultz*. Dept.of Neurology Research, U. Southern California and Childrens Hospital of L A., Los Angeles, CA 90027.

Corticotropin Releasing-Hormone (CRH) has recently been demonstrated in avian and rat retina. This neuropeptide forms an important link in the organism's stress response; it functions as both a hypothalamic neurohormone and as a neuromodulator in other central nervous system (CNS) regions. Its role in the retina has not been defined.

We have detected CRH in frog retina. CRH-like immunoreactivity was present in retinal homogenates subjected to radioimmunoassay utilizing antiserum to rat/human CRH. (35.5± 2.4 pg/retina). Furthermore, immunofluorescence, enhanced by an avidin-biotin complex and visualized with phycoerythrin (Vector, CA), has allowed the visualization of CRH-containing retinal cells. Specificity was ascertained by the use of normal serum instead of antibody, and by the elimination of fluorescence after pre-adsorption of the CRH-antiserum with an excess of the peptide.

Study of the role of CRH in frog retina may provide information about the mechanisms of neuropeptide actions in the CNS, for which the retina is a well defined, relatively simple model.

19.14

LOCALIZATION OF cAMP IN THE TELEOST RETINA FOLLOWING EXPOSURE TO DOPAMINE (DA). L.H.Y. Young and J.E. Dowling. M.E.E.I. and The Biological Labs., Harvard, Boston/Cambridge, MA.

Many studies have shown that the lateral inhibitory effects mediated by the horizontal cells (HCs) in the outer plexiform layer are modulated by the interplexiform cells and DA. In 1981, using both homogenates of carp retina and enriched HC fractions, Dowling and coworkers provided evidence that DA receptors on the HCs are linked to adenylate cyclase. The present study provides information on the localization of cAMP in the white perch retina by immunohistochemical and autoradiographic methods. Using a goat anti-cAMP antiserum with an immunofluorescent staining technique, retinas incubated in DA showed a marked increase in staining in the distal inner nuclear layer where HCs are located. Autoradiography of whole retinas incubated in 20 μM tritiated ATP showed minimal radioactivity distributed homogeneously throughout the retinal layers. However, in the presence of DA, there was enhanced radioactivity in the HC layer. Tissue culture HCs also showed increased labeling following exposure to DA. These experiments provide further evidence that DA receptors on the HCs are linked to adenylate cyclase and that their activation results in the accumulation of cAMP.

19.15

IMMUNOHISTOCHEMICAL LOCALIZATION OF GABA$_A$ RECEPTORS IN THE RETINA OF THE PRIMATE *Saimiri sciureus*. [1]T.E. Hughes, [2]R.G. Carey, [3]J. Vitorica*, [3]A.L. de Blas, and [1]H.J. Karten. [1]Dept. of Neurosci., UCSD, La Jolla, CA 92093, [2]Div. of Neurobiol., St. Joseph Hosp. and Med. Centr., Barrow Neurol. Inst., Phoenix AZ 85013, [3]Dept. of Neurobiol. & Behav., SUNY Stony Brook, NY 11794.

Some amacrine cells in the retina are thought to use gamma-aminobutyric acid (GABA) as an inhibitory neurotransmitter, yet little is known about which neurons express the receptors for GABA. Monoclonal antibodies against the GABA$_A$ receptor have provided a means to identify some of these neurons (Mariani et al., Brain. Res., 415:152, 1987; Richards et al., J. Neurosci., 7:1866, 1987). In the squirrel monkey, such an antibody labeled somata in the inner nuclear (INL) and ganglion cell (GCL) layers. The staining was confined to the cell's plasmalemma and cytoplasmic granules. The labeled neurons in the INL had small somata at the vitreal edge of the layer. Most arborized only in the inner plexiform layer, but a few also had labeled processes that extended into the inner half of the outer plexiform layer. The labeled cells in the GCL were among the largest present, and their arbors were, at any given eccentricity, similar in appearance. Many were ganglion cells which were retrogradely labeled following injections of Fluro-Gold into the optic tract. The antibody labeled their axons, suggesting that GABA$_A$ receptors move anterogradely to the retinal terminal fields. Sections labeled for both L-glutamic acid decarboxylase (GAD), or GABA, and the receptor revealed that GAD/GABA was distributed much more widely in the IPL than was the receptor. This variance may reflect either the presence of other GABA receptors in the IPL or GABA in portions of the IPL where it is ineffective as a neurotransmitter. Supported by NEI EY06004 to TEH, BRSG RR05872 to RGC, NIH NS17708 to J.V & A.L.d., EY06890 to HJK.

19.16

IMMUNOLOGICAL AND ELECTROPHYSIOLOGICAL IDENTIFICATION OF SUBSETS OF RETINAL GANGLION CELLS. B.A.Barres, D.P.Corey, and L.L.Y.Chun*, Program in Neuroscience, Harvard Medical School, Howard Hughes Medical Institute, and Dept. of Neurology, Mass. General Hospital.

In cell suspensions from postnatal day 8 rat retinas labelled retrogradely with granular blue injections in the superior collicular brachium, nearly 100% of retinal ganglion cells (RGCs) were labelled (Perry, 1983). We observed that 100% of RGCs could also be labelled by the Thy-1 antibodies 2G12 and MRC-OX-7, as previously reported (Barnstable and Drager, 1984). However, we observed an approximately equal number of Thy-1-labelled cells that were *not* retrogradely labelled. These cells are probably not RGCs because they did not express voltage-dependent sodium or calcium currents while 100% of retrogradely labelled cells did.

We observed that one Thy-1.1-specific antibody, T11D7 (Lake and Clark, 1979) stained only 1/3 of retrogradely labelled RGCs. Size histograms revealed two peaks: 2/3 of cells were 12 um in diameter and T11D7-negative, while 1/3 of cells averaged 13.5 um and were T11D7-positive. Further evidence that these cells represent a genuine subset of RGCs was obtained by whole-cell patch recordings. Acutely dissociated RGCs were electrophysiologically heterogeneous. For all cells the maximal sustained firing rate was limited by a spike adaptation mechanism, but among cells we observed a continuum of spike adaptation. The T11D7-positive cells fired at the highest sustained frequencies and spike adapted only with large currents, half of T11D7-negative cells fired at a slower rate, and the other half spike adapted at all currents injected. The mechanism of spike adaptation in these cells cannot be accounted for by the recruitment of an "M" current (Adams *et al.*, 1982) or a K_{Ca} current (Madison and Nicoll, 1984).

These RGC subsets may have developmental significance; preliminary experiments suggest that T11D7 labelling fails to correlate with cell size in the adult.

19.17

IMMUNOCYTOCHEMICAL LOCALIZATION OF GLYCINE IN THE TURTLE RETINA. W. D. Eldred and K. Cheung*. Department of Biology, Boston University, Boston, MA 02215.

Previous workers have localized ^{3}H-glycine uptake within amacrine cells in the turtle retina. We have examined glycine-like immunoreactivity (GLY-IR) to provide more detail on its localization in the turtle retina. We used a rabbit antiserum directed against glycine (gift of R. Wenthold) and employed standard fluorescent and avidin-biotin labeling techniques. There were isolated immunoreactive somata in the ganglion cell layer (GCL) and numerous immunoreactive somata in the inner nuclear layer (INL), including several types of amacrine cells. One amacrine cell type had somata adjacent to the inner plexiform layer (IPL) which gave rise to processes which arborized primarily at the border of strata 1 and 2 and in stratum 3 within the IPL. A second type of labeled amacrine cell had smaller somata in the central INL, which gave rise to processes which arborized more diffusely within the IPL. A third cell type resembled interplexiform cells in that it gave rise to extensive arborizations in the outer plexiform layer and the IPL. In the GCL some of the immunoreactive cells were apparently true ganglion cells because they gave rise to labeled axons. The present localization of GLY-IR within several amacrine cell types, possible interplexiform cells and ganglion cells, indicates that glycine may be involved in many diverse synaptic interactions in both the IPL and OPL of the turtle retina. This research supported by EY04785 to WDE.

19.18

SYNAPTIC ORGANIZATION OF ENKEPHALIN-LIKE IMMUNOREACTIVE AMACRINE CELLS IN THE GOLDFISH RETINA. Carl B. Watt. Alice R. McPherson Laboratory of Retina Research , Center for Biotechnology, Baylor College of Medicine, The Woodlands, Texas 77381.

A total of 194 synaptic relationships were observed that involved the immunostained processes of enkephalin (enk)-like immunoreactive amacrine cells in the goldfish retina. Corresponding to light microscopic studies, the large majority of these enk-processes were observed in sublayer 5 of the inner plexiform layer. In greater than 95% of the synaptic relationships, the enk immunostained profile served as the presynaptic element. Fifty-nine percent of the time, enk profiles synapsed onto amacrine cell processes, while 30% of their synapses were onto profiles that lacked synaptic vesicles. They also occasionally formed synaptic contacts onto the somas of cells located along the outer border of the inner plexiform layer and in the ganglion cell layer. Enk profiles received synaptic input only from amacrine cells (4% of the time), while no direct synaptic interaction was observed between enk profiles and bipolar cells. However, enk processes were found to synapse onto amacrine cell profiles that in turn formed synaptic contacts onto large bipolar endings in sublamina b of the inner plexiform layer.

This work was supported by NIH grant EY05622 and by the Retina Research Foundation (Houston).

19.19

ANTISERUM TO ASPARTATE AMINOTRANSFERASE LABELS RETINAL GABA-ERGIC NEURONS. C. Brandon. Dept. Cell Biology & Anatomy, Chicago Medical School, North Chicago, IL

Aspartate aminotransferase (AAT) may be a marker for aspartate/glutamate neurons, but may also be present in some GABAergic neurons (Donoghue, J. Neurosci. 5, 2597 (1985)). AAT has been identified in retinas from rat, guinea pig, and primate (Brandon, PNAS 80, 5117 (1983); Mosinger, JCN 233, 255 (1985)). We have used immunocytochemistry to compare AAT-IR and GABAergic neuronal populations in the ferret retina.

In the ferret, cones were AAT-IR while rods were not labeled. Some horizontal cells were strongly AAT-IR; these may be cone horizontal cells. A small number of regularly-spaced bipolar cells were AAT-IR; these may be cone bipolar cells. Several types of amacrine cell bodies were AAT-IR; their processes branched in three sublaminae of the IPL, at depths of 17%, 36%, and 61%. Most neurons in the ganglion cell layer were AAT-IR.

GABAergic neurons were identified with an antiserum to rabbit brain GAD. Amacrine cells were the only cells that were GAD-IR. Their labeled processes branched in exactly the same three IPL sublaminae that contained AAT-IR processes.

We conclude that, in the ferret retina: 1) glutamate or aspartate may be cone transmitters; 2) some horizontal cells may use glutamate as a transmitter; and 3) antiserum to AAT stains GABAergic amacrine cells. The simultaneous labeling of AAT-IR cone bipolar cells and GABAergic amacrine cells may facilitate the study of the synaptic interactions between these two neuronal populations. Supported by USPHS.

19.20

TRANSIENT EXPRESSION OF CRF-LIKE IMMUNOREACTIVITY BY HORIZONTAL CELLS IN THE DEVELOPING RAT RETINA. L. Trojanczyk*, D. Zhang and H.H. Yeh (SPON: M.L. Blair). Dept. Neurobiology and Anatomy, Univ. Rochester School of Medicine, Rochester, NY 14642.

In the developing rat and mouse retina, horizontal cells have been reported to express transiently GAD-like immunoreactivity. Here, we report a similar phenomenon, one which involves the transient expression of a peptidergic phenotype and one which was uncovered in the course of studying the emergence and maturation of corticotropin releasing factor like immunoreactivity (CRF-LI) in the rat retina.

Examination of 4-um epon-embedded transverse sections revealed that, between postnatal days(PD)-4 and -15, faint CRF-LI staining could be observed in cells that were aligned at regularly-spaced intervals along the developing inner nuclear layer-outer plexiform layer junction. In tangential sections through this stratum, they exhibited large, round somata and appeared to be evenly distributed in a regular mosaic pattern. These features are typical of horizontal cells in their location and morphology. Tangential sections further revealed a central-to-peripheral gradient, being more prevalent in the central retina on PD-4 and then in the periphery by PD-10. By eye-opening (PD-15), CRF-LI in horizontal cells were no longer detectable. Birth dating using [^{3}H]thymidine indicated that these cells were generated between embryonic day 14 and 18, slightly earlier than those CRF-LI cells that are destined to become amacrine or displaced amacrine cells.

Although the mammalian horizontal cell transmitter(s) remains to be established, our finding is consistent with the notion of a switch in transmitter phenotype by developing, pluripotential horizontal cells.

Supported by PHS grant NS24830 and the Rochester Eye Bank.

19.21

HISTOGENESIS OF CRF-LIKE IMMUNOREACTIVE NEURONS IN THE RAT RETINA. D. Zhang[1], M. Gallagher*[1,2], C.D. Sladek[1,2] and H.H. Yeh[1], Depts. of Neurobiology and Anatomy[1] and of Neurology[2], Univ. Rochester School of Medicine, Rochester, NY 14642.

Work from our laboratory has demonstrated a system of corticotropin releasing factor-like immunoreactive (CRF-LI) amacrine and displaced amacrine cells in the rat retina. During development, CRF-LI can be detected by postnatal day(PD)-3 and assumes adult-like pattern of distribution by PD-19. Here, we examined the histogenesis of CRF-LI retinal neurons using a double-label approach combining [^{3}H]thymidine autoradiography and immunohistochemistry and also measured CRF-LI levels throughout retinal development by radioimmunoassay (RIA).

The bulk of CRF-LI neurons appear to cease mitotic activity prenatally between embryonic day(ED)-16 and 20. Within this population, approximately 20% are "born" on ED-18. No specific accumulation of autoradiographic label has been detected following exposure to [^{3}H]thymidine beyond the day of birth (PD-0). Preliminary results of RIA indicate that CRF-LI is present on PD-3 at threshold levels (5-10 pg/retina) of our detection system, increases dramatically between PD-7 and -9, reaches peak levels between PD-13 and -15 (eye-opening) and, thereafter, declines gradually to reach adult levels.

Overall, CRF-LI amacrine and displaced amacrine neurons are generated 5-7 days prior to their detection by immunohistochemistry. In addition, consistent with the developmental profiles of CRF-LI cell number and distribution, our RIA data lead us to postulate that increased synthetic activity rather increased number of CRF-LI cells may account for the transient surge in CRF-LI around eye-opening.

Supported by PHS grants DK19761 (CDS) and NS24830 (HHY).

20.1

DEVELOPMENT OF GLIAL CELL LAMINATION IN THE FERRET LATERAL GENICULATE NUCLEUS (LGN). Vivien A. Casagrande and James B. Hutchins, Dept. of Cell Biology, Vanderbilt Univ. Sch. Med., Nashville TN 37232.

We have shown that glial cell lamination precedes neuronal cell lamination in the tree shrew LGN. We now present evidence that this result is, if not ubiquitous, at least present in both tree shrews and ferrets. Ultrastructural immunohistochemistry confirms that antibodies to glial fibrillary acidic protein (GFAP) label glial cell intermediate filaments early in development. GFAP+ processes are found in bands in the tree shrew at birth, and in ferrets at about three weeks after birth. In both cases, banding of glial cells is only evident several days before the first clear interlaminar spaces form in the nucleus. No banding and only radial glia are seen during the early postnatal period of retinal afferent segregation. Glial cell banding is also absent in adults of both species. However, while few GFAP+ perikarya are present in the adult tree shrew LGN, a significant number of GFAP+ cell bodies and their processes persist in the adult ferret LGN. Differences also exist in the pattern of GFAP and vimentin immunoreactivity in the developing LGN of these two species, but the arrangement of fine, filamentous glial cell processes appears to consistently precede neuronal cell lamination. These results suggest that glial cells may acts as guides during the process of LGN cell layer development.
Supported by NIH EY05038, NSF BNS8708429 (VAC) and Pfeiffer Foundation (JBH).

20.2

PERSISTENCE OF RETINAL TERMINALS IN CAT DORSAL LATERAL GENICULATE NUCLEUS FOLLOWING KAINIC ACID LESIONS. H.E. Pearson. Dept. of Anatomy, Temple Univ. School of Medicine, Philadelphia, PA 19140.

Kainic acid (KA) lesions of the CNS can provide useful models for studying the effects of rapid cellular degeneration, particularly in the adult where the response to axotomy is protracted. However, the possibility that KA disrupts the integrity of afferents to the lesion site must also be addressed. HRP was injected intraocularly in six adult cats, three of which had received multiple injections of KA into dLGN one week earlier. After a survival of 72 hr. adjacent sections of each brain were processed for microscopy. Thionin staining revealed large areas of degeneration within dLGN after KA injection. These areas were characterised by the absence of neurons and increased numbers of glia. Sections reacted for HRP showed dense terminal and preterminal labelling within these regions of degeneration, indicating that retinal ganglion cell terminals remain intact. At the ultrastructural level, neuron loss and gliosis were confirmed, as was the presence of RLP terminals, believed to be characteristic of those of retinal origin. These findings confirm the validity of KA as a model for rapid neuron loss in the dLGN of the adult cat and permit further investigation of the long-term effects of target removal on retinal ganglion cells.
Supported by NS25196.

20.3

MORPHOLOGY AND DISTRIBUTION OF RETINAL TERMINALS IN THE DORSAL LATERAL GENICULATE NUCLEUS (LGBd) OF THE HAMSTER. S. Jhaveri*, R.S. Erzurumlu and G.E. Schneider, Dept. Brain & Cognitive Sciences, M.I.T., Cambridge, MA, 02139.

The morphology and distribution of retinal terminals within the LGBd were studied in normal and monocularly enucleated adult hamsters by labeling optic tract (OT) axons with HRP and processing the tissue with DAB. Analysis included use of both camera-lucida tracings and computer-aided reconstructions. Three types of morphologically distinct retinogeniculate terminals were distinguished: Type R1 terminals have very large, ovoid swellings and are most commonly associated with large-caliber axons. Type R2 terminals emerge from fine-caliber axons and are in the form of small varicosities. Frequently, three or more preterminal axons contribute to the formation of rosette-like clusters of R2 terminals. Type R3 terminals have medium-sized boutons and display considerable variability in morphology and configuration. Type R3 terminals may be comprised of more than one subpopulation.

Type R1 terminals arise from both the ipsi- and contralateral retinae and are distributed predominantly within a medial core of the LGBd. Type R2 terminals originate exclusively from the contralateral retina and are located laterally and ventrally, forming an outer laminar shell. Type R3 terminals arise from both retinae and have a widespread distribution within their respective terminal zones. We are currently using the M.I.T. *Neurotrace* System for 3-D reconstruction and quantification to evaluate the relationship between these terminal types and single, parent axons in the OT.

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20.4

CHOLINERGIC PROFILES IN THE LATERAL GENICULATE NUCLEUS (LGN): LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS. James B. Hutchins and Vivien A. Casagrande, Vanderbilt University School of Medicine, Dept. of Cell Biology, Nashville, TN 37232.

In order to better understand the role of cholinergic input to the LGN, we have used histochemical, immunohistochemical, and autoradiographic techniques to characterize the ultrastructure of cholinergic contacts in the tree shrew LGN. At the light microscopic level, choline acetyltransferase (ChAT) immunocytochemistry reveals thin, varicose processes in all six LGN cell layers and in interlaminar zones. No ChAT+ cell bodies are seen in the adult LGN. At the electron microscopic level these ChAT+ terminals are seen as densely filled thin processes. Although ChAT immunohistochemistry obscures the ultrastructure of the terminal, the profiles appear to have round vesicles and sometimes contain dark mitochondria. EM histochemistry of acetylcholinesterase reveals reaction product surrounding apparent dendrites as well as round and flat vesicle-containing profiles. Preliminary EM autoradiography of muscarinic cholinergic receptors labels asymmetric synapses between profiles with round vesicles and dark mitochondria and an apparent dendrite. These data point to a consistent cholinergic terminal and synaptic morphology in the LGN which is probably shared by several other species.
Supported by Pfeiffer Foundation (JBH) and NIH EY05038 and NSF BNS-8708429 (VAC).

20.5

CHOLINERGIC INNERVATION OF THE LATERAL GENICULATE NUCLEUS: COLLATERAL PROJECTIONS TO OTHER THALAMIC AND MIDBRAIN STRUCTURES IN TREE SHREW. A. Jacobs*, D. Fitzpatrick, D. Raczkowski, and I.T. Diamond. School of Medicine and Departments of Neurobiology and Psychology, Duke University, Durham, NC 27710.

The lateral geniculate nucleus (GL) receives a dense projection from cholinergic neurons located in the ponto-mesencephalic reticular formation. These projections are not limited to the GL; they also terminate in other thalamic nuclei and in the superior colliculus (SC). Here the goal was to determine whether individual cholinergic neurons that project to the GL send axon collaterals to other structures. In each experiment WGA-HRP was injected into the GL on one side and rhodamine beads were placed in another thalamic nucleus or the SC. Brainstem sections were processed to demonstrate both retrograde transport and ChAT immunoreactivity in the same neurons. The results show that cholinergic neurons projecting to the GL send collaterals to every other injected structure, but the percentage of individual neurons containing both tracers varied from 35% to 1% depending on the target. The highest percentages (20-35%) were found following injections of rhodamine beads into the SC and contralateral GL, while the lowest (<5%) were found following injections of the ventral posterior nucleus, anteroventral nucleus, and the central lateral and medial dorsal nuclei.
Supported by BNS8411964, BNS8519709, EY06821, and MH04849.

20.6

QUANTITATIVE IMMUNOGOLD EVIDENCE FOR GLUTAMATE AS A NEUROTRANSMITTER OF RETINAL AND CORTICAL TERMINALS IN THE LATERAL GENICULATE NUCLEUS OF THE MACAQUE. V.M.Montero. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53705.

Current neurochemical evidence suggests that the neurotransmitters in the retinal and cortical pathways to the lateral geniculate nucleus (LGN) might be excitatory amino acids. In this study I have used a postembedding quantitative immunogold procedure on ultrathin sections (Somogyi et al., Neurosci. 19:1045, 1986) to measure the intensity of immunoreactivity to an antibody against glutamate (R. Wenthold, NIH) over profiles of retinal, cortical, and F type terminals, and of glial cells in the parvo- and magnocellular layers of LGN of the rhesus monkey. In two samples of 160 electronmicrographs taken from each of the two main subdivisions of LGN the mean gold particles densities per μm^2 ± SEM over the different profiles were as follows: Parvocellular layers, cortical terminals 23.6 ± 0.67; retinal terminals 16 ± 0.37; F terminals 7.3 ± 0.42; glial cells 5.8 ± 0.49. Magnocellular layers, cortical terminals 28 ± 0.78; retinal terminals 19.9 ± 0.5; F terminals 10 ± 0.46; glial profiles 9.6 ± 0.58. The results thus revealed significantly higher concentrations of glutamate in the retinal and cortical terminals than in the F terminals (demonstrated to be immunogold GABA+ in the same material) and glial cells ($p \leq 0.0001$), which probably reflect glutamate levels in the "transmitter pool" of this amino acid. The lower gold particles densities over the F and glial profiles, which were not significantly different, probably reflect glutamate levels in the "metabolic pool". These results support the notion that glutamate is a neurotransmitter in cortical terminals and in M and P retinal ganglion cells terminals in LGN of the macaque.
Supported by NIH grants EY02877 and HD03352.

20.7

THE RETINAL EPSP IS VOLTAGE DEPENDENT IN GENICULATE Y (BUT NOT X) CELLS OF CATS. F.-S. Lo* and S.M. Sherman. Dept of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

The dependence of retinal EPSP amplitude on membrane voltage (V_m) in geniculate X and Y cells was studied in anesthetized, paralyzed cats. We evoked EPSPs by optic chiasm stimulation and altered V_m by passing current through the intracellular recording electrode. For 18 of the 20 X cells, EPSP amplitude correlated negatively and linearly with V_m over the tested V_m range of -120mV to -10mV. This is expected for conventional synapses: EPSP size decreases as V_m nears the EPSP reversal potential. However, EPSP amplitude in each of the 18 Y cells (and the other 2 X cells) dramatically increased with membrane depolarization over a wide V_m range (roughly -120mV to -40mV); further depolarization of V_m reduced EPSP amplitude. We saw no changes in input resistance (e.g., anomalous rectification) that could explain the voltage dependency of EPSP amplitude in Y cells. Instead, this dependency is remarkably similar to that reported for NMDA receptors. Perhaps the retinogeniculate Y synapse is glutamatergic and uses many NMDA receptors, while the X pathway does not prominently do so. Whatever its explanation, this voltage dependency affords a mechanism by which retinogeniculate transmission can be gated: various nonretinal inputs (e.g., corticogeniculate axons) that can affect V_m in Y cells can thereby control the gain of the retinogeniculate EPSP and thus the efficacy of signal transfer to the visual cortex.

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20.8

EFFECTS OF BRAINSTEM STIMULATION ON X AND Y CELLS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT. D.J. Uhlrich, S.A. Bloomfield & S.M. Sherman. Dept. Neurobiology, SUNY, Stony Brook, NY 11794.

We studied the intra- and extracellular responses of geniculate cells in cats to electrical stimulation of the parabrachial region of the brainstem. Brainstem stimulation consisted of pulse trains of 16msec at 250Hz delivered through bipolar electrodes. The typical response to such stimulation is depolarization followed by hyperpolarization. However, some X cells respond with depolarization only, and some Y cells with hyperpolarization only. Similar effects on spontaneous activity are seen with extracellular recording, although the time course of responses may be extended compared to the intracellular responses. When brainstem stimulation precedes visual stimulation, the former can affect responses to the latter (i.e., amplitude, duration, and direction) nonlinearly: that is, these interactive effects cannot be predicted from the effects of brainstem stimulation on spontaneous activity alone. For some cells, brainstem stimulation affected the responses to visual stimulation for up to 1sec or more. On average, X cells show such nonlinear effects more strongly than do Y cells. Y cells, however, often show differential effects depending on whether the visual stimulus is a small spot limited to the receptive field center or one large enough to cover the surround as well; X cells rarely show such a difference depending on stimulus size.

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20.9

TEMPORAL RESPONSE PROPERTIES OF LAGGED AND NON-LAGGED CELLS IN THE CAT LATERAL GENICULATE NUCLEUS. A.B. Saul and A.L. Humphrey, Dept. of Neurobiology, Anatomy, and Cell Science, U. of Pittsburgh, Pittsburgh PA 15261.

Two major groups of X-relay neurons in the A-laminae of the cat's LGN have recently been described[1,2]. These lagged (X_l) and non-lagged (X_n) cells have similar spatial response properties, but differ dramatically in their temporal behavior. Responses of these cells were studied by presenting small counterphasing spots and drifting gratings, which induce responses dominated by the component at the stimulus temporal frequency. We measured the amplitude and phase of the first harmonic response component over a range of temporal frequencies. X_l-cells have lower preferred temporal frequencies and weaker responses to high frequencies than do X_n-cells. The clearest differences appear in the timing of responses, however. At 1Hz, X_n-cells respond slightly ahead of the stimulus, with a phase lead of about .06 cycles. Y-cells respond similarly, leading the stimulus by about .1 cycles. X_l-cells, however, lag the stimulus by about .23 cycles at 1Hz. This quarter-cycle difference between lagged and non-lagged units could play a functional role in providing quadrature pairs as inputs to direction-selective cortical cells[3]. However, the phase difference between X_n- and X_l-cells does not remain constant as temporal frequency is varied. Response phase increases linearly with temporal frequency. The slope of the phase-frequency curve is response latency. X_l-cells have latencies of 100-300msec measured by this slope, as compared to 40-100msec for X_n- or Y-cells. Therefore, at higher frequencies the phase difference between lagged and non-lagged cells increases. At about 4 Hz X_l-units fire ¼ cycle after X_n-units of the same center-type.

These differences in tuning and timing of responses correspond to the prolonged, partly inhibitory effect of a visual stimulus on lagged cells as opposed to the predominantly excitatory, transient effect on non-lagged cells. The inhibition on X_l-cells is of intrageniculate origin[1,2]. The present findings support the suggestion[1,2] that a major function of intrageniculate inhibition is to tranform retinal signals in the temporal domain.

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20.10

RESPONSES OF X AND Y CELLS IN THE CAT LATERAL GENICULATE NUCLEUS TO MOVING BARS AND EDGES. Y.H.Kwon*, M.Esguerra, A.W.Roe, and M.Sur (SPON: W.Richards). Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

One proposal for the function of X and Y cells in early vision requires that X cells in the retina and LGN provide the cortex with a convolution of image intensity with a receptive field function and that Y cells provide a time-derivative of the same (Marr and Ullman, Proc.R.Soc.Lond. B211, 151-180, '81). To test this notion we measured the responses of X and Y cells in the cat LGN to "thin" light and dark bars (with widths 0.1-0.25 times receptive field center widths) and edges both moving at constant velocity. We also obtained spatiotemporal contrast sensitivity functions and visual latencies, and located zero-crossings and peaks in the bar and edge reponses for comparison with predicted crossings and peaks.

The response of X cells (n=6) to a thin bar moving at low velocity is, as expected, similar to the line weighting function of their receptive fields. The X cell response to an edge is well-predicted by convolving their receptive fields with a step function. The response of Y cells (n=7) to an edge also agrees well with the predicted time-derivative of a step convolution. The response of Y cells to thin bars, however, is often broader than the time-derivative of the line weighting function.

This suggests that the thin bars elicit additional responses from Y cells, such as from small, spatially dispersed, subfields that add to or rectify the differentiated (dipole) responses to thin bars (cf. Hochstein and Shapley, J.Physiol. 262, 265-284, '76). Stimuli such as moving edges that effectively engage the linear components of Y cell receptive fields yield time-derivative (transient) responses that are consistent with the band-pass temporal characteristics of these cells, just as the X cell responses follow from their low-pass temporal transfer functions.

Supported by NIH grant EY 07023 and NIGMS grant T32GM07484-11.

20.11

EXTRATHALAMIC CONTROL OF X AND Y CELL ENCOUNTER RATES IN THE LGN OF THE MONOCULARLY PARALYZED CAT. R.J. Moore*, J.W. Vaughan, W.L. Salinger, H.H. Willis, III, and L.R. Cole*. Dept. of Psychology, Univ. of North Carolina at Greensboro, Greensboro, NC 27412.

The lateral geniculate nucleus (LGN) is the target of extrathalamic influences, including those that are derived from proprioceptive and binocular processes. The operation of these processes is revealed in the LGN by monocular paralysis (MP), which results in a decrease in the encounter rate of X cells relative to Y cells. It is clear that this effect is a manifestation of binocular and proprioceptive interactions because it is seen in the LGN layers innervated by the mobile as well as the paralyzed eye, and it can be reversed by visual or proprioceptive deafferentation of the mobile eye.

In the present experiments we have continued our assessment of the role of visual and proprioceptive interactions in producing and maintaining the effects of MP by lesioning key extrathalamic structures in anesthetized subjects. To accomplish this, LGN cells with receptive fields within the central 5 degrees of visual space were classified as X or Y using a standard battery of tests before and immediately after lesioning the target structure. Cryogenic blockade or aspiration of visual cortex ipsilateral (but not contralateral) to the recorded LGN reversed the MP effect (i.e., increased the encounter rate of X cells relative to Y cells) in both laminae A and A1 ($P < .05$, 10 cats, 643 cells). In addition, aspiration of the cerebellum produced a similar effect ($p < .05$, 4 cats, 188 cells), but lesions of the superior colliculus did not (4 cats, 128 cells). These results reveal that the extrathalamic circuit, which controls the X and Y cell encounter rates in the LGN and is sensitive to binocular and proprioceptive signals, includes the cerebellum and visual cortex, but not the superior colliculus.

20.12

ECCENTRICITY-SPECIFIC ANESTHESIA EFFECTS ON RELATIVE ENCOUNTER RATES FOR X- AND Y-CELLS IN ADULT CAT LGN. H.H. Willis,III, W.L.Salinger, J.W. Vaughan, R.J. Moore*, & L.R.Cole*. Dept. of Psych., UNCG, Greensboro NC 27412

The relative recording probabilities of X- and Y-cells in regions representing central visual space in the cat lateral geniculate nucleus (LGN) have been shown to be dynamically controlled by mechanisms which are sensitive to levels of anesthesia and to disruption of normal binocular interactions. In the present experiments, we have extended the analysis of these mechanisms to more peripheral representations within the LGN A and A_1 laminae of binocularly paralyzed adult cats.

Using painless head restraint, we recorded LGN cells, classifying 298 as X or Y. Matched pairs of penetrations were made at various eccentricities, with animals either anesthetized or sedated. Although the average number of cells per pass was unchanged between them, the two conditions differed dramatically. In penetrations when the cats were sedated (n=148 cells), there were roughly equal numbers of X- and Y-cells, with no systematic changes in X/Y ratios across the representation of the central 18 degrees of visual space. In marked contrast, when animals were anesthetized, recordings in the matched penetrations (n=150 cells) yielded a surprisingly steep and tight exponential fit ($R^2>.92$, $p < .0001$), declining from near 100% X-cells at area centralis to under 23% within 18 degrees of eccentricity.

One of these paired sets of observations could reflect anatomic distributions of LGN X and Y cells. However, the other must reflect activities of physiological processes powerful enough to affect relative encounter rates for X and Y cells, and doing so in opposite directions in central and peripheral representations of visual space. This raises questions about the functional role and perceptual consequences of such processes.

Supported under an NSF Graduate Fellowship to H.H.W.

20.13

EFFECTS OF DORSAL RAPHE STIMULATION ON NEURONAL ACTIVITY IN THE RAT LATERAL GENICULATE NUCLEUS.
Y. Kayama[1], S. Shimada*, Y. Hishikawa* and T. Ogawa*.
Dept. of Physiology, Akita Univ. Sch. of Med., Akita 010; [1]Fukushima Medical College, Fukushima 960-12, Japan.

The dorsal raphe nucleus (DR) of rats anesthetized with urethane was penetrated by a stimulating electrode, whose position was confirmed histochemically after experiment. During repetitive stimulation of the DR at 200 Hz for several to 10 seconds, spontaneous firing of lateral geniculate relay neurons changed variably from neuron to neuron. However, their firing was consistently depressed for 20 seconds to 2 minutes after the cessation of DR stimulation. This depression was seen in many cases concurrently with strengthening of slow activity in cortical EEG, but in others without any change in EEG. The DR-induced depression was blocked by methysergide applied ionophoretically. In some cases the depressive effects of DR stimulation disappeared after several neurons were tested, possibly because of depletion of serotonin. In such cases, intraperitoneal administration of 5-hydroxy-tryptophan could recover the depression.

These results show that the serotonergic system arising from the DR exerts an inhibitory influence on relay neurons. Together with the noradrenergic and cholinergic systems, both of which give excitatory influences to relay neurons, thalamic neuronal activity is controlled by triple diffuse projection systems from the brainstem.

20.14

PHYSIOLOGICAL AND MORPHOLOGICAL PROPERTIES OF TECTO-RECIPIENT CELLS IN THE HAMSTER'S DORSAL LATERAL GENICULATE NUCLEUS.
W.H. Rohrer, R.D. Mooney and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

The stratum griseum superficiale of the superior colliculus (SC) projects to the dorsal lateral geniculate nucleus (LGNd), but relatively little is known about the morphology or receptive field properties of the neurons that receive this input. We characterized, filled with horseradish peroxidase, and recovered 27 LGNd cells that were tested for responsivity to SC stimulation. We obtained electrophysiological data from another 8 cells in which "spills" or incomplete fills provided histological verification of a recording site in the LGNd. Of these 35 cells, 54% (N=19) were excited by SC shocks. The average response latency was 3.7 ms. Seven of the SC driven cells had X-like receptive fields, 4 had Y-like receptive fields, 4 were unresponsive, and 4 were not completely tested. Seven of the cells activated by SC shocks were projection neurons and 10 could not be antidromically activated from visual cortex. Two cells were not tested. The somas and dendritic arbors of projection neurons were larger than those of interneurons. Surprisingly, the soma and dendritic areas of cells with X-like receptive fields tended to be larger than those with Y-like receptive fields. Supported by BNS 85-00142, EY 04170, and NS 07229.

20.15

EFFECTS OF REMOTE STIMULI ON VISUAL RESPONSES OF LATERAL GENICULATE NEURONS: SPATIAL PARAMETERS.
Cérat, A., S. Molotchnikoff and P. Rouillon. Département de Sciences biologiques, Université de Montréal, Montréal, Québec. H3C 3J7.

We have previously demonstrated that a conditioning stimulus (CS) presented in the periphery of the visual field (VF) modifies the visual responses of a geniculate cell to a LED. This effect is mediated by the superior colliculus (SC) as its inactivation suppresses the interaction. This investigation analyzes the spatial parameters of these interactions. Anesthetized rabbits were prepared for extracellular recordings of LGN cells. In addition, a lidocaine hydrochloride filled micropipette was lowered in to the SC. A LED was positioned in the center of the geniculate receptive field (RF) while a moving slit was presented in the periphery of the VF. Several conclusions emerge from these results. 1) The CS produces a larger attenuation of the visual responses of tested neurons if the RF is eccentric. Conversely, the CS increases the discharges of cells whose RF are close to the optic axis. There is also a positive correlation between the amplitude of the effects and the size of the RF. 2) It seems that there is an optimal interstimuli distance where the effects are largest. 3) In most cases, the effects are abolished if the SC is rendered inactive.

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20.16

MODIFICATION OF VISUAL INPUT AT LGN BY CORTICAL AND BRAINSTEM FEEDBACKS.
K. P. Unnikrishnan and *E. Harth*. AT&T Bell Laboratories, Molecular Biophysics Research Department, Murray Hill, NJ 07974 and Physics Department, Syracuse University, Syracuse, NY 13244.

Through computer simulations, we have investigated the role of feedback pathways in sensory information processing. We show that at the mammalian dorsal lateral geniculate nucleus (dLGN) feedback from cortex and brainstem reticular formation is capable of modifying retinal input in feature specific ways. In the presence of visual input, incomplete information is filled in and in the absence of input quasi-sensory images are created. GABAergic interneurons in the perigeniculate (PGN) with the NMDA receptors in the LGN (for which there is recent evidence) can carry out the necessary computations in the model.

ALCOHOL I

21.1

THE EFFECT OF GASTRIC VAGOTOMY ON VOLUNTARY ETHANOL INTAKE IN THE RAT.
P. Toth*, L.A. Grupp, E. Perlanski*, S. Harding* and M.A. Linseman. Dept. of Pharmacology, Univ. of Toronto, and Addiction Research Foundation of Ontario, Toronto, Ont., Canada M5S 1A8.

Ethanol as a drug acts on the central nervous system; however, it also has food properties (i.e., calories, taste) and is consumed orally. Given that gastric vagal afferents have been shown to be important in the control of feeding, we investigated the effect of gastric vagotomy (GVX) on ethanol intake. Free-fed GVX and sham-operated control rats were offered ethanol using the limited access procedure which is known to produce a robust level of ethanol intake and detectable blood ethanol levels. Compared to the sham group, the GVX animals drank less ethanol at the two concentrations (3%, 6% w/v) that were offered ($p<0.01$). These findings suggest that in a procedure that elicits consumption of pharmacologically and behaviorally significant amounts of ethanol, intake can nevertheless be influenced by a manipulation that has been shown to alter feeding behavior. Supported by the Labatt Brewing Company.

21.2

BEHAVIORAL INTERACTIONS WITH ETHANOL (ET) AND CNS EFFECTS OF ICV ADMINISTERED DILAZEP AND ITS METABOLITES IN MICE.
M.S. DAR, Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858.

Dilazep (ip), a coronary vasodilator and an uptake inhibitor of adenosine (Ad), dose dependently potentiated acute ethanol-induced motor incoordination (EIMI) in mice. In view of peripheral cardiovascular depressive effects of dilazep, the effect of icv dilazep (25, 50 & 75ug), and its metabolites BHPD (15, 31 & 62ug) and TBPD (62 & 125ug) on EIMI was studied. Dose related potentiation of EIMI was noted with dilazep and its metabolites. Whereas dilazep (ip) produced no apparent CNS effects, by icv route it caused tonic-clonic seizures. AD uptake inhibition, Ca^{++} entry blockade or direct activation of AD receptors was ruled out as the mechanism of seizures because dipyridamole, verapamil or R-PIA, respectively, did not produce these. The CNS excitation was minimal with BHPD and none with TBPD. Theophylline pretreatment partially blocked potentiation of EIMI by dilazep and BHPD and none by TBPD. Data suggested dilazep-induced potentiation of EIMI is partly due to AD receptor mechanism and partly due to other yet unknown mechanism(s) and further supported our earlier reports about AD involvement in the CNS effects of ET. Data also suggested that dilazep(icv)-induced seizures are due to mechanism(s) other than AD uptake inhibition, Ca^{++} entry blockade or direct AD receptor activation.

21.3

SPECIFICITY OF RESPONSE STRATEGIES IN BEHAVIORAL TOLERANCE TO ETHANOL. D.A. King* and F.A. Holloway. Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.

Chronic exposure to ethanol (ETOH) results in the development of persistent tolerance to ETOH's rate-decreasing effects on rat operant behavior. Such data are consistent with a functional interpretation of tolerance in which animals acquire, through "intoxicated practice" (IP), some compensatory response strategy specific to the task demand characteristics. The present study attempted to directly assess the nature of this hypothetical learned response strategy in rats given IP on an FR task. Male Sprague-Dawley rats were trained on a DRL-15 s (a.m.) and a FR-30 task (p.m.) to respond for food reinforcers. Half of the rats received 30 days of pre-session ETOH injections prior to the FR-30 task only, while the other half received p.m. saline (SAL) injections. If rats acquire tolerance to ETOH's rate-decreasing effects on FR performance by developing a "learned rate-increasing strategy," then such an effect may be reflected in these rats' DRL performance by significant response-rate increases. The results confirm this expectation. Dose-effect analysis indicated the development and retention of significant tolerance on the FR task in the ETOH-treated rats which was absent in the SAL-treated controls. On the DRL task, SAL-treated controls evidenced no change in ethanol sensitivity. As anticipated, however, ETOH-treated rats demonstrated significant increases in response rates on the DRL task. Supported by NIAAA AA06351.

21.4

THE 5-HT$_3$ ANTAGONIST GR38032F DECREASES ALCOHOL CONSUMPTION IN RATS. E.M. Sellers*, H.L. Kaplan*, M.O. Lawrin*, G. Somer*, C.A. Naranjo* and R.C. Frecker* (SPON: W.G. Tatton). Depts. of Pharmacol. and Med., Univ. of Toronto, and Clin. Psychopharmacol. Program, Toronto, Ontario M5S 2S1.

Ethanol consumption is regulated by multiple neural systems. Effective ways to decrease alcohol consumption in humans are needed. A micro-drinking monitoring system detects decreases in rat ethanol drinking at doses of fluoxetine (a serotonin uptake inhibitor) 20-50 fold lower than reported using a 24-hour preference paradigm, e.g. 500-1000 µg/kg (Sellers, E.M., et al, Clin. Pharmacol. Ther. 43(2): 187, 1988). The drinkometer may have predictive value since the effects correspond with results from clinical studies with zimelidine, citalopram, viqualine and fluoxetine (Naranjo, C.A. and Sellers, E.M. In: Recent Developments in Alcoholism, Volume VII. Galanter, M. (ed.), Plenum Publishing Corporation, New York, 1988).

Male Wistar rats (200 g, n = 5-6/group) with a > 50% free-choice preference for 6% v/v ethanol received GR38032F, a specific selective 5-HT$_3$ antagonist (Glaxo), 1, 10 and 100 µg/kg i.p. once 1.0 hour prior to the 12-hour dark cycle. Differences between first treatment and last baseline day showed GR39032F at 1 µg/kg decreased ethanol intake and minutes in which drinking occurs by 40% and 33% over 12 hours respectively (ANOVA $p < 0.05$). Effects were less pronounced at higher doses suggesting an inverted "U" shaped dose-effect relation. In another study, decreases in ethanol consumption volume were found at lower doses, e.g. 0.01 µg/kg, however the effects did not persist after 3 days of treatment.

These data are the first to suggest 5-HT$_3$ receptors may modulate consummatory behaviour. GR38032F may be a new clinical lead for treatment of alcohol abuse.

21.5

GENETICALLY SELECTED LINES OF HIGH- AND LOW-ALCOHOL-DRINKING RATS: OPERANT STUDIES. A.D. Levy, W.McBride, J.Murphy, L.Lumeng, & T.-K.Li. Inst Psych Res, Indiana Univ Sch Med, VA Med Ctr, Regenstrief Inst, Indianapolis, IN 46223

Lines of high- and low-alcohol-drinking rats (HAD and LAD) have been selectively bred from a heterogeneous N/Nih stock. This study compared ethanol (EtOH) reinforcement between HADs and LADs in an operant task, similar to studies with Wistar-derived P and NP lines of rats selected for EtOH preference (PBB 8:475, 1978).

HAD and LAD male rats (6/line), were trained to bar press for sweetened milk on an FR5 schedule during daily 1 hr sessions, until reaching criteria of 100 reinforcements for 3 consecutive days. Rats were then tested for bar pressing for varying concentrations of EtOH (2-30% v/v). LADs exhibited few responses for EtOH, while moderate rates were obtained for the HAD's across all EtOH concentrations (reaching approximately 0.8 g/kg for 30% EtOH, $p<.05$). Similar to the P line, the HAD line clearly demonstrated that they will work to obtain EtOH without food or water restriction, suggesting that EtOH is reinforcing in these rats. (Supported by AA-03243 and AA-07462).

21.6

EFFECTS OF ALCOHOL ON THE SEXUAL BEHAVIOR OF FEMALE RATS. J.P.J. Pinel and J.G. Pfaus. Dept. Psychology, Univ. British Columbia, Vancouver, B.C. V6T 1Y7 Canada.

Much of the experimental evidence for a disruptive effect of alcohol on sexual behavior comes from the study of male rats and dogs; no studies had examined the effect of alcohol on the sexual behavior of female laboratory animals. Accordingly, we examined the effects of a wide range of doses of alcohol (0.125 to 2.0 g/kg) on the sexual behavior of ovariectomized Sprague-Dawley rats (N = 12) rendered **moderately** sexually receptive with SC injections of 10 ug estradiol benzoate 48 hr and 250 ug progesterone 4 hr before each test. The female rats were tested at 4-day intervals with sexually active male rats in small Plexiglas testing chambers during the dark phase of their circadian light cycle. Doses of ethyl alcohol (25%, v/v) or saline were injected IP 1 hr before each test in a latinized, repeated measures design. The highest dose of alcohol (2 g/kg) completely abolished lordosis behavior and a moderate dose (1 g/kg) significantly reduced both lordosis quotients and magnitudes. In contrast, a low dose (.5 g/kg), which had no effect on lordosis behavior, significantly increased the incidence of proceptive behaviors (i.e., hopping, darting, and ear-wiggling). These results are consistent with anecdotal reports that alcohol can have both facilitatory and disruptive effects on human sexual behavior.

21.7

RAPID TOLERANCE TO THE DISRUPTIVE EFFECTS OF ETHANOL ON HIGH-SPEED REACTION TIME IN YOUNG AND OLD RATS. R. D. Mayfield*, M. Grant*, D. Lowe*, W. W. Spirduso[a] and T. Schallert[b]. Institute for Neurol. Sciences, Depts. of Kinesiology & Health Education[a] and Psychology[b], Univ. Texas at Austin, Austin, TX 78712

We have recently reported that rapid tolerance develops to the detrimental effects of ethanol (EtOH) on reactive capacity (RC) in rats. The RC model is a high-speed reaction time task that measures the overall success in avoiding mild footshock (percent avoidance) as well as the response latency of the successful avoidances (measured in msec). The purpose of this study was to examine the effect of age on the development of tolerance to the effects of EtOH using the RC model. Young (8 - 11 mo) and old (24-26 mo) Fischer 344 rats were shaped to release a lever in response to an auditory/visual stimulus. Shaping was continued until the animals could reliably release the lever in less than 500 msec. The animals were given blocks of ten trials at 5, 10, 20, 45, 65, and 90 min post-EtOH/or -saline. Animals of each age either received RC sessions while intoxicated (Behavior While Intoxicated; BWI) or while sober (Behavior While Sober; BWS). The BWS group was administered EtOH immediately after the RC session except on Day 5 when they received EtOH immediately before the RC test session. EtOH (17-20% w/v; 1.6-2.5 g/kg) was administered to both the BWI and BWS groups 3 times per day at 8 hr intervals for 5 days via intragastric intubation. EtOH doses were increased slightly each day so that blood EtOH concentrations (BECs) were greater than or equal to the BECs of the previous day. The performance of both the young and the old animals in the BWI group was significantly impaired after the first EtOH challenge (Day 1), as measured by both percent avoidance and response latency. Percent avoidance scores significantly improved in both age groups by Day 5 in spite of higher BECs. The same degree of improvement was not observed for response latencies. In general, the old animals were more impaired than the young on Days 1 through 4; however, by Day 5 there were no significant age-related differences in performance. The performance of the BWS animals (young and old) was impaired as measured by both percent avoidance and response latency. There were no clear age-related differences in performance in the BWS animals on Day 5. GRANTS: AA06761 and NS20827.

21.8

ETHANOL-INDUCED MOOD CHANGES CORRELATE WITH CHANGES IN REGIONAL CEREBRAL METABOLISM. J.T. Metz, H. de Wit*, J.B. Brunner*, S.J. Gatley*, M.D. Cooper*. University of Chicago, Chicago, IL

This study used positron emission tomography (PET) to examine the effects of ethanol on regional cerebral metabolism (rCM) and subjective mood.

Nine adult males were tested on two occasions each. On one day, subjects consumed 0.5 mg/kg of ethanol dissolved in a citrus flavored beverage; on the other day, they received the vehicle beverage only. They were injected with 6-8 mCi 2FDG and for 45 minutes performed a visual monitoring task. Before the beverage and 20 and 40 minutes after 2FDG injection, subjects completed a Profile of Mood States (POMS). rCM was determined using Sokoloff's model with standard rate constants and fourteen ROIs obtained from tomographic metabolic images.

Although all subjects attained marked blood levels of ethanol, across subjects average metabolic rate for the whole brain did not change in response to ethanol (9.06 mg/100g/min with placebo, vs. 8.71 with ethanol). No region showed a consistent metabolic change in response to ethanol.

Changes in rCM relative to whole brain metabolic changes after ethanol, however, were related to changes in mood. Changes in three positive moods (Vigor, Friendliness, and Elation) had strong negative correlations ($r > 0.70$ in absolute value) with changes in relative rCM in left temporal cortex; they had comparable positive correlations with changes in left parietal cortex. Changes in three negative moods (Anxiety, Depression, and Anger) had strong positive correlations with changes in relative rCM in left temporal cortex and strong negative correlations with changes in left parietal cortex. Homologous regions on the right had similar correlations. Other regions were uncorrelated with mood changes.

These data suggest that changes in rCM may be related to ethanol's mood-altering effects. Individual differences in mood associated with the drug may underlie differential risk for alcohol abuse. The data also show that PET may be useful in the study of mood.

21.9

DIFFERENTIAL ETHANOL AND HABITUATION EFFECTS ON OPEN-FIELD ACTIVITY MEASURES. H.L. June*, T.O. Moore*, K.L. Edwards* and M.J. Lewis. Dept. of Psychology, Howard University, Washington, D.C. 20059.

Ethanol (E) has been found to produce differential effects on habituation of exploration and locomotion (Lister, R.G., Psychopharm., 92: 78, 1987). To further investigate E and habituation effects on activity, the present study examined E-induced stimulation effects and habituation in rats selected for low spontaneous activity.

Male Charles River rats who were selected for low-wheel running activity were habituated to the open field over a six week period by repeated testing. Rats received E (0.75 g/kg) or saline 5 minutes prior to being placed in a Digiscan activity apparatus. Horizontal (H) and nonhorizontal (NH) activity were measured for 10 minutes. Saline treated rats showed initial higher levels of activity which declined with subsequent testing, indicating habituation. A similar profile occurred with E-treated rats in H activity, although their activity levels were higher than saline controls. In contrast, E-treated rats showed increases in NH activity with subsequent testing. The E group showed higher levels of both activities in comparison with saline controls. These results suggest that E may be differentially affecting qualitative components of locomotor activity and their habituation.

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21.10

ETHANOL INDUCED MOTOR BEHAVIOR IN NORMAL AND ACATALASEMIC MICE. C.M.G. Aragon*, C. Pesold*, F. Rogan*, and Z. Amit (SPON: Z.W. Brown). Center for Studies in Behavioral Neurobiology, Concordia Univ., Montreal, Quebec H3G 1M8.

The role of brain catalase in modulating the psychopharmacological effects of ethanol was investigated by examining ethanol induced locomotor activity in normal, C3H-N, and a corresponding colony of acatalasemic C3H-A, mice. Following administration with i.p. injections of one of three doses of ethanol (0.8, 1.6 and 3.2 g/kg) or saline, mice were placed in open field chambers and locomotor activity was measured during a 10 minute testing period. A significant increase in locomotor activity was recorded in both mice at lower doses of ethanol, while the higher dose produced a marked depression. Normal mice demonstrated more locomotor activity than acatalasemic at all ethanol doses. No differences in blood ethanol levels were observed between the two strains. Acatalasemic brain and liver residual catalase activity was found to be 40% of normal mice. These results suggest a role for brain catalase in ethanol effects and support the notion that centrally formed acetaldehyde is a factor mediating some of ethanol's psychopharmacological effects.

21.11

INTERACTIONS OF THE ALPHA$_2$-ADRENOCEPTOR ANTAGONIST, ATIPAMEZOLE, WITH ETHANOL ON EXPLORATION AND LOCOMOTOR ACTIVITY. M.J. Durcan, R.G. Lister and M. Linnoila. Laboratory of Clinical Studies, NIAAA, Blg10 3C218, 9000 Rockville Pike, Bethesda, MD 20892, USA.

Atipamezole (Farmos Ltd.) is a highly potent and selective alpha$_2$-adrenoceptor antagonist. In this study its behavioral effects and its interactions with ethanol were explored in mice using a holeboard test of directed exploration and locomotor activity. When administered alone, atipamezole (0.1-3.0 mg/kg i.p.) had no effect on either exploratory head-dipping or locomotor activity 30 min after administration. However the drug completely antagonized the effects of the alpha$_2$-adrenoceptor agonist medetomidine (0.1 mg/kg). In the ethanol study, atipamezole was administered at the same time as a contralateral i.p. injection of 2 g/kg ethanol 30 min prior to testing. Ethanol significantly reduced exploratory head-dipping ($p<.01$) and increased locomotor activity ($p<.001$). Atipamezole (1 and 3 mg/kg) significantly antagonized the ethanol-induced reduction in exploratory head-dipping ($p<.05$); no change in ethanol's locomotor stimulant property was seen. We have found that atipamezole antagonizes the ataxic effects of ethanol (Lister et al., this meeting). These results suggest alpha$_2$-adrenoceptor mediation of some of the behavioral effects of ethanol.

21.12

ODOR AS A CUE FOR ALCOHOL AVERSION LEARNING IN RATS LACKING GUSTATORY NEOCORTEX. S.W. Kiefer, N.S. Morrow, and C.W. Metzler*. Department of Psychology, Kansas State University, Manhattan, KS 66506.

The hypothesis that the normal alcohol aversion learning found in rats lacking gustatory neocortex (GN) is based on the odor cue of alcohol was tested. Control rats (n=23) and GN rats (n=17) were trained to drink for 5 min from a modified drinking spout with an odor disc attached. On training day, half the rats from each group drank water in the presence of the odor of alcohol; the remaining rats consumed 5% (v/v) alcohol from the drinking tube (experiencing the odor and taste of alcohol). Illness was induced after a delay of 30 min. All rats were then tested for drinking water in the presence of the alcohol odor. Results showed that both control rats and GN rats given alcohol in the drinking tube during training showed strong avoidance of drinking in the presence of the alcohol odor, an avoidance which extinguished after four test sessions. Rats given odor-illness pairings failed to show avoidance of the odor. The results support the hypothesis that rats lacking GN are capable of using the odor of alcohol as a salient cue in developing learned alcohol aversions.

Supported by NIAAA grant AA05898

21.13

TEMPERATURE DEPENDENCE OF ETHANOL DEPRESSION IN MICE: DOSE RESPONSE. R.L. Alkana, D.A. Finn,* G.G. Galleisky,* M. Bejanian and B. Jones.* University of Southern California, School of Pharmacy, Los Angeles, CA 90033.

Previous studies indicate that body temperature alters brain sensitivity to ethanol in C57BL/6 and BALB/C mice following injection of 3.6 g/kg ethanol. The current study tested the generality of this effect across a range of hypnotic ethanol doses. Drug naive, male C57BL/6J mice were injected with 3.2, 3.6 or 4.0 g/kg ethanol (20% w/v) and were exposed to one of six designated temperatures from 15 to 34 ^{o}C. Ambient temperature significantly affected wake-up rectal temperatures, sleep-times and wake-up blood and brain ethanol concentrations at all three doses. Wake-up temperatures were significantly positively correlated with sleep-times and significantly negatively correlated with wake-up blood and brain ethanol concentrations at all three doses. The sleep-time duration was dose dependent at each ambient temperature, but the degree of body temperature change and the wake-up blood and brain ethanol concentrations did not appear to be dose-dependent. The latter finding suggests that the threshold ethanol concentration for return of function was not influenced by dose. These results demonstrate the generality of temperature dependence across hypnotic doses and emphasize the importance of body temperature as a variable in ethanol research. (Supported by research grant RO1 AA05234, NIAAA, ADAMHA).

21.14

GANGLIOSIDES, OR SIALIC ACID, ANTAGONIZE ETHANOL INTOXICATION. R. Boyles,* J. Mathew,* L. Cherian,* and W. R. Klemm. Dept. Vet. Anat., Texas A&M U., Col. Sta., TX 77843.

Because ethanol had a dose-dependent effect on hydrolysis of brain sialogangliosides, we tested the possibility that injected gangliosides might antagonize ethanol (3.9-4.5 gm/kg). Clear anti-intoxication effects were seen at 24 hr post-injection of 125-130 mg/kg of mixed gangliosides, but not at lower or higher doses. Sleep time was reduced on the order of 50%, and roto-rod agility was significantly enhanced. Sialic acid (SA) similarly antagonized ethanol, but not ceramide or the prescursor of SA, N-acetyl-D-mannosamine (100 mg/kg each).

As intoxication level directly correlates with brain sialoconjugate hydrolysis, any compound that interferes with SA hydrolysis might reduce intoxication. Thus, injection of ganglioside or SA might prevent/compensate for at least part of ethanol-induced SA hydrolysis. The results show that SA is a key component in mediating ethanol effects. Changes in ganglioside not only correlate with intoxication, but we must also consider that they cause many signs of intoxication.

21.15

MUSCARINIC CHOLINERGIC INFLUENCES ON ETHANOL SENSITIVITY IN LONG-SLEEP AND SHORT-SLEEP MICE. V.G. Erwin and B. Jones.* Alcohol Research Center, School of Pharmacy, University of Colorado, Boulder, CO 80309.

Sensitivity to the hypnotic effects of ethanol were selectively increased by central administration of muscarinic agonists. Carbachol or oxotremorine, but not nicotine, icv, enhanced hypnotic sensitivity to ethanol, in short-sleep (SS) but not long-sleep (LS) mice. Likewise, the acetylcholinesterase inhibitor, neostigmine, icv, differently enhanced hypnotic sensitivity to ethanol in these mouse lines. LS and SS mice were equally sensitive to the hypothermic effects of oxotremorine, icv. The muscarinic antagonists, atropine or pirenzepine, icv, were without effect on ethanol sensitivity, but at doses of 0.5 µg to 1.5 mg these compounds effectively antagonized oxotremorine- or neostigmine-enhanced ethanol sensitivity in SS mice. Pirenzepine, an M_1 selective antagonist, produced a parallel shift in the oxotremorine dose-response curve, indicating that the enhanced hypnotic sensitivity to ethanol may be due to interaction of oxotremorine with M_1 muscarinic receptors. The results suggest that LS and SS mice differ genetically in neuronal processes activated by specific muscarinic agonists and are consistent with the hypotheses that ethanol acts in part via membrane receptor coupling to intracellular processes known to mobilize intracellular Ca^{++}. (This research was supported in part by USPHS grants AA03527 and AA07330.)

21.16

ROLE OF PHARMACOKINETIC FACTORS IN CONDITIONED TOLERANCE TO ETHANOL HYPOTHERMIA. M. Singh*, A.D. Le*, J.M. Khanna and H. Kalant*. Department of Pharmacology, University of Toronto and Addiction Research Foundation of Ontario, Toronto, Canada.

The role of pharmacokinetic factors in conditioned tolerance to ethanol (EtOH) was examined using a counterbalanced design. One group of rats received EtOH in a distinctive environment (DE) and an equal volume of saline in their home environment (HE), on alternate days. The DE located 3 rooms away from the HE was characterized by dim light and static noise. The other group received EtOH in their HE and saline in the DE. Control animals received saline injections in both environments. After 12 EtOH training sessions, rats treated with EtOH in the HE showed less hypothermia than their controls when tested in both environments. However, those treated with EtOH in the DE showed tolerance only when tested in the DE. Rats which previously received EtOH in the DE absorbed EtOH more slowly than their saline controls when both were tested in the DE, but not when tested in the HE. However, those treated with EtOH in the HE absorbed EtOH at the same rate as their controls when tested in either environment. These results show clearly that conditioned tolerance to EtOH can occur in the absence of change in pharmacokinetics.

Supported by NIAAA grant AA0 7003-01.

21.17

SEIZURE SUSCEPTIBILITY IN MICE GENETICALLY SELECTED FOR ETHANOL WITHDRAWAL SEVERITY: SENSITIVITY TO GABA-BZ-CL-COMPLEX DRUGS IN WSP AND WSR MICE. A. Kosobud, D.A. Feller and J. Crabbe. VA Med. Center and Oregon Health Sci. Univ., Portland, OR 97201 USA

WSP mice have been genetically selected to express severe handling induced convulsions (HIC) following ethanol withdrawal, and WSR mice to show minimal HIC. Other differences between lines should be due to the influence of genes affecting EtOH withdrawal severity. We tested the hypothesis that WSP mice were more sensitive than WSR to drugs affecting the GABA-benzodiazepine-picrotoxin receptor/chloride ionophore (GABA-BZ-CL) complex. WSP and WSR mice were tested for the severity of HIC at several times before and after ip bicuculline, picrotoxin, pentylenetetrazole, RO15-4513, and strychnine. In each case, WSP mice showed either a greater or a longer lasting stimulation of HIC than WSR mice after drug treatment. Thus, some genes acting at the GABA-BZ-CL complex may be implicated in ethanol withdrawal severity. That strychnine also differentiated WSP and WSR mice suggests that some more general seizure mechanisms may also be implicated. Threshold seizure susceptibility was also determined by tail-vein infusion of several compounds including the above. A different pattern of results from the two seizure testing methods was seen in the WSP and WSR lines. Supported by Grants AA05828, AA06243, AA06498, NIDA Contract No. 271-87-8120, and by a grant from the VA.

21.18

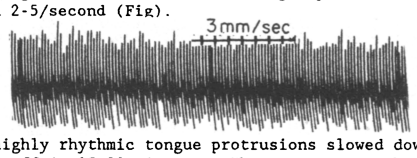
ETOH EFFECTS ON LOCOMOTOR ACTIVITY IN MOUSE LINES SELECTED FOR ETOH HYPOTHERMIA AND ACTIVATION. T.J. Phillips*, D. Baxter*, and J.C. Crabbe (SPON: D.J. Feller). VA Med. Center and Oregon Health Sci. Univ., Portland, OR 97201.

We examined locomotor activity (ACT) after ethanol (ETOH) in 2 sets of selectively bred mouse lines. HOT and COLD lines were selected for degree of ETOH hypothermia, and FAST and SLOW lines were bred for high and low ETOH-induced ACT. ACT was recorded for 30 min. after i.p. saline or 2.0 g/kg ETOH. HOT and COLD mice were similarly activated by ETOH in the first 5 min., however, ACT of HOT mice returned to control levels by 15 min. after injection while COLD mice were depressed between 15 and 30 min. FAST mice were stimulated for the first 10 min., and then their ACT approached control levels. SLOW mice exhibited no activation, and their ACT was depressed at 10 min. and later. These sets of mice, selectively bred for different ETOH responses, are heterogeneous in the relative locomotor activity differences they display to ETOH. Further characterizations of locomotor activity responses and detailed examinations of correlated responses to ETOH and their genetic associations are in progress.
Supported by the Vet. Admin. and by Grants AA05828, AA06243, and AA06498.

21.19

REMARKABLY RAPID AND RHYTHMIC TONGUE PROTRUSIONS PRODUCED BY ACUTE HYPNOTIC DOSES OF ETHANOL IN FISCHER F344 RATS. M.K. Menon. Psychopharmacol. Res. Lab., V.A. Med. Ctr, Sepulveda, CA 91343 and Dept. Psychiat. UCLA. Sch. Med. Los Angeles, CA 90024.

Male Fischer F344 rats (NIH/NCI, 180-220g) were injected with ethanol diluted with isotonic NaCl solution. (1 ml/100g body wt, i.p.). Soon after the animal lost its righting reflex it was placed on its back on a board, the tongue was attached to a strain gauge and the movements recorded on a Grass polygraph. In rats treated either with 23 percent (1.81 g/kg) or with 25 percent (1.97 g/kg) ethanol, the rate of tongue jerks varied between 2-5/second (Fig).



These highly rhythmic tongue protrusions slowed down and tapered off in 15-30 minutes. These movements, however, could be re-initiated by touching the tongue with a saline-soaked cotton swab. Rats treated with 28 percent ethanol (2.2 g/kg) showed either a weak response or were non- responsive. The influence of drug treatments on this remarkable acute effect of ethanol is being investigated. Supported by the Veterans Administration.

21.20

DEVELOPMENTAL THERMOREGULATION IN LONG- AND SHORT-SLEEP MICE FOLLOWING PRENATAL EXPOSURE TO ALCOHOL. D.M. Gilliam, L.E. Kotch*, and E.P. Riley*. Center for Behavioral Teratology, SUNY Albany, Albany, NY 12222.

Long-Sleep (LS) and Short-Sleep (SS) mice, selectively bred for differences in ethanol-induced narcosis, provide a tool to investigate how genetic differences in alcohol sensitivity influence susceptibility to prenatal alcohol effects. The ontogeny of core body temperature regulation was measured in offspring of LS and SS mice, following prenatal exposure to alcohol. LS and SS dams were intubated twice per day, 6 hrs apart, with either ethanol (3.0 or 4.0 g/kg) or an amount of maltose-dextrin made isocaloric to the 4.0 g/kg ethanol dose on days 7 through 18 of pregnancy. Non-intubated control groups were maintained for each line. Litters were fostered at birth. One male and female from each litter were tested every other day from post-natal day 7 through 21. On each test day, rectal temperatures were taken at 0, 60, and 120 min after isolation from the nest. Results show that LS and SS offspring prenatally exposed to the 8.0 g/kg/day ethanol dose had lower body temperatures at 60 and 120 min, but not at 0 min, following isolation from the nest compared to offspring from all other treatment groups ($p's < .05$). These data suggest prenatal exposure to alcohol results in thermoregulatory deficits in offspring of both alcohol sensitive and insensitive mice.

Supported in part by grant AA06939 to D.M.G. and by AA00077 to E.P.R.

22.1

BEHAVIORAL TOLERANCE AND SENSITIZATION TO CGS 19755, A COMPETITIVE N-METHYL-D-ASPARTATE RECEPTOR ANTAGONIST.
C. A. Boast, G. Pastor, S. C. Gerhardt and J. M. Liebman. Res. Dept., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Competitive N-methyl-D-aspartate (NMDA) receptor antagonists, including CGS 19755, show a variety of behavioral effects when administered acutely. To evaluate the possibility of tolerance or sensitization, a high dose of CGS 19755 (54 mg/kg i.p./injection) was administered to mice twice daily for 14 days (CGS group); a separate group of mice received vehicle (VEH group). One day after the last repeated injection, mice were challenged with vehicle or one of several doses of CGS 19755 (10, 30, 54 or 100 mg/kg) and were tested for ataxia (righting and traction reflexes), spontaneous locomotor activity and the threshold i.v. dose of NMDA required to induce convulsions. In the VEH group, challenge doses of 54 and 100 mg/kg CGS 19755 reduced motor activity. In contrast, the CGS group markedly increased motor activity in response to challenge doses of 30 and 54 mg/kg. A comparison of the VEH and CGS groups showed only slight tolerance to CGS 19755-induced ataxia. Neither the threshold dose of NMDA required to induce seizures, nor the elevation of this threshold dose by acute challenge with CGS 19755, differed significantly between groups. The divergent effects of repeated CGS 19755 in each of these measures suggest that each may be mediated by distinct neuronal systems.

22.2

MK 801 INCREASES LOCOMOTOR ACTIVITY AND INDUCES PCP-LIKE BEHAVIORS: COMPARISON OF SYSTEMIC, INTRA-ACCUMBENS AND INTRA-FRONTAL CORTEX ADMINISTRATION.
R. Gerber, G. Pastor* and J. M. Liebman. Res. Dept., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901.

The drug, MK 801, has been characterized as a noncompetitive NMDA receptor antagonist with a potentially unique site of action (Foster and Wong, Br. J. Pharmac. 91:403-409, 1987). The present experiments compared its behavioral effects in rats with those of a competitive NMDA receptor antagonist, CPP, and a dissociative anesthetic-type noncompetitive NMDA receptor antagonist, PCP, using Omnitech Digiscan animal activity analyzers. When administered i.p., MK 801 markedly increased motor activity at 0.3 mg/kg with a peak effect at 50 min post-injection, and at 1.0 mg/kg with a slower onset to peak activity. Abnormal motor behaviors induced by MK 801 resembled those associated with PCP, which also increased motor activity. In contrast, CPP at doses as high as 30 mg/kg i.p. induced only a small increase in motor activity and no abnormal behaviors other than ataxia. Intra-accumbens or intra-frontal cortex adminis-tration of MK 801 (10 ug) did not increase motor activity; solubility limitations precluded administration of higher doses. Previous studies have shown that CPP is effective at 0.3-1.0 ug but that doses of 100-300 ug PCP are required. These results associate the behavioral effects of MK 801 with those of the noncompetitive, rather than the competitive, NMDA receptor antagonists.

22.3

HYPERACTIVITY INDUCED BY INTRA-FRONTAL CORTEX INJECTIONS OF NMDA RECEPTOR ANTAGONISTS: EVIDENCE FOR PHARMACOLOGICAL SPECIFICITY AND DOPAMINE MEDIATION.
K. A. O'Neill, G. Pastor* and J. M. Liebman (SPON: B. Petrack). Res. Dept., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901.

A distinctive motor hyperactivity syndrome is induced by microinjections of competitive N-methyl-D-aspartate (NMDA) receptor antagonists, such as CPP, into the medial prefrontal cortex of rats (O'Neill and Liebman, Brain Res. 435: 371-375, 1987; Pastor et al., this meeting). The pharmacological selectivity of this effect was examined. Rats were anesthetized and bilaterally implanted with stainless steel cannulae in medial prefrontal cortex. At least one week later, they were placed in Omnitech Digiscan animal activity chambers and, following a 1 hour adaptation period, drugs were injected in 0.5 µl solution. No motor hyperactivity was induced by the local anesthetic lidocaine (10 µg), the mixed sigma and PCP agonist, SKF 10047 (30 and 100 µg) or the putative sigma receptor ligand, BW 234U (30 and 100 µg). At high doses (2.0 and 20 µg), NMDA also did not cause motor hyperactivity. Thus, it seems unlikely that this effect of CPP is mediated by local anesthetic effects, NMDA receptor-mediated neurotoxicity, or sigma or PCP binding sites. Haloperidol, at a dose (0.03 mg/kg i.p.) having minimal effects on spontaneous motor activity, markedly attenuated the effects of CPP, suggesting dopaminergic mediation of these behaviors.

22.4

LOCALIZATION OF MOTOR STIMULANT EFFECTS INDUCED BY INTRA-FRONTAL CORTEX MICROINJECTIONS OF CPP, A COMPETITIVE NMDA RECEPTOR ANTAGONIST.
J. M. Liebman, K. A. O'Neill and G. Pastor (SPON: R. G. Browne). Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Microinjections of competitive NMDA receptor antagonists, such as AP-7 or CPP, into rat frontal cortex (FC) induce an unusual pattern of locomotor activation (O'Neill and Liebman, Brain Res. 435: 371-375, 1987). The present experiments assessed the neuroanatomical selectivity of this effect. Under surgical anesthesia, rats were bilaterally implanted with stainless steel cannulae. In behavioral experiments, rats were microinjected with CPP (1 ug in 0.5 µl) or saline vehicle after 60 min of baseline motor activity recording, then were immediately replaced in the chambers and behavioral observations were initiated. Short-latency (within 20 min) increases in motor activity, and episodic darting behaviors, were associated with placements in medial FC. Slightly posterior placements, located in the anterior margins of the nucleus accumbens and corpus striatum, induced short-latency hyperactivity but no darting behaviors. Ataxia was produced by placements in nucleus accumbens and in cerebral cortex dorsal to the genu of the corpus callosum, but the latter placement yielded no increases in motor activity. In conclusion, the effects of intra-frontal cortex CPP microinjections show a high degree of neuroanatomical specificity.

22.5

ACTIVATION OF BIOGENIC AMINE TURNOVER BY MK-801.
T.A. Pugsley, S. Z. Whetzel* and C. R. Clark. Department of Pharmacology, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

The novel anticonvulsant MK-801, like phencyclidine (PCP) and ketamine (K), has been described as a non-competitive antagonist of N-methyl-D-aspartate (NMDA). These drugs exert part of their action via PCP binding sites linked to the activation of NMDA receptors. PCP and its analogs are known to act as indirect DA agonists. This study examines the effects of MK-801 on biochemical indices of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) turnover in rat brain. MK-801 (0.2-6.6 mg/kg i.p.) caused dose-dependent increases in the levels of the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) in striatum (ST), mesolimbic (ML) and frontal cortex (FC) regions as well as levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA). High doses of PCP (10 mg/kg i.p.) and K (50 mg/kg i.p.) elevated HVA levels in ML and ST, while DOPAC levels were elevated only in FC and 5-HIAA levels were generally not altered except for increases in FC. MK-801 (0.2 mg/kg i.p.) also increased the rate of DA synthesis in ST and NE synthesis in brain stem. These findings indicate that MK-801 *in vivo* accelerates biogenic amine turnover resembling somewhat agents such as amfonelic acid. However, unlike the latter, these effects of MK-801 may be mediated by NMDA receptor antagonism.

22.6

ACUTE EFFECTS OF THE ANTIDEPRESSANT/ANXIOLYTIC TRIAZALO-BENZODIAZEPINES ON CRF CONCENTRATIONS IN RAT BRAIN.
M.J. Owens*, G. Bissette and C.B. Nemeroff. Duke Univ. Med. Ctr., Durham, NC 27710.

Preclinical and clinical evidence indicates that corticotropin releasing factor (CRF) integrates the endocrine, autonomic and behavioral responses of an organism to stress and may be involved in the pathophysiology of major depression. We postulated that clinically efficacious antidepressants and anxiolytics may alter neuronal CRF.

Male Sprague-Dawley rats received i.p. injections of: vehicle, a classical tricyclic antidepressant (imipramine 10 mg/kg), or the anxiolytic/antidepressant triazalobenzodiazepines (alprazolam 1 mg/kg, or adinazolam 10 mg/kg). Animals were killed one hour later and trunk blood collected for radioimmunoassay of ACTH and corticosterone concentrations. The frozen brains were dissected into 18 brain regions. The tissues were radioimmunoassayed for CRF utilizing ^{125}I-Tyr^{0}-CRF prepared in our laboratory.

Treatment with either alprazolam or adinazolam produced significant reductions in plasma concentrations of ACTH when compared to either controls or imipramine treated rats. In agreement with this, both the alprazolam and adinazolam-treated animals had correspondingly increased hypothalamic concentrations of CRF. In addition, the concentration of CRF was significantly reduced in the locus coeruleus, amygdala, piriform cortex and cingulate cortex in both the alprazolam and adinazolam-treated animals.

We have previously demonstrated that stress decreases hypothalamic but increases locus coeruleus CRF concentrations. Thus the two triazalobenzodiazepines exert effects on CRF concentrations opposite to those of stress. The present findings support the possibility that the therapeutic anxiolytic and purported antidepressant properties of these benzodiazepines may be the result of their direct or indirect actions on CRF neurons. (Supported by NIMH MH-42088).

22.7

BRIGHT ARTIFICIAL LIGHT ADMINISTERED DURING THE REGULAR PHOTOPERIOD PRODUCES SUBSENSITIVITY TO NICOTINE. S. C. Dilsaver. Psychopharmacology Program, Department of Psychiatry, Neuroscience Program, The Ohio State University, Columbus, Ohio 43210-1228.

Eleven (11) adult, male Sprague-Dawley rats were treated with bright artificial light during the regular photoperiod for 14 days. This treatment was associated with blunting of the thermic response to nicotine.

Mean core temperature prior to the first (before treatment) and second nicotine challenges was 37.4 ± 0.7°C (n = 12) and 37.4 ± 0.2°C. Data were analyzed by assessing the change in thermic response during each of the two 60-minute periods comprising the 120-minute nicotine challenge. The mean thermic response to nicotine during the first 60-minute period was -0.47 ± 0.48°C before treatment compared to -0.07 ± 0.48°C following two weeks of phototherapy ($p < 0.02$, $t = 2.86$). the mean thermic response during the second 60-minute period before chronic administration of bright artificial light was -0.85 ± 0.68°C compared to -0.13 ± 0.59°C afterwards ($p < 0.003$, $t = 3.99$).

The effect of bright artificial light measured in this experiment is consistent with the effects of traditionally used antidepressants. The results may be related to the mechanism of action of bright light in the treatment of seasonal depression.

22.8

LACTIC ACID HAS AN ANTIDEPRESSANT-LIKE EFFECT ON RATS PERFORMING UNDER A DIFFERENTIAL-REINFORCEMENT-OF-LOW-RATE 72-SECOND SCHEDULE. J.E. Lim*, A. Li*, and L.S. Seiden. Dept. Pharm/Phys. Sci., Univ. of Chicago, Chicago, IL 60637.

The differential-reinforcement-of-low-rate 72-second (DRL 72-s) schedule is an operant procedure that has been demonstrated to be a behavioral screen for antidepressant drugs that is both sensitive and specific. Antidepressants and electroconvulsive shock, but not drugs from any other class, cause an increase in reinforcement rate and improvement in temporal discrimination in rats performing under this schedule (O'Donnell & Seiden, 1982; Seiden et al., 1985; Seiden & O'Donnell, 1985). In the present study, we examined effects of lactic acid administration on DRL 72-s performance. Lactic acid is often used as a vehicle for dissolving drugs being tested in behavioral experiments. We found that lactic acid significantly increased reinforcement rate ($F(3,9)=5.321$, $p<0.05$) in a dose-dependent manner while it did not have an effect on response rate. Lactic acid also enhanced temporal discrimination. On the other hand, neither acetic acid nor pyruvic acid, two metabolites of lactic acid, affected reinforcement rate, response rate or temporal discrimination. These results indicate that lactic acid has some direct antidepressant-like activity on the DRL 72-s behavioral screen. This research was supported by NIMH MH-11191; RSA MH-10562 (L. Seiden), and Short Term Training Grant NIH 5 T35GM08140-02 (J. Lim).

22.9

METHIONINE ENHANCES THE ANTIDEPRESSANT EFFECT OF CLENBUTEROL ON THE DIFFERENTIAL-REINFORCEMENT-OF-LOW-RATE 72-SECOND SCHEDULE. R. Dunn* and L. Seiden (SPON: R. McCrea). Dept. Pharm/Phys. Sci., U. of Chicago, Chicago, IL 60637.

Antidepressants increase the reinforcement rate and decrease the response rate of rats performing on a DRL 72-s operant schedule. Drugs from other psychopharmacological classes do not produce this same effect (Seiden et al., Psychopharmacol. 86:55, 1985). Clenbuterol, the lipophilic beta-2 adrenergic agonist produces effects on the DRL 72-s schedule similar to those produced by antidepressants (O'Donnell, J. Pharmacol. Exp. Ther. 241:68, 1987). We have found that agents affecting the one carbon cycle augment the effects of clenbuterol on the DRL 72-s schedule. Thus, methionine, betaine, and guanidoacetic acid (GAA) were found to significantly enhance clenbuterol increases in reinforcement rate and decreases in response rate. Methionine loading will increase the concentration of S-adenosylmethionine (SAM) in rat brain (Baldessarini, Biochem. Pharmacol. 15:741, 1966). Betaine is trimethylglycine, and may also increase brain levels of SAM. GAA, however, is methylated in the liver to form creatine and decreases the availability of methyl groups for the one carbon cycle. Elucidation of the biochemical basis for the enhancement of the antidepressant effect of clenbuterol on the DRL 72-s schedule by agents affecting the one carbon cycle is being pursued. This research was supported by PHS MH-14274 Training Grant; RSA MH-10562 (L. Seiden) and PHS MH-11191.

22.10

CHRONIC ANTI-DEPRESSANT TREATMENT ALTERS ELECTROPHYSIOLOGICALLY RECORDED β-ADRENERGIC BUT NOT 5-HT RESPONSES IN RAT HIPPOCAMPAL SLICES. S.G. Beck and W.P. Clarke, Department of Pharmacology, Mount Sinai School of Medicine of CUNY, New York, NY 10029-6574.

Chronic anti-depressant treatment down regulates β-adrenergic receptors, decreases β-adrenergic stimulation of adenylyl cyclase, and has been reported to increase sensitivity of 5-HT inhibition of neuronal firing in hippocampus. We investigated the effects of chronic anti-depressant treatment on electrophysiologically recorded responses mediated by 5-HT and β-adrenergic receptors in area CA1 of rat hippocampal slices in vitro. Sprague Dawley rats were treated for two weeks with 10 mg/kg imipramine or saline by i.p. injections or osmotic mini-pumps implanted subcutaneously. In contrast with previous reports we found no change in the concentration-response curves for the decrease in the extracellularly recorded population spike amplitude (PSA) mediated by the 5-HT_{1A} receptor or the increase in PSA elicited by the β-adrenergic receptor in control and anti-depressant treated animals. Intracellular recordings revealed no change in the hyperpolarization or reduction in AHP elicted by 5-HT. However, the reduction of the AHP mediated by the β-adrenergic receptor was significantly less, even though the depolarizing response was not altered. These results contrast with the acute and short term effects of imipramine on 5-HT and β-adrenergic responses (Halloran, P. & Beck, S.G., this meeting). These results are consistent with the biochemical evidence of a down-regulation of β-adrenergic receptors after chronic anti-depressant treatment. Supported by MH 41917.

22.11

EFFECTS OF ACUTE AND SHORT TERM ADMINISTRATION OF ANTIDEPRESSANTS ON ELECTROPHYSIOLOGICALLY RECORDED 5-HT AND β-ADRENERGIC MEDIATED RESPONSES. P.M. Halloran* and S.G. Beck (SPON: C.J. Molineaux), Department of Pharmacology, Mount Sinai School of Medicine of CUNY, New York, NY 10029.

Imipramine, a tricyclic antidepressant, blocks the reuptake of 5-HT and NE. Imipramine's effect as a neuronal uptake inhibitor is seen immediately whereas its antidepressant action takes several weeks. The acute and short term effects of imipramine on responses elicited by 5-HT and β-adrenergic receptors are being investigated using intracellular recording in rat hippocampal slices (CA1 region) in vitro. Acute administration involves addition of antidepressant into the perfusion medium after pyramidal cell penetration; short term administration involves injection of antidepressant (10 mg/kg) s.c. 24 hours prior to the experiment. Concentration response curves for the 5-HT mediated hyperpolarization were shifted to the left by acute imipramine treatment. Short term administration of imipramine did not alter the hyperpolarization or reduction in after hyperpolarization (ahp) mediated by 5-HT receptors or the depolarization and reduction in ahp mediated by β-adrenergic receptors. The acute and short term effects of imipramine differ from those responses following chronic administration. (See Beck et. al., this meeting). Supported by USPHS grant MH 41917.

22.12

ANTIDEPRESSANT SPECIFICITY IN THE REVERSAL OF PERFORMANCE DEFICITS IN INTRACRANIAL SELF-STIMULATION FROM MESOLIMBIC AND MESOCORTICAL SITES IN THE DBA/2J MOUSE STRAIN. Pamela Baillie*, Cindy Wolfe*, Glenda MacNeil*, Marilyn Kasian* and Robert M. Zacharko, Dept. of Psychology, Carleton Univ., Ottawa, Ont., CANADA

Previous work in this laboratory revealed that the performance alterations induced by uncontrollable stressors on intracranial self-stimualtion (ICSS) vary as a function of the strain of animal examined, the chronicity of the stressor employed as well as the brain region under investigation. We also noted that the reversal of ICSS deficits in various strains of mice was dependent on the antidepressant employed. We now report that ICSS deficits following uncontrollable footshock from both mesolimbic and mesocortical sites (i.e., frontal cortex, nucleus accumbens, ventral tegmental area) in the DBA/2J mouse strain are resistant to the effects of desmethylimipramine (DMI). While DMI appeared to be marginally effective, and in some cases detrimental, to the reversal of ICSS deficits in DBA/2J mice, amitriptyline and trazadone rapidly reinstated responding. This profile appeared consistent with both prophylactic or therapeutic administration regimens. The differences in pharmacological specificity afforded by amitriptyline and trazadone over DMI, with respect to 5-HT, presumably underlie such an effect. This interpretation would be consistent with the proposed neurochemical substrate mediating ICSS in the DBA/2J mouse.

22.13

ANTIDEPRESSANT AND OPIATE ANTAGONISTIC EFFECTS OF MIF-1 IN AN ANIMAL MODEL OF DEPRESSION. M.F. Pignatiello*, G.A. Olson, A.J. Kastin, R.H. Ehrensing*, J.H. McLean, and R.D. Olson. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148 and V.A.M.C., New Orleans, LA 70146

Given the relationship of stress and depression to the endogenous opiate system (EOS), MIF-1 was tested in Katz's chronic stress model. In this model, 70-day-old rats received either no stressors or a chronic daily protocol of a variety of stressors, during which time daily injections of MIF-1, naloxone, or control were given. After three weeks, rats were tested in an open field for activity and defecation. Five doses (0.01 to 10.0 mg/kg) of both MIF-1 and naloxone were tested. MIF-1 (0.1 and 1.0 mg/kg) showed significant antidepressant effects. No dose of naloxone was significant. MIF-1 (10.0 mg/kg) and naloxone (1.0 mg/kg) exacerbated the effects of the chronic stress. Tail-flick tests showed less analgesia with increasing doses of both compounds and more analgesia in chronically-stressed rats. In a second study, in which several compounds (MIF-1, Tyr-MIF-1, naloxone, and imipramine) were given after the chronic stress, spontaneous remission resulted for all groups within two weeks. Thus, low doses of MIF-1 showed antidepressant effects. Results also indicated that these effects of stress may be mediated by the endogenous opiate system.

22.14

EFFECTS OF THYMOLEPTIC DRUGS ON SEROTONERGIC FUNCTION IN AFFECTIVE DISORDER PATIENTS. L.H. Price, D.S. Charney, P.L. Delgado,* G.R. Heninger, Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Abnormal serotonergic (5HT) function may be involved in the pathogenesis of affective disorders. Depressed patients show blunted prolactin (PRL) responses to the 5HT precursor i.v. tryptophan (TRP), indicating diminished 5HT function. Long-term treatment with the tricyclic antidepressants (TCAs) desipramine (DMI) and amitriptyline, and short- and long-term treatment with the monoamine oxidase inhibitor (MAOI) tranylcypromine, enhance net PRL responses to i.v. TRP in depressed patients. We used the PRL response to i.v. TRP to assess the effects of short- and long-term treatment with 3 thymoleptic drugs on 5HT function in affective disorder patients. METHOD: A total of 84 patients participated after meeting DSM-III criteria. After ≥3 weeks psychotropic drug-free, patients received TRP 7 grams i.v. infused over 20 minutes. Samples for plasma PRL were obtained at intervals before and after the TRP infusion. The test procedure was repeated after <1 week and after >3 weeks of treatment with lithium (LI) (n=23), DMI (n=25), or the selective 5HT reuptake inhibitor fluvoxamine (FLUV) (n=36). PRL was measured by RIA. RESULTS: TRP significantly increased PRL. The PRL response was significantly enhanced after short-term, but not long-term, LI treatment. DMI enhanced the PRL response after long-term, but not short-term, treatment. FLUV markedly enhanced the PRL response after both short- and long-term treatment. CONCLUSION: These findings are consistent with evidence that enhanced 5HT function may be a necessary, but not sufficient, condition for efficacy for some antidepressants, such as TCA's, MAOI's, and 5HT reuptake inhibitors. Homeostatic responses of the 5HT system to long-term LI treatment may limit that drug's antidepressant efficacy. The mechanism of action of some atypical antidepressants which do not enhance the PRL response to i.v. TRP, such as trazodone and mianserin, remains to be determined.

22.15

CHRONIC ADMINISTRATION OF SERTRALINE DESENSITIZES SEROTONIN AUTORECEPTORS. J. Heym and L.S. Reynolds . Central Research Division, Pfizer Inc., Groton, CT 06340.

Sertraline is a member of a new class of antidepressants characterized by potent and selective inhibition of 5HT uptake. We previously demonstrated that sertraline inhibits 5HT neuron firing in the dorsal raphe nucleus (DRN) when administered acutely to anesthetized rats (Neurosci. Abstr. 12, 473, 1986). This effect presumably is due to elevated synaptic levels of 5HT producing negative feedback inhibition of 5HT neurons via somatodendritic autoreceptors. De Montigny et al. (Neuropharmacol. 23, 1511, 1984) have proposed that the latency for antidepressant efficacy of 5HT uptake inhibitors observed in the clinic may reflect 5HT autoreceptor desensitization, a process which permits greater enhancement of 5HT neurotransmission to be achieved over time. The present studies were conducted to see if chronic exposure to sertraline produces an alteration of 5HT autoreceptor sensitivity.

Male Sprague-Dawley rats were implanted with osmotic pumps set to deliver either sertraline (10 mg/kg/day) or vehicle by continuous s.c. infusion for 14 days. On day 14 electrophysiological recording from the DRN was carried out under chloral hydrate anesthesia using conventional extracellular techniques. Despite continued exposure to sertraline, spontaneously active units matching the criteria for 5HT cells were found. When the inhibitory effect of the 5HT autoreceptor agonist 8-OH-DPAT was assessed by i.v. injection, 5HT neurons recorded from sertraline treated animals were 3 - 5 fold less sensitive than those recorded from rats receiving vehicle infusion.

These data suggest that chronic exposure to sertraline does desensitize 5HT autoreceptors thereby attenuating negative feedback inhibition of 5HT neurons. Under such circumstances a maximal enhancement of 5HT function in forebrain can be achieved which may be critical for antidepressant efficacy.

22.16

DOWN-REGULATION OF β-ADRENOCEPTORS IN RAT BRAIN BY SERTRALINE: EFFECTS OF CO-ADMINISTERING SEROTONERGIC AGENTS. B. K. Koe and L. A. Lebel*. Central Research Division, Pfizer Inc., Groton, CT 06340.

Recent studies demonstrated that sertraline, a new antidepressant and selective 5-HT uptake blocker, desensitizes the cyclic AMP generating system of limbic forebrain (Koe et al., 1983, 1987) and down-regulates β-adrenoceptors of cerebral cortex (Byerley et al., 1987; Koe et al., 1987). Moreover, these effects of sertraline appeared to be facilitated by co-administration of the 5-HT agonist quipazine. Our present studies included the co-administration of other serotonergic compounds with sertraline, such as 8-HO-DPAT (5-$HT1_A$ agonist), TFMPP (5-$HT1_B$ agonist), ritanserin (5-HT_2 antagonist), methiothepin (5-HT releaser), racemic norfenfluramine (5-HT releaser) and gepirone (5-$HT1_A$ agonist). Experiments consisted of i.p. injection of test drug plus sertraline to rats, b.i.d., for 4 days and determining K_D and B_{max} of [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding in homogenates of anterior cortex one day after dosing. We found that down-regulation of β-adrenoceptors by sertraline was neither facilitated nor antagonized by co-administered 8-HO-DPAT, TFMPP or ritanserin. On the other hand, methiothepin, norfenfluramine or gepirone, in combination with sertraline (17.8 μmol/kg; non-down-regulating dose), elicited a decrease in receptor density without affecting affinity of [^{3}H]DHA binding. Our findings indicated that other ways of enhancing serotonergic activity can facilitate down-regulation of β-adrenoceptors by sertraline.

22.17

EFFECTS OF IMIPRAMINE ON THE NOCTURNAL BEHAVIOR OF OLFACTORY BULBECTOMIZED (OB) RATS R.J. Radek* and W.J. Giardina Dept. of Pharmacology, Abbott Laboratories, Abbott Park, IL 60064.

After removal of olfactory bulbs, rats show a marked increase in locomotor activity in novel environments. The chronic administration of imipramine and other antidepressants reduces this hyperactivity. The present study assessed the effect of imipramine on OB rat activity in the undisturbed home cage environment. Seven days after surgery, sham operated and OB rats were placed in Omnitech Animal Activity Monitor cages (42x42x30cm) and maintained on a 13 hour light - 11 hour dark cycle. Food and water were provided ad lib. Locomotor activity was recorded hourly for up to 18 days. OB rats were significantly more active during the dark phase than sham operated rats. The dark phase hyperactivity persisted throughout the observation period. Both sham and OB rats were inactive during the light phase. After 7 to 10 daily injections of imipramine at 10.0 or 15.0 mg/kg, i.p., activity of OB rats was significantly reduced during the dark phase. This reduction persisted until imipramine was withdrawn. Imipramine (10.0 mg/kg, i.p.) had no effect on the activity of sham rats. The delayed effect of imipramine in OB rats mimics its delayed antidepressive effect in man. This experimental approach may be useful in evaluating the onset of action of antidepressants.

22.18

DOWN-REGULATION OF BETA-ADRENERGIC AND SEROTONINERGIC RECEPTORS IN OLFACTORY BULBECTOMIZED RATS FOLLOWING SUB-CHRONIC IMIPRAMINE ADMINISTRATION. A.A. Hancock, R.J. Radek*, W.J. Giardina, and J.F. Debernardis*. Department of Pharmacology, Abbott Laboratories, Abbott Park, IL 60064.

Chronic treatment of normal rats using various antidepressants has been shown to reduce the concentration of both beta-adrenergic (B) and serotoninergic ($5HT_2$) receptors. We have evaluated B and $5HT_2$ receptors in sham operated and olfactory bulbectomized (OB) rats using 10 concentrations each of either ^{125}I-Cyanopindolol or ^{3}H-Ketanserin. After 10 days administration of imipramine (10 mg/kg, i.p.) both sham operated and OB rats possessed 10 and 15% fewer B receptors, respectively, while $5HT_2$ receptors were decreased 25 and 20%, respectively. No changes were observed in the affinity of the radioligands for their receptors. R. J. Radek and W. J. Giardina reported (see "Effects of Imipramine on the Nocturnal Behavior of OB rats", this proceedings) that 7-10 daily injections of imipramine (10 mg/kg, i.p.) reversed the hyperactivity of OB rats in the home cage environment. Together, these two studies suggest that it may be possible to relate changes in receptor concentrations with the onset of behavioral changes in the OB rat after antidepressant treatment.

23.1

COMPARISON OF NMDA ANTAGONISTS ON LOCAL CORTICAL BLOOD FLOW IN THE RAT. M.E. Mann, P.A. Boxer and F.W. Marcoux. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

The dissociative anesthetic ketamine has been shown to increase cerebral glucose utilization and blood flow unlike most other centrally acting anesthetics which are metabolic depressants. We compared the effects of ketamine and MK-801 on local cortical blood flow (LCBF) in Sprague-Dawley rats (350-450 g). Rats were instrumented with a chronically implanted platinum-iridium electrode (125μ diameter) in the anteroparietal cortex. LCBF was measured by H_2 clearance in unanesthetized, freely moving rats housed in plexiglass chambers. Ketamine (IP) at the subanesthetic 25 mg/kg dose produced LCBF increases which were of short duration (<90 min). The increase in LCBF was less with the 62.5 mg/kg dose and the anesthetic 150 mg/kg dose. MK-801 (IP) produced LCBF increases of long duration (>2 hrs) which were equivalent at 0.2, 0.66 and 2.0 mg/kg doses; gross behavioral disruption, but not anesthesia, was noted at these doses. Ketamine at 4.2 mg/kg and MK-801 at 0.02 mg/kg had little or no effect on LCBF. Both of these noncompetitive NMDA antagonists increased LCBF, but with different profiles of activity, which may be related to their different anesthetic properties.

23.2

CHOLINERGIC MODULATION OF FRONTAL CORTICAL BLOOD FLOW IN RATS. J.J. Kinsora*, C. Bay-Gemmill* and R.E. Davis (SPON: J.P. Symons). Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Cholinergic agents have been shown to increase local cortical blood flow (LCBF). It is not known, however, if cholinergic-induced changes in LCBF are mediated by central or peripheral cholinergic action. In addition, the brain areas mediating central cholinergic alterations in LCBF also are not known. However, because basal forebrain neurons provide a major source of cholinergic afferents to the cortex, this area is likely to contribute to cholinergically-driven changes in LCBF. The purpose of this experiment was to determine the effects of centrally and peripherally acting cholinergic agents on LCBF and to determine the role of basal forebrain cholinergic neurons on LCBF under basal and cholinergically- driven conditions.

LCBF was measured indirectly using the H2 clearance technique from bilaterally or unilaterally implanted platinum-iridium electrodes placed in the frontal cortex of male, Long-Evans rats. At least 4 weeks prior to measurement of LCBF some rats were infused unilaterally with the excitotoxin ibotenic acid (1.0ug in 1.0ul) or an equivalent volume of vehicle (0.9% saline, sham operated) through cannulae directed at basal forebrain cholinergic neurons (BF). Two 15 minute baseline LCBF measurements were taken prior to administration of a variety of cholinergic agents. Following administration of these agents LCBF was measured every 15 minutes for the next 1 to 2 hrs.

Cholinomimetics induced increases in LCBF which were blocked by scopolamine but not by methylscopolamine. This suggests that these agents act centrally to increase LCBF. Baseline LCBF was not altered by BF lesions. In sham operated rats, cholinomimetics induced bilaterally symmetrical increases in LCBF in the frontal cortex. Similar increases in LCBF were induced by these agents on the side contralateral to the BF lesion in unilaterally lesioned animals. In contrast, there was a dose dependent decrease in the ability of cholinesterase inhibitors but not direct agonists to increase LCBF on the side ipsilateral to the BF lesion.

23.3

EFFECTS OF YOHIMBINE ON REGIONAL CEREBRAL BLOOD FLOW IN PATIENTS WITH PANIC DISORDERS. SW Woods, K Koster*, JH Krystal, EO Smith*, IG Zubal*, PB Hoffer*, DS Charney. Clinical Neuroscience Research Unit, Yale University School of Medicine, New Haven, CT 06508

Previous studies have demonstrated increased behavioral, cardiovascular, and biochemical responses in patients with panic disorder (PD) to the alpha-2 adrenergic antagonist yohimbine (YOH). In order to examine possible alterations in regional blood flow (rCBF) induced by YOH in PD, 4 drug-free patients (PTS) with PD (mean ± SD = 26 ± 2 yrs) and 4 healthy subjects (HS) (24 ± 2 yrs) in an ongoing study each gave informed consent and received intravenous YOH 0.4 mg/kg and saline placebo (PLA) over 10 minutes in random sequence on separate days, followed immediately by injection of the rCBF radiopharmaceutical Tc-99m d,l- hexamethylpropyleneamine oxime (20 mCi). Injections were performed with eyes closed in a dimly lit, quiet room. One hour later subjects underwent single photon emission computed tomography scanning. Using a neuro-dedicated multicrystal camera, ten transaxial images spaced 10 mm apart and starting from and parallel to the orbitocanthal-meatal line were obtained per study. Seven bilateral regions of interest (ROI), (frontal,parietal, temporal, and visual cortices, striatum, thalamus and cerebellum) were identified independently by two investigators, blinded to drug or placebo, using CT scans and a brain atlas of identical cerebral levels. Reported values are the mean of the two determinations. Ratios of ROI/cerebellum and asymmetry indices were calculated on the average counts per pixel. Greater increases in self-rating scales of anxiety occurred after YOH than after PLA in all PTS and one HS. Decreases in frontal cortex to cerebellar bloodflow ratios were observed in all PTS on the YOH day relative to PLA (10.8 ± 9.7 and 11.2 ± 7.7 % on left and right, respectively. In contrast, frontal rCBF on YOH and PLA days was similar in the HS. YOH-induced shifts in thalamic blood flow symmetry were also observed consistently in the PTS but not in the HS. No robust changes were identified in other cortical areas or striatum in either group. These preliminary data may suggest roles for altered noradrenergic regulation of frontal cortical and thalamic function or perfusion in the pathophysiology of PD.

23.4

EFFECT OF HYPOCAPNIA ON REGIONAL CEREBRAL GLUCOSE UTILIZATION. Satwant K. Samra, M.D.*, Patricia Turk*. (SPON: W.D. Willis, Jr., M.D., Ph.D.). Department of Anesthesiology, University of Texas Medical Branch at Galveston, Texas 77550-2778.

Deliberate hypocapnia is a common clinical practice in the management of patients with raised intercranial pressure. While effect of hypocapnia on cerebral blood flow has been well studied, only two recent studies have looked at effect of blood carbon dioxide tension ($PaCO_2$) on regional cerebral glucose utilization (rCMRg). Both looked at the effect of *hypercapnia* only and reported a significant decrease in rCMRg with *hypercapnia*. This study reports the effect of *hypocapnia* on rCMRg. Institutional approval of the protocol was obtained and guidelines of NIH for the care and use of laboratory animals were followed. Fourteen Sprague-Dawley male rats were randomly assigned to either normocapnia (n=7, $PaCO_2$=40±2mm) or hypocapnia (n=7, $PaCO_2$=25±2mm) group. Rats were anesthetized with N_2O and oxygen and mechanically ventilated to achieve desired $PaCO_2$. rCMRg was measured by the [^{14}C] deoxyglucose method described by Sokoloff et al. rCMRg in 26 brain structures was studied. Values of rCMRg in normocapnic group were comparable to those previously reported by Sokoloff in awake rats. Hypocapnic group showed slightly higher values in all structures studied but in none of the structures was the difference statistically significant.

23.5

DEVELOPMENT OF EARLY COLLATERAL BLOOD FLOW TO CEREBRUM IS COMPROMISED IN CHRONIC HYPERTENSION. P. Coyle. Dept. of Anatomy and Cell Biology, The University of Michigan, Ann Arbor, MI 48109.

The objective of this study was to measure collateral blood flow to cerebrum by laser Doppler flowmetry during 20-30 seconds (sec) of ipsilateral common carotid artery occlusion in stroke-prone spontaneously hypertensive rats (SHRSP) and normotensive Wistar rats (NW). Blood pressure (BP) was measured bilaterally in the external carotid artery. Blood flow (ml/minx100gm) and BP (mm Hg) results:

Strain	n	Before (control)		During (15sec)		After (15sec)	
		flow	BP	flow	BP	flow	BP
SHRSP	11	111±5	180±9	57±4*	90±9*	120±5	176±10
NW	10	118±3	122±4	98±12	53±5*	129±4	123±5

BP and blood flow ipsilateral to the occlusion fell to minimal levels within seconds of the occlusion. Blood flow escaped to control levels by 15 sec of occlusion in NW, but in SHRSP blood flow failed to escape ($*p < 0.05$). Treatment with eserine prior to occlusion produced escape in SHRSP and accelerated escape in NW. Atropine blocked the escape in NW and was without significant effect in SHRSP. After chronic occlusion the anastomosing collaterals were enlarged. Thus after common carotid artery occlusion in chronic hypertension, blood flow through collateral vessels to cerebrum is impaired but there is sensitivity to a cholinergic vasodilatory mechanism.

23.6

USE OF [6-^{3}H]DEOXYGLUCOSE IN THE MEASUREMENT OF LOCAL CEREBRAL METABOLIC RATE FOR GLUCOSE . H. Nakanishi*, B. Agranoff, C.Kennedy, C.B. Smith and L.Sokoloff. LCM, NIMH, Bethesda, MD & NSL, MHRI, U. of Mich., Ann Arbor, MI

The autoradiographic deoxyglucose (DG) method for the determination of LCMRglu usually employs [^{14}C]DG as the radiolabeled tracer. [^{18}F]Fluorodeoxyglucose and [^{3}H]DG have also been used, particularly in conjunction with [^{14}C]DG in a sequential double label experimental design in which an experimental animal may serve as its own control. For quantification it is essential that the 2 isotopic forms of DG are metabolized identically. In the present studies, mixtures of [1-^{14}C]DG and [1,2-^{3}H]DG were injected IV into rats, and the time course of the plasma [^{3}H]/[^{14}C] ratio was determined. The ratio increased with time, reaching 112% of the zero time value by 60 min. If samples were dried before counting, the ratio at 60 min approached the zero time level. A possible explanation is that there is an exchange of ^{3}H from the C2 position of DG with water and that clearance of [^{3}H]water from plasma is slower than that of DG. To circumvent this problem, similar experiments were carried out in which [6-^{3}H]DG (custom-synthesized by ARC, St. Louis, MO.) and [1-^{14}C]DG were simultaneously injected. The plasma ^{3}H/^{14}C ratio remained constant throughout the 60 min time course. These results show that in sequential double label experiments the use of [6-^{3}H]DG is preferable to any DG with ^{3}H in the C2 position.

23.7

DIRECT ESTIMATION OF REGIONAL LUMPED CONSTANT BY DYNAMIC POSITRON TOMOGRAPHY WITH LABELED FLUORODEOXYGLUCOSE IN HUMANS. *A. Gjedde,* H. Kuwabara**. (SPON: *G.W.Dauth*) McConnell Brain Imaging Unit, Montreal Neurological Institute, McGill University, Montreal, Canada H3A 2B4

The lumped constant (Λ), ratio between net brain uptake of positron-labeled fluorodeoxyglucose (FDG) and glucose, must be known to calculate the glucose utilization by the FDG method. Assuming symmetrical transport across the blood-brain barrier and constant ratios for transport (τ) and phosphorylation (π) of FDG and glucose, $\Lambda = \tau\rho + \pi(1-\rho)$ where ρ is the ratio of net to unidirectional uptake of FDG (K/K_1). Michaelis-Menten kinetics indicated that the ratio between transport rates in the two directions across the BBB (K_1/k_2) must be the same for FDG and glucose. Thus, $k_2 + k_3 = (K_1 + [K\tau\gamma/\Lambda])/[(1-\rho)V_d]$ where γ is the ratio between the plasma glucose concentration and Michaelis constant, and V_d the brain water content. The relationship allowed us to reduce the number of the parameters in the operational equation from 4 to 3, i.e. K, K_1, and plasma volume V_p. In seven healthy elderly males (age 63 ± 6), cortical regions were analyzed assuming $\tau = 1.1, \pi = 0.25, K_t = 4.8$ (mM), and $V_d = 0.77$ (ml/g). Despite the constraint, the fit was as good as that of four parameters. Λ averaged 0.55 ± 0.04 and varied from 0.49 to 0.60 between subjects. The transfer coefficients averaged: K_1 0.09±0.02 (ml/g/min), k_2 0.21±0.04 (min^{-1}), k_3 0.12±0.04 (min^{-1}). V_e, which often exceeded the physiological limit (0.77) in the four-parameter fits, was 0.44±0.03 and strikingly uniform.

23.8

BRAIN SLICE 2-DEOXYGLUCOSE (2DG) METABOLISM. G. Newman, F. Hospod, C. Patlak. Depts. of Neurology and Neurological Surgery, SUNY, Stony Brook, NY 11794.

Our kinetic analysis of brain slice 2DG metabolism differs from prior *in vivo* studies insofar as we incubate at constant glucose and 2DG, measure 2DG and 2DG6P separately rather than total radioactivity, and obtain 8 to 10 slices (and time points) per animal. Hypothalamic or hippocampal brain slices are pre-incubated in K-R, and then either exposed to 0.3 μCi of ^{14}C-2DG for 3 to 90 min followed by a very brief rinse for uptake experiments or exposed to radioactive tracer for 60 min followed by 0.5 to 300 min rinses for washout experiments. Frozen slices are extracted with perchloric acid and the 2DG and 2DG6P separated on anion exchange resin before scintillation counting. The four resulting data curves (2DG and 2DG6P, uptake and washout) are then analyzed by simultaneous non-linear least squares analysis using the solutions of differential equations for kinetic models based upon particular biochemical hypotheses of the fate of the 2DG tracer.

Our results reveal a previously unidentified kinetic compartment. Entry into the compartment follows phosphorylation of 2DG to 2DG6P with a rate constant similar to that usually ascribed to the phosphatase reaction in prior *in vivo* 2DG studies. However, efflux from this compartment is sufficiently slow that the radioactivity is effectively trapped in the tissue. The chemical form of the trapped material is unknown at present but appears in our assays as 2DG following acid extraction of the tissue. De-phosphorylation with rapid efflux from the tissue appears negligible and so that the model can be reduced to a 5 parameter model which closely fits all of the data for 1000μ hypothalamic or hippocampal slices and 540μ hypothalamic slices. An alternative five parameter model based upon sequential organellar transport of 2DG6P and de-phosphorylation with rapid efflux of 2DG fits the data poorly.

23.9

PHOTIC STIMULATION CAUSES ENHANCED GLYCOLYSIS IN THE SUPERIOR COLLICULUS. W.P. Pulsinelli, R.P. Kraig. Cornell Univeristy Medical College, New York, N.Y. 10021

Recent studies (Fox and Raichle PNAS, 1986; Collins et al., J. Neurochem., 1987) question the classic teaching that the energy needs of physiologic cerebral activity are met largely by oxidative-phosphorylation. We addressed this question by comparing lactate (Lac) production in the superior colliculi (S.C.) of rats subjected to unilateral photic stimulation. The left eye of halothane anesthetized, mechanically ventilated adult rats was stimulated photically at 20 Hz while the right eye was patched. These conditions caused a significant increase of glucose metabolism in the right superior colliculus. The brains of these animals (n= 10) were frozen in situ and lactate, ATP and phosphocreatine (PCr) levels were measured in 5 sets of pooled tissue dissected from the superior gray zone of the left and right superior colliculi.

	Left S.C.	Right S.C.	Paired t-test
Lac	1.98±0.24	2.71±0.29	p = 0.0006
ATP	2.13±0.12	1.95±0.28	p = 0.2096
PCr	5.04±0.15	4.59±0.61	p = 0.5239

The results demonstrate significantly higher levels of lactate in the stimulated superior colliculus, but no change in ATP or PCr levels. We conclude that increased energy demands in the superior colliculus associated with physiologic stimuli are at least partially met by enhanced glycolysis not coupled to oxidative phosphorylation.

23.10

CEREBRAL MATURATION OF OXIDATIVE METABOLISM IN THE CAT. *D. A. Hovda, H. T. Chugani*, B. Badie*, M. E. Phelps, and J. R. Villablanca, Depts. of Psychiat., Anat., Neurol., and Div. of Nuc. Med. and Biophys., UCLA Sch. Med., Los Angeles, CA 90024*

Optical density readings were obtained from cat brain tissue processed for cytochrome oxidase histochemistry at postnatal days 7, 30, 45, 60, 90, 120, 180 and adulthood. The results indicated: (1) most structures reached adult values by 30d with some motor structures (i.e., cerebellum, s. nigra, VPL thalamus, and red n.) exhibiting adult values by 7d; (2) thereafter, a number of structures exceeded adult values as follows: e.g., motor cortex and basal ganglia (by 24% peaking at about 90d); red n. (by 48% at about 30d); s. colliculus (by 37% at about 60d); visual cortex and lateral geniculate n. (by 8% peaking at 45-90d); (3) some structures did not show a substantial increase over adult rates (e.g., limbic system areas, dentate n., s. nigra). These results indicate an earlier maturation of cerebral oxidative metabolism than we previously described for glucose utilization (Soc. Neurosci. Abst., 13:1139, 1987). However, like for glucose utilization, some structures exceed adult values of oxidative metabolism during development. (NIH, PO1 NS 15654-09S1, HD 05 958.)

23.11

POSITRON EMISSION TOMOGRAPHY MEASUREMENT OF HUMAN REGIONAL CEREBRAL GLUCOSE UTILIZATION FOLLOWING 48 HOURS OF SLEEP DEPRIVATION. M. Thomas*, H. Sing*, G. Belenky, D. Thorne*, T. Balkin*, D. Penetar*, D. Redmond*, J. Norcross*, & P. Newhouse. Department of Behavioral Biology, Walter Reed Army Institute of Research, Washington, DC 20307-5100; H. Mayberg, H. Holcomb, B. Sadzot*, R. Dannals*, J. Links*, & H. Wagner*. Divisions of Nuclear Medicine and Radiation Health Sciences, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

We measured regional cerebral glucose utilization using Fluorine-18 2-deoxyglucose (FDG) and positron emission tomography (PET) in six right-handed normal male subjects (Ss), ages 21-29, in two conditions: 1) after 48 hours during which the Ss were totally deprived of sleep; and 2) after 48 hours during which the Ss were permitted 7 hours of sleep each night. Magnetic resonance imaging (MRI) scans were obtained on all Ss and used to register the PET images. Ss served as their own controls, and were scanned twice, in counterbalanced fashion, once while sleep deprived and once while rested. During the injection and uptake of the FDG, Ss performed a continuous performance task and were instrumented for polysomnographic evaluation (EEG, EMG, and EOG) of level of alertness. We are currently analyzing the data with regard to between condition differences in: 1) regional cerebral glucose utilization; 2) performance on the continuous performance task; and 3) polysomnographically defined level of alertness.

23.12

SENSORY-MOTOR ACTIVATION OF BRAIN AREAS IN NORMALS AND SCHIZOPHRENICS AS MEASURED BY PET. J. D. Brodie*, E. Bartlett*, F. Barouche*, S.L. Dewey, A. P. Wolf*, (P. Brink, Spon), NYU Medical Center, New York, NY 10016; Brookhaven National Laboratory, Upton, NY 11973

Brain areas activated by specific sensory-motor functions were identified in normal and schizophrenic subjects using positron emission tomography and ^{11}C-2-deoxyglucose to measure changes in cerebral glucose metabolism. In normals, the SIMPLE finger-tapping produced significant ($p<0.01$) increases from BASELINE metabolism in the contralateral sensory-motor hand area left and right basal ganglia and thalamus. COMPLEX finger movement produced significant increases in the supplementary motor area as well as contralateral sensory-motor hand area, left and right basal ganglia and thalamus. The schizophrenics showed a normal pattern of cortical activation during simple finger-tapping, but showed higher than normal metabolism in thalamus. During the complex finger movement, the schizophrenics failed to show activation in the supplementary motor area. These results are consistent with known patterns of metabolic dysfunction in schizophrenia (i.e., hypofrontality; increased subcortical metabolism) and demonstrate the potential effectiveness of these sensory-motor tasks in eliciting metabolic patterns that may prove to be pathognomic for the disease. Research supported by DOE, OHER, NIH Grant MH-42647.

23.13

ENERGY METABOLISM, HEMOGLOBIN SATURATION, AND CYTOCHROME c OXIDASE REDOX RESPONSES IN THE BRAIN OF NICOTINE TREATED RATS. Avis L. Sylvia. Dept. of Physiology, Duke University Medical Center, Durham, N.C. 27710

Anesthetized rats were subjected to acute iv injection or constant infusion of nicotine (dose range 2-1000μg/kg). Differential wavelength visible spectrophotometry was used to measure changes in cytochrome oxidase (CYT a,a3) redox state, hemoglobin saturation (HbO2/Hb), and blood volume in the parietal cortex of skull intact animals. EEG activity and systemic arterial pressure were concurrently monitored. In vitro measurements of changes in cortical metabolites (glucose, pyruvate, lactate, PCr, ATP, ADP) were made in brain samples from normoxic (30%O2/70%N2)rats subjected to constant iv nicotine infusion. Doses of nicotine were tested in normoxic, hyperoxic (100%O2), and hypoxic (12%O2/balance N2) rats. Findings from these studies include: 1) Nicotine does not significantly alter cerebrocortical bioenergetics as evaluated by direct metabolite measurements; 2) Low doses of nicotine cause increased CYT a,a3 oxidation and Hb oxygenation while high doses produce increased CYT a,a3 reduction, Hb deoxygenation, and elevated blood volume; 3) These responses to nicotine are independent of increases in arterial pressure and are O2-dependent in vivo; 4) Constant iv nicotine infusion appears to cause down regulation of CNS nicotinic receptors resulting in inhibition of both the cytochrome redox and microvascular responses. (Supported by STRC Grant-0096).

23.14

CROSSED CEREBELLAR AND UNCROSSED BASAL GANGLIA DIASCHISIS IN ALZHEIMER'S DISEASE BY POSITRON EMISSION TOMOGRAPHY. P.L. McGeer, H. Akiyama*, R. Harrop*, E.G. McGeer, R. Peppard* and the UBC-TRIUMF PET Team. Kinsmen Lab, Dept Psychiatry, Univ of British Columbia, Vancouver, Canada, V6T 1W5

We analyzed PET-fluorodeoxyglucose scans for diaschisis on a series of 26 consecutive demented patients meeting the clinical and radiological criteria of Alzheimer's disease (SD) and 10 age-matched controls. The local cerebral metabolic rates for glucose (LCMR) were calculated for corresponding left and right areas in numerous regions of interest (ROI) in the cerebral cortex, as well as the basal ganglia, thalamus and cerebellum. An asymmetry index (AI) was calculated for each ROI: AI = $[LCMR_L-LCMR_R]/[LCMR_L+LCMR_R]$. The correlation between the AI for each cortical area and the AI for each subcortical area was calculated by linear regression analysis. In SD significant negative correlations ($p<0.0005$ in each case), suggesting crossed diaschisis, were found between AIs for the frontal, parietal, angular and temporal cortices and AIs for cerebellar hemispheres. A significant positive correlation ($p<0.001$), suggesting uncrossed diaschisis, was found between AIs in the frontal cortex and AIs in the basal ganglia. Autopsy examination established SD in 5 of the cases and demonstrated that the depressed LCMR in the basal ganglia and cerebellum was not due to primary degeneration, but was a genuine result of diaschisis. (Supported by grants from the MRC of Canada and the Alzheimer Society of B.C.)

23.15

EFFECT OF DEγE (ORG 5878) ON REGIONAL CEREBRAL GLUCOSE UTILIZATION IN FREELY MOVING RATS. P. Room*, J.A.D.M. Tonnaer and Th. de Boer*. Dept. CNS Pharmacology, Organon International B.V., Oss, The Netherlands.

The effect of the potential antipsychotic non-opioid β-endorphin (βE) fragment desenkephalin-γ-endorphin (DEγE, βE_{6-17}) has been studied on local cerebral glucose utilization (LCGU). Two modifications were introduced to the classical ^{14}C-Deoxyglucose technique as described by Sokoloff (J. Neurochem. 28:897,1977). First, the animals were cannulated one week prior to the experiment to allow blood sampling and/or drug administration under unrestrained conditions. The second modification involved the image analysis program and included superposition of autoradiogram and identical histologically-stained section in order to improve the neuroanatomical resolution. Male Wistar rats acutely received either DEγE (20 μg/kg, i.v.) or saline and LCGU was measured in about 150 brain structures. The majority of brain areas were unaffected by DEγE, including areas of the cortex, the thalamus and the nigro-striatal system. However, significant reductions were observed in the medial septum and diagonal band complex, the hippocampal formation, the ventral tegmental area (VTA) and in the cerebellum. These data indicate that, whereas the nigrostriatal, serotonergic and cortical/thalamic systems remained unaffected by the dose of DEγE used, parts of the cholinergic and mesolimbic dopaminergic systems showed reduced glucose utilization, indicating that DEγE's action on LCGU is distinct from those of antipsychotics currently used in the clinic.

BIOLOGICAL RHYTHMS: NEUROANATOMICAL ASPECTS

24.1

CROSS-SPECIES TRANSPLANTATION OF FETAL HYPOTHALAMIC TISSUE RESTORES CIRCADIAN LOCOMOTOR RHYTHM TO SCN-LESIONED HOSTS. P.J. Sollars and D.P. Kimble, Institute of Neuroscience, University of Oregon, Eugene, OR 97405

It has long been recognized that complete lesions of the hypothalamic suprachiasmatic nucleus (SCN) abolish the circadian locomotor rhythm in hamsters, and it has recently been reported by several laboratories that homografts of fetal hypothalamic tissue into SCN-lesioned hosts restore the circadian rhythm of locomotor activity. We have attempted a cross-species transplantation of whole tissue grafts using fetal mouse (Mus musculus) hypothalamus as donor tissue and SCN-lesioned hamsters (Mesocricetus auratus) as hosts, in order to examine the possibility that the resultant rhythm manifests a species-specific characteristic (i.e., period length) determined either by the donor or by the host species.

While these are on-going experiments, we report herein the results of two completed preliminary trials. The heterograft tissue has survived in the third ventricle of the host brain for up to two months. A circadian rhythm of locomotor activity has developed between five and thirty days after the implantation surgery. In all cases to date, the resultant rhythm has had a period shorter than 24 hrs. This period is similar to that expressed by the intact mouse and by homograft transplants of dispersed cell suspensions, and is distinct from the period expressed by intact hamsters and by hamster whole tissue homografts. Supported by the Medical Research Foundation of Oregon.

24.2

IS THE FETAL SCN SUFFICIENT TO INDUCE RHYTHM RECOVERY WHEN TRANSPLANTED TO SCN-LESIONED HAMSTERS? R.A.Aguilar-Roblero*, L.P. Morin and R.Y.Moore. (SPON: R. Salceda). Depto. de Neurociencias, IFC, UNAM, México and Depts. of Neurology and Neurobiology, HSC SUNY at Stony Brook, USA.

Transplantation of fetal suprachiasmatic nuclei (SCN)induce restoration of circadian rhythms in previously arrhythmic SCN-lesioned hamsters. The present study attempted to obtain additional information regarding the mechanisms involved in restoration of rhythmicity. Fifteen male golden hamsters (90-110 gr) which sustained electrolytical lesion aimed to the SCN showed loss of the wheel running circadian rhythm (CR). Fetal anterior hypothalamic tissue (E-15) was transplanted to the third ventricle of 9 of the SCN-lesioned animals, the remaining 6 were used as long term lesion controls.

Restoration of CR after transplantation of hypothalamic tissue was found in 6 animals, in these cases the SCN within the graft was identified by immunohystochemistry. The grafted tissue was found either in the third or the lateral ventricles. Three animals did not recovered CR in spite that 2 of these showed clear development of the SCN in the graft. In these 3 cases the graft was found in the third ventricle. No spontaneous recovery of rhythmicity was found in SCN-lesion animals.

The present results indicate that recovery of rhythmicity does not depends only of the presence of the SCN in the graft nor the placement of the graft in the third ventricle. (Supported by NIH grant NS-16304 to RYM).

24.3

TIME COURSE OF PEPTIDERGIC EXPRESSION IN SUPRACHIASMATIC NUCLEUS TRANSPLANTED INTO 3rd VENTRICLE AND IN AGE-MATCHED INTACT HAMSTERS. M.-T. Romero and R. Silver. Psychology Department, Barnard College of Columbia University, New York, NY 10027.

Fetal tissue containing the SCN implanted into the 3rd ventricle of hamsters made arrhythmic by SCN lesions restore free-running rhythms of locomotor activity (Lehman, M.N., et al., J. Neurosci.. 7:1626, 1987). The grafted tissue of recovered animals express neuropeptides characteristic of the normal SCN. Behavioral recovery usually occurs 2 to 6 weeks post-implantation.

We used immunocytochemistry to determine the time course of peptidergic expression in the SCN grafted into the 3rd ventricle of adult hosts compared to the SCN in situ. VIP, VP and NPY immunoreactivity was examined at regular intervals post-implantation and in same age neonatal SCN. Preliminary results indicate that the grafted SCN expresses some neuropeptides within 2 to 3 weeks after implantation, which is within the time frame of behavioral recovery. (Supported by NIH grant NS24292 to RS.)

24.4

NEUROGENESIS OF THE SYRIAN HAMSTER SUPRACHIASMATIC NUCLEUS. F.C. Davis, R. Boada* and J. LeDeaux*. Dept. of Biology, Northeastern Univ., Boston, MA 02115 and Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

The relationship between the initiation of circadian pacemaker function and the development of the suprachiasmatic nucleus (SCN) is not understood. In the hamster, entrainment of a circadian pacemaker appears to occur on or before day 14 of gestation. Crossland and Uchwat (Develop. Brain Res. 5:99, 1982) reported that SCN neurogenesis occurs only 1-3 days earlier. The present study was done to confirm and extend this finding. Pregnant hamsters (Mesocricetus auratus) were given single injections of 3H-thymidine at 7 times from 10.5 to 13.5 days postfertilization. Brains of 33 pups were processed for autoradiography. Labelled cells were mapped by superimposing a 9x10 grid on the SCN. Greatest labelling occured on days 11, 11.5 and 12. Maps of labelled cells indicated that the ventrolateral SCN was formed before the dorsomedial SCN. This gradient suggests that functionally distinct cell populations are produced at different times (e.g. VIP vs VP cells) and that the fetal cell population selected for transplantation or for culture might be biased by the precise time when tissue is obtained. The results indicate that either the SCN need not be formed for entrainment to occur or that entrainment can occur within only two days after SCN neurogenesis. Supported by NIH grant HD18686 to FCD.

24.5

DEVELOPMENT OF THE RETINOHYPOTHALAMIC TRACT IN THE RAT. Joan C. Speh and Robert Y. Moore. Depts. of Neurology and Neurobiology, State University of New York, Stony Brook, NY 11794-8121,

The retinohypothalamic tract (RHT) is a visual pathway from the retina to the suprachiasmatic nucleus (SCN) of the hypothalamus which participates in the entrainment of circadian rhythms. Recent studies using cholera toxin conjugated to HRP (CT-HRP) indicate that the rat RHT has three components: 1.a dense projection to the ventrolateral SCN with some extension into the dorsomedial portion of the nucleus, 2.a projection to the anterior hypothalamic area (AHA) and retrochiasmatic area (RCA) and 3.a projection to the lateral hypothalamic area (LH). In this study we examined the development of the RHT projections using the CT-HRP method. All components of the projection achieve an adult pattern by P15. The LH projection is the first to develop. It is present on E21 and increases strikingly between P1 and P6. At this point the projection is more dense and more extensive than in the adult and the projection is gradually reduced to the adult pattern between P6-P15. The projection to the SCN first appears as scattered varicosities ventrally at P1. These gradually increase in number until P4 and thereafter the projection develops rapidly until the adult pattern appears at approximately P10. As with the LH innervation, the projection to the SCN is initially more extensive than in the adult and is restricted between P6-P10. The projection to the AHA and RCA first appears at P2-P3 and gradually becomes more extensive to approximate the adult pattern by P15.

Thus, the development of the RHT is complex with components appearing at different times with different patterns of development that indicate specialized interactions of the developing axons with the neurons to be innervated. Supported by NIH grant NS-16304.

24.6

FLUORESCENT LATEX MICROSPHERES INJECTED INTO THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) LABEL GANGLION CELLS IN THE GOLDEN HAMSTER RETINA. G.E. Pickard. Dept. of Anatomy, Health Sciences Center, West Virginia University, Morgantown, WV 26506.

A direct retinal projection to the hypothalamic suprachiasmatic nucleus (SCN) is well established. In addition, several laboratories have described retinal fibers in the periventricular zone dorsal to the SCN. HRP injections into this region dorsal to the SCN label hamster retinal ganglion cells (Pickard, G.E., J. Comp. Neurol., 211:65, 1982). Recently, retinal fibers have been described in the hamster which course through the SCN and continue to the caudal PVN (Youngstrom et al., Brain Res. Bull., 19:747, 1987). In the present study, fluorescent microspheres (0.25 ul) were unilaterally injected into the PVN of male golden hamsters. After 24 -48 hr, animals were perfused with physiological saline followed by 10% buffered formalin, retinae were prepared as whole mounts, and brains were sectioned at 50 um with a cryostat. A small number of labeled ganglion cells were observed in the ipsilateral and contralateral retinae. To assess whether labeling was a result of microspheres diffusing into the retinal terminal field of the SCN, another cell group afferent to the SCN, the intergeniculate leaflet (IGL), was examined for labeled neurons; no labeled cells were noted in the IGL.

Supported by NIH grant NS 21165.

24.7

RETINAL AND NEUROPEPTIDE Y INNERVATION OF THE HAMSTER SUPRACHIASMATIC NUCLEUS: LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS. L.M. Wyatt, R.B. Norgren, Jr.* and M.N. Lehman. Dept. of Anat. & Cell Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH 45267.

Photic information which entrains the suprachiasmatic nucleus (SCN) reaches it directly via the retinohypothalamic tract (RHT), and indirectly from the intergeniculate leaflet (neuropeptide Y [NPY] fibers). We have used double label immunocytochemistry at both a light and EM level to examine the distribution of hamster retinal and NPY input, particularly with respect to vasoactive intestinal polypeptide (VIP) cells of the SCN. Adult male hamsters received intraocular injections (10 ul) of 0.1% unconjugated cholera toxin (CT), subunit (List Biological). After 1 day survival, hamsters were perfused with 4% paraformaldehyde; for light microscopy, a combined immunoperoxidase/immunofluorescence procedure was used to visualize VIP cells and either CT- or NPY-labelled fibers; a double label EM technique recently developed in our lab was used to analyze NPY input to VIP cells. At a light microscopic level, the densest concentration of NPY fibers in the hamster SCN closely overlaps the location of VIP cells in the ventral SCN. In contrast, heavy accumulations of CT-labelled retinal varicosities extend over a much wider extent of the SCN, particularly in a rostral and dorsolateral direction. EM analysis of the ventral SCN revealed NPY axon terminals in direct axosomatic contact with both VIP and non-identified cells. Several adjacent NPY axon terminals were sometimes seen synapsing upon a single non-immunoreactive dendrite. We are currently examining the synaptic arrangements of retinal afferents with VIP cells and non-identified neurons, and comparing them to those of NPY inputs. [Supported by NIH NS24642 to M.N.L.]

24.8

STRUCTURAL ORGANIZATION OF THE RAT INTERGENICULATE LEAFLET. J.P. Card, R.P. Meade* and R.Y. Moore. Medical Products Dept., E.I. du Pont de Nemours and Co., Wilmington, Delaware 19898 and Neurology Dept., SUNY @ Stony Brook, Stony Brook, New York 11790.

The intergeniculate leaflet (IGL) of the thalamus is a distinct subdivision of the lateral geniculate complex which has recently been implicated in the neural control of circadian rhythmicity. In the present study we have analyzed the cytoarchitecture, structure and circuitry of the IGL. The cytoarchitectural boundaries of the IGL are most effectively demonstrated by retrograde transport of fluorescent dyes from the suprachiasmatic nuclei and/or the contralateral IGL as well an anterograde transport of CT-HRP from the eye. These data demonstrate that the IGL consists of a 10 mm lamina of neurons interposed between the dorsal and ventral geniculate nuclei which also extends ventromedially to the zona incerta. Neurons exhibiting NPY and mENK immunoreactivity are distinguished by their projections (NPY projects to the SCN; mENK neurons project to the contralateral nucleus), but show no differential distribution within the nucleus. Golgi impregnations of IGL neurons reveal small, bipolar neurons with 2-3 primary dendrites confined to the nucleus. Examination of retinal afferents with electron microscopy demonstrates that these afferents terminate within distinct glomeruli which are presynaptic to apical dendrites and axons. These data demonstrate that the IGL is both structurally and functionally distinct from other subdivisions of the lateral geniculate complex and provide further information on the means through which this thalamic nucleus exerts its control over circadian rhythms.

24.9

NPY, GFAP, GAD, 5-HT AND ENKEPHALIN IN THE INTERGENICULATE LEAFLET AND SUPRACHIASMATIC NUCLEI OF THE HAMSTER. L.P. Morin, L. Smale, K. Michels, R. Johnson and R.Y. Moore. Depts. Psychiatry and Neurology, HSC, SUNY, Stony Brook, NY 11794

A retinohypothalamic tract projects directly to the suprachiasmatic nucleus and provides photic information to a circadian clock. There is also a retinal projection to the intergeniculate leaflet (IGL) of the thalamus. The IGL projects to the SCN through a geniculohypothalamic tract, cells of which contain neuropeptide Y (NPY). The present research has identified the IGL using cholera HRP localization of retinal projections and compared its location with immunohistochemically identified glial fibrillary acidic protein (GFAP), glutamic acid decarboxylase (GAD), 5-HT and enkephlin (ENK) in the IGL and SCN of the hamster.

The rostral IGL starts in the lateral dorsal thalamus, ventrolateral part, in contact with the horizontal cerebral fissure. The middle half of the IGL lies medial to the optic tract, between the dorsal and ventral lateral geniculate nuclei. In the caudal quarter, the IGL is in contact with the lateral thalamic surface, ventral to the medial geniculate nucleus and dorsal to the cerebral peduncle. At the caudal extreme, the IGL is contiguous with the lateral terminal nucleus (LTN) of the accessory optic tract. A distinction can be made between the IGL and LTN during evaluation of cholera-HRP product, but not based on immunoreactivity of NPY. The general morphological features of the IGL are the same whether derived from material reacted with NPY, GFAP or ENK antibody by the location of retinal efferents. GAD and 5-HT are also evident in the IGL throughout its length, but do not delineate it as clearly. All the antigens are localized in the hamster SCN where ENK is co-extensive with NPY. The data are consistent with available functional studies of the IGL and SCN with respect to circadian rhythm regulation. Supported by grant NS22168.

24.10

PHOTIC SENSITIVITY OF HAMSTER GENICULATE NEURONS THAT PROJECT TO EITHER THE SUPRACHIASMATIC NUCLEI OR THE CONTRALATERAL GENICULATE. D.X. Zhang and B. Rusak, Dept. of Psychology, Dalhousie Univ., Halifax, Canada B3H 4J1.

Cells in and near the intergeniculate leaflet (IGL) of the Syrian hamster are known to project to the suprachiasmatic nuclei (SCN); they appear to play a role in mediating photic responses of circadian rhythms. Other cells in this region project to the contralateral IGL. We are investigating the photic responsiveness of cells that project to these targets. Hamsters were anesthetized with urethane and prepared for recording from the area of the intergeniculate leaflet (IGL). Stimulating electrodes were aimed at an analogous site in the contralateral geniculate and at the SCN. Photic responses of cells in the IGL and adjacent areas were assessed with diffuse binocular illumination. Cells were also tested for antidromic activation from the two targets. Most cells identified as projecting to the SCN (by high-frequency following, collision tests and constant latencies of 1.7-16.7 ms) showed a sustained, intensity-dependent activation to retinal illumination. A few cells were suppressed or phasically activated by retinal illumination. The few cells so far identified as projecting to the contralateral geniculate did not project to the SCN and showed little photic sensitivity. Cells that form the projection to the contralateral geniculate appear to differ from those projecting to the SCN in their responses to illumination. Supported by MRC and NSERC of Canada and Dalhousie RDFS.

24.11

THE AFFERENT AND EFFERENT ORGANIZATION OF THE RAT LASCN. D.M. Murakami* and C.A. Fuller. Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

To further examine the possibility that the region lateral to the SCN and dorsal to the optic chiasm (LASCN) may play a role in circadian function, this study examines the morphological distinction of the LASCN from the surrounding hypothalamus. Coronal sections through the hypothalamus were either nissl stained with thionin or impregnated with the Golgi Kopsch technique. Soma profiles were sampled from the LASCN and medial preoptic region (MPO) in nissl stained sections, and measured for mean soma diameter and soma shape (ratio of long and short diameters). Golgi stained neurons were examined for dendritic morphology and orientation. LASCN neurons had smaller soma diameters ($x=10.82\pm2.11, n=100$) than MPO neurons ($x=12.09\pm1.85$, $n=109$) The LASCN neuron soma profile ($x=2.70 \pm 0.99$) was more elongated than that of MPO neurons ($x=1.93 \pm 0.59$). LASCN neurons typically exhibit two bipolar dendrites oriented parallel to the chiasm, and very few extending dorsally into the MPO or anterior hypothalamus. MPO neurons typically exhibit multipolar arrangements of dendrites with many extending ventrally into the LASCN. LASCN neurons closest to the SCN extend a dendritic arbor that will intermix with a plexus of SCN dendrites at the outer border of the SCN. SCN dendrites rarely extend into the LASCN. These morphological features suggest that the LASCN may be a distinct region from the surrounding hypothalamus. [Supported by NIH Grant MH41477, Smokeless Tobacco Res Council Grant 0167 and NASA Grant NAG 2-349.]

24.12

THE MORPHOLOGY OF NEURONS IN THE RAT LASCN. J.R. Nayduch*, D.M. Murakami* and C.A. Fuller (SPON: J. Miller). Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

Previously we suggested that the region lateral to the SCN and dorsal to the optic chiasm (LASCN) may play a role in circadian function. To further investigate the potential role of the LASCN in circadian function, this study examines the afferent and efferent connections of the LASCN, particularly the neural interconnections with the SCN, a nucleus known to play a major role in circadian function. HRP injections were made into either the LASCN or the SCN as a retrograde marker. Following injections, the animal was sacrificed and the brain prepared for histology. Coronal sections through the brain were reacted with either TMB or Hanker-Yates reagents. Injections into the SCN demonstrated that neurons located in the LASCN were labelled with HRP. Injections into the LASCN resulted in a high density of labelled neurons in the ventral portion of the SCN. The dorsal SCN exhibited a low density of labelled neurons. Other regions that contained well labelled HRP neurons include the locus coeruleus, dorsal raphe, paragigantocellular reticular, anterior hypothalamic, medial preoptic region, diagonal band and paraventricular nuclei. The distinct pattern of interconnections between the LASCN and SCN further suggests that the LASCN may play a role in circadian function and recieves neural input from a variety of neural regions that may modulate this function. [Supported in part by NIH Grant MH41477, Smokeless Tobacco Res. Council Grant 0167 and NASA Grant NAG 2-349.]

24.13

DISRUPTION OF FEEDING BUT NOT WHEEL-RUNNING CIRCADIAN RHYTHMS BY HYPOTHALAMIC KNIFE-CUTS AND EFFECTS OF GANGLIOSIDES. J.D. Levine*, A.M. Rosenwasser, J.A. Witcher*, J.A. Yanovski*, R.R. Miselis and N.T. Adler (SPON:D. Levitt). Dept Anat. and Psych. Univ. Pennsylvania, Phila. Pa. 19104

Knife-cuts passing through or around the SCN have been used to study the relationship between neural connectivity and behavioral circadian rhythms. In this ongoing investigation, wheel-running and feeding rhythms were monitored concurrently. Following entrainment to LD12:12, female rats were maintained in constant dim red light. While free-running, some animals received coronal knife-cuts aimed at the mid-SCN. Chi-square periodogram analysis revealed that the knife cuts resulted in disruption of feeding but not wheel-running rhythms. We are also looking at variation in recovery of function and its possible acceleration by exogenous administration of purified GM-1 gangliosides: in two pilot animals, it appeared that vehicle+ganglioside treatment led to an earlier and more complete recovery of the rhythm than vehicle only.

These preliminary results suggest that output pathways from the circadian pacemaker underlying the expression of these two rhythms may be dissociated, and that neural support of at least one rhythm, feeding, possesses considerable potential for functional plasticity. (Supported by BRSG-RR-07083-21 to N.T.A., NIH-HD04522 to N.T.A. and Purified GM-1 was a gift of FIDIA)

24.14

CIRCADIAN RHYTHMS IN HAMSTERS AFTER SCN ISOLATION. H.J. Hakim* and R. Silver, Psychology Department, Barnard College of Columbia University, New York, NY 10027.

Transection of most efferents from the suprachiasmatic nucleus (SCN) results in the loss of circadian activity patterns (Nishio, T. et al., Psyhiol & Behav, 23, 1979; Stephan, FK & Nunex AA, Beh Biol, 20, 1977) suggesting that, at least in rats, SCN efferents are necessary for the expression of circadian rhythmicity.

We examined locomotor activity in male golden hamsters after isolating the SCN using a Halasz wire microknife. Hamsters were housed in constant dim red illumination (LL) for at least ten weeks. Brains were immunocytochemically stained for SCN peptides, VIP and VP, and antisera to glial fibrillary acidic protein (GFAP) was used to delimit the border of the knife cut.

Preliminary results indicate that free running rhythms persist in hamsters in which the knife substantially isolated and/or slightly damaged the SNC (N=9). These animals show both VIP and VP immunoreactivity within the island. Hamsters showing arrhythmic activity sustained severe damage to the SCN (N=4). Animals with cuts that missed the SCN (N=2) and all sham operated animals (N=4) maintained circadian rhythmicity. (Supported by NIH grant NS24292)

24.15

LESIONS DORSAL TO THE SUPRACHIASMATIC NUCLEUS (SCN) ABOLISH SPLIT RHYTHMS IN HAMSTER RUNNING-WHEEL ACTIVITY. M.E. Harrington, G. Eskes, P. Dickson* and B. Rusak. Dept. of Psychology, Dalhousie Univ., Halifax, NS B3H 4J1 Canada.

Constant light exposure (LL) can result in "splitting" of circadian rhythms into two components coupled about 12 h apart. Splitting has been interpreted as evidence for the presence of two main oscillators underlying circadian rhythms. Abolition of splitting after unilateral SCN ablation suggested that each SCN could correspond to one of these component oscillators.

We examined whether lesions outside the SCN would abolish split activity rhythms of hamsters in LL. Wheel-running activity was recorded for at least 3 months after surgery. Damage was assessed by Kluver-Barrera staining. Large bilateral lesions of the medial aspect of the paraventricular nucleus (with little or no SCN damage) generally abolished the split condition immediately. Bilateral damage to the anterior SCN or complete unilateral SCN ablation also abolished the split pattern. Other behavioral effects of surgery included phase shifts and period changes of both components of the split rhythm.

These results indicate that destruction of one SCN is not essential for the elimination of split rhythms since lesions dorsal to the SCN or partial bilateral SCN damage are also effective. Damage to SCN efferents and/or indirect lesion effects on SCN function may account for the loss of the split condition. (Supported by NSERC and MRC of Canada and Dalhousie RDFS.)

24.16

THE EFFECTS OF KNIFE CUTS IN THE HYPOTHALAMIC SUB-PARAVENTRICULAR ZONE OF THE FEMALE RAT ON ESTROGEN-INDUCED DIURNAL RHYTHMS OF PROLACTIN (Prl) AND LUTEINIZING HORMONE (LH), AND CIRCADIAN WHEEL RUNNING ACTIVITY. A.G. Watts, W.J. Sheward* & D. Whale*. MRC Brain Metabolism Unit, Edinburgh, U.K.

The efferent projections of the suprachiasmatic nucleus (SCh) have been traced, using various techniques, to areas within the hypothalamus and forebrain. A substantial terminal area was identified in the region between the SCh and the paraventricular nucleus (PVH)—the sub-paraventricular zone (Watts et al., JCN, 258:204, 1987). In an attempt to determine the importance of this area in the maintenance of SCh generated rhythms, knife cuts were made in the sPVHz region in ovariectomized female rats given an oestradiol-17β (E2) capsule; here, surges of plasma LH and Prl can be seen every afternoon at a time corresponding to those present on the afternoon of pro-oestrus in intact animals. In addition, the effect of the knife cuts on circadian wheel running activity was investigated. At the end of the experiment sections from all knife cut animals were stained for VIP-immunoreactivity (IR) to check for the effects of the knife cut on SCh efferents to the sPVHz and to the paraventricular nucleus of the thalamus (PVT). In animals where the sPVHz VIP-IR was absent the incremental rise in both LH and Prl were markedly reduced for at least 14 days following the knife cut. A similar result was obtained when animals were grouped according to presence or absence of VIP-IR in the PVT. The incremental rise in both LH and Prl was significantly lower in animals with deafferented PVT when compared to intact animals. When animals were grouped according to presence or absence of damage to the preoptic area, there were no significant differences between the 2 groups. When a knife cut was given to animals whose circadian activity cycles were monitored, no substantial disruption was observed in either the ability to synchronize to the light/dark cycle or to free-run in a constant light environment. These results show that the efferent pathway which leaves the SCh in a dorsocaudal direction to innervate the sPVHz is capable of influencing the magnitude of incremental rise in both plasma LH and Prl concentrations. The knife cut did not disrupt any rostrally directed fibres from the SCh, e.g., the SCh derived NP-II innervation of the anteroventral periventricular nucleus was unaffected by knife cuts which disrupted VIP-IR to the sPVHz. When animals were grouped according to presence or absence of damage to the preoptic area, there were no significant differences between the 2 groups. These results suggest that any rostrally directed fibres do not play as significant a role in maintaining diurnal LH and Prl release as do the dorsocaudally directed fibres.

24.17

KNIFE CUTS DORSAL TO THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS DISINHIBIT GONADOTROPIN SECRETION IN MALE GOLDEN HAMSTERS KEPT IN LONG OR SHORT DAYS. L.L. Badura, C.L. Sisk, and A.A. Nunez, Psychology Dept. and Neuroscience Prog., Michigan State University, E. Lansing, MI 48824

Male hamsters housed in a long-day photoperiod show a rise in plasma levels of gonadotropins following castration. Exposure to a short-day photoperiod attenuates the post-castration rise in LH and FSH. The present experiment was designed to determine if manipulations that abolish gonadal responses to photoperiod also abolish the short-day induced inhibition of gonadotropin release following castration.

Testicular regression was induced in male hamsters by exposure to short days (6L:18D) for 10 weeks. Half of the animals then received a horizontal knife cut aimed just dorsal to the PVN; the remaining hamsters received sham surgery. Following surgery, half of the animals were transferred to long days (16L:8D). Blood samples were drawn from all animals via cardiac puncture 1 week before surgery, and again at 10, 20, and 30 days post-surgery. Plasma levels of FSH in each sample were determined by radioimmunoassay.

The post-castration rise in FSH was lower and more protracted in sham-operated animals in 6L:18D than those in 16L:8D. At 10 and 20 days post-surgery, knife cut animals in both photoperiods had higher plasma levels of FSH than sham-operated animals in either photoperiod, although FSH was slightly higher in knife cut animals in 16L:8D than in 6L:18D. By 30 days post-surgery, all groups showed similar levels of FSH. These findings indicate that knife cuts dorsal to the PVN cause a disinhibition of FSH that is attenuated by exposure to short days.

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24.18

PARASAGITTAL KNIFE CUTS LATERAL TO THE PARAVENTRICULAR NUCLEI OF THE HYPOTHALAMUS BLOCK REPRODUCTIVE RESPONSES TO PHOTOPERIOD IN FEMALE GOLDEN HAMSTERS. K.K. Kelly*, L.L. Badura, and A.A. Nunez, Psychology Dept. and Neuroscience Prog., Michigan State University, E. Lansing, MI, 48824.

In female hamsters, interruption of the dorsally-projecting fibers of the hypothalamic paraventricular nuclei (PVN) is not sufficient to block short-day-induced estrous acyclicity and uterine regression. The purpose of the present study was to investigate the functional significance of the laterally-projecting fibers of the PVN in photoperiodic control of female reproductive physiology. Cycling female hamsters were maintained in a long-day (16L:8D) photoperiod. One group received bilateral parasagittal knife cuts aimed lateral to the PVN; the other group received sham surgery. After a two week recovery period, half the animals were moved to a short-day (6L:18D) photoperiod. Vaginal discharge was evaluated daily until all sham-operated animals in 6L:18D exhibited discharges charateristic of anestrus. The animals were then sacrificed, their uteri weighed, and the brains prepared for histological evaluation.

Animals with bilateral knife cuts placed through the lateral one-third of the PVN continued to show regular 4-day estrous cycles and to maintain stimulated uteri in both long and short days; thus, parasagittal cuts prevented the effects of short days on reproductive physiology. This finding suggests that the lateral efferent projections from the PVN represent an important component of the neural pathway that mediates photoperiodism in female hamsters.

Supported by NIMH grant MH 37877 to A.A.N.

24.19

PARAVENTRICULO-SPINAL PATHWAYS AND RESPONSES TO PHOTOPERIOD IN THE SYRIAN HAMSTER (*Mesocricetus auratus*). T.G. Youngstrom & A.A. Nunez. Psychology Dept. and Neuroscience Prog., Michigan State Univ., E. Lansing, MI. 48824.

An efferent paraventricular nucleus (PVN) projection to the spinal cord has been implicated in the control of seasonal reproductive cycles in the Syrian hamster. Horizontal knife cuts placed dorsal to the PVN in male, but not female, hamsters prevent short-day induced gonadal regression (Brain Res. Bull. *16*:705, '86; Brain Res. *370*:102, '86; Soc. Neurosci. Abstr. *13*:865 '87). To examine the course of PVN efferent fibers to the spinal cord, injections of Cholera toxin conjugated to horseradish peroxidase (CT-HRP) were aimed at the intermediolateral cell column and lateral spinal cord at the level of C7 to T2. Animals were perfused and the tissue processed using a previously published procedure (J. Histochem. Cytochem. *28*(11):1255, '80). Labeled neurons were present throughout the rostro-caudal extent of the PVN, but primarily in the parvocellular subdivisions of the nucleus. Labeled fibers that originated from these neurons formed two bundles, one immediately dorso-medial above the third ventricle and a second that exited the PVN laterally. The bundle projecting laterally divided into two components. A prominent ventro-lateral tract appeared to join the medial forebrain bundle, while a second smaller set of fibers turned dorso-laterally. While some horizontal knife cuts placed above the PVN may sever the dorsal bundle just above the PVN and third ventricle, knife cuts placed further dorsally (Brain Res. *370*:102, '86) should leave PVN-spinal connections intact; yet those dorsal cuts are also effective in preventing testicular regression. In males, knife cuts dorsal to the PVN result in elevated serum levels of follicle stimulating hormone (FSH) regardless of photoperiod (Badura *et al.*, Soc. Neurosci. Abstr. *14* '88). Thus, due to the increase in FSH, animals in short days may maintain large testicles even though the neural pathway that mediates photoperiodism is not damaged by the knife cuts. Supported in part by NIMH Grant MH 37877 to AAN.

24.20

EVIDENCE FOR RETINAL INPUT TO THE BASAL FOREBRAIN IN THE HAMSTER THAT MAY INFLUENCE RESPONSES TO PHOTOPERIOD. A.A. Nunez, T.G. Youngstrom & M.L. Weiss. (SPON: G. Richmond) Psychology Dept. and Neuroscience Prog., Michigan State Univ., E. Lansing, MI. 48824.

In this study, retinal projections to the brain of the Syrian hamster (*Mesocricetus auratus*) were examined using intraocular injections of Cholera toxin conjugated to horseradish peroxidase (CT-HRP). Following a 24-72 hour survival period, male and female Syrian hamsters were perfused and the tissue processed using a previously published procedure (J. Histochem. Cytochem. *28*(11):1255, '80). Areas previously reported to receive direct retinal input were labeled including the suprachiasmatic nucleus (SCN), the paraventricular nucleus (PVN), the sub-PVN area and the supraoptic nucleus (SON) (Soc. Neurosci. Abstr. *13*:211, '87). In addition labeled fibers were seen in the preoptic area where a diffuse bundle continued into the diagonal band of Broca. A distinct fiber tract was followed into the superficial layer of the piriform cortex where putative terminals were seen. Extra-geniculate input to the thalamus was primarily to the nucleus reuniens and dorsal thalamus. A portion of this projection appeared to terminate in either the caudal bed nucleus of the stria terminalis or rostral thalamus. Many areas of the basal forebrain that receive direct retinal input participate in the control of reproductive behavior or physiology and may play a role in photoperiodism. Supported in part by NIMH Grant MH 37877 to AAN and NRSA Postdoctoral Grant NS 08125 to MLW.

25.1

CHOLINERGIC AND CATECHOLAMINERGIC SUPPRESSION OF NERVUS TERMINALIS GANGLION CELL ACTIVITY IN THE ELASMOBRANCH. J. White* and M. Meredith. Dept. of Biol. Sci., Florida State Univ., Tallahassee, FL 32306-3050.

Our previous observations suggest that impulses from the brain suppress nervus terminalis (NT) ganglion cell activity in the bonnethead shark (Sphyrna tiburo), probably through inhibitory synaptic potentials. We are currently conducting in vitro pharmacological studies on the bonnethead NT to investigate the synaptic interactions responsible for this suppression. Multi-unit activity from NT ganglion cells was recorded extracellularly from central nerve trunks with suction electrodes. Electrical stimulation of central or peripheral nerve trunks suppresses this activity. Bath application of acetylcholine (ACh), norepinephrine (NE), dopamine, and epinephrine (EPI) at 1 to 100 uM also suppressed ganglion cell activity. These agonists were effective when applied under zero-calcium conditions to block synaptic transmitter release, suggesting that each was acting directly on the NT ganglion output cells and not through interneurons or presynaptic mechanisms. Experiments with neurotransmitter antagonists further suggest that ACh may be acting via muscarinic receptors and EPI via alpha-adrenergic receptors. Furthermore, ACh and NE suppression appeared to be due to different mechanisms: ACh prevented further suppression by electrical stimulation, while NE application did not. The functional significance of this difference is unknown and is currently under study.

Supported by NSF Grants 8412141 and 8615159.

25.2

HORN SHARKS POSSESS SEPARATE HOMOLOGUES OF NUCLEUS GENICULATUS LATERALIS AND NUCLEUS ROTUNDUS. E. Fiebig* and R. G. Northcutt (SPON: M. S. Northcutt). SIO Neurobiol. Unit & Dept. of Neurosci's., UCSD, La Jolla, CA 92093.

A dorsolateral optic complex is said to exist in sharks (Ebbesson, Brain Behav. Evol., 6:75, 1972) and to have given rise to a lateral geniculate nucleus and n. rotundus by parcellation (Ebbesson, Behav. Brain Sci's., 7:321, 1984). Injections of WGA or WGA-HRP into the eye in horn sharks, Heterodontus francisci, revealed no retinal projections to the dorsal posterior thalamic nucleus. However, injections of PHAL into the optic tectum (4 cases) revealed extensive tectofugal projections to this nucleus. While the projections of this nucleus are not known in Heterodontus, only a few cells of the dorsal posterior thalamic nucleus project to the tectum following injections of WGA-HRP into the tectum (5 cases), and that in a related genus, Platyrhinoidis, the dorsal posterior nucleus and a more rostral retino- and tectorecipient thalamic nucleus (anterior nucleus) project to the telencephalon (E. Fiebig and H. Bleckmann, in press). The topography and connections of the dorsal posterior nucleus support the hypothesis that this nucleus is the homologue of n. rotundus. Therefore, at least some elasmobranchs have separate homologues of the geniculate and rotundal nuclei, and the condition in these species does not support a hypothesis of phylogenetic parcellation.

25.3

LOCUS COERULEUS AND SUBCOERULEUS IN AN ELASMOBRANCH: TELENCEPHALIC, CEREBELLAR AND SPINAL CORD CONNECTIVITIES AND IMMUNOHISTOCHEMISTRY. W.L.R. Cruce, E. Fiebig*, S.L. Stuesse, and H. Bleckmann*. NEOUCOM, Rootstown, OH, UCSD, La Jolla, CA, and U. of Bielefeld, FRG.

HRP or WGA-HRP was injected into spinal cord, cerebellum, or basal forebrain bundle of the thornback guitarfish, Platyrhinoidis triseriata. Mesencephalic cells showing retrograde label were compared to cells showing 5HT and tyrosine hydroxylase (TH) immunoreactivity. All cells are small (10x30u) and oval. Locus coeruleus (LC) cells are scattered along the ventricular border medial to the sulcus limitans for a short distance below the caudal pole of the trigeminal motor nucleus; some cells lie dorsolateral to the sulcus limitans as well. Major dendritic processes are parallel to the ventricular surface. Subcoeruleus pars alpha (SCA) is found at the same level as LC and merges with that nucleus in a dorsomedial direction. Ventrolaterally SCA merges with pars beta (SCB). SCA cells can be distinguished from cells of LC and SCB by their dorsomedial to ventrolateral dendritic orientation. SCB is a tight group of cells (probably homologous to the mammalian Kolliker-Fuse n.) found at the same level as LC but lying at the lateral edge of the brainstem between nucleus F dorsally and nucleus B ventrally. SCB merges medially with SCA but can be distinguished from it by the mediolateral major dendritic axis of SCB cells. All three nuclei have a predominantly ipsilateral projection to spinal cord and telencephalon. LC and SCA but not SCB project to cerebellum bilaterally. All three nuclei contain cells immunoreactive to TH but only LC contains 5HT immunoreactive cells.

25.4

LEUCINE-ENKEPHALIN-LIKE IMMUNOREACTIVITY IN THE FOREBRAIN OF THE THORNBACK GUITARFISH, WITH COMPARISONS TO A SQUALOMORPH SHARK. J.G. New, Neurobiology Unit, Scripps Inst. of Oceanography, UCSD, La Jolla, CA 92093.

The telencephalon of the thornback (Platyrhinoidis triseriata) represents an intermediate level of complexity between the primitive condition of squalomorph sharks and the large and complex telencephalon of galeomorph sharks and myliobatoids. The purpose of this study is to localize L-ENK+ neurons in the forebrain of Platyrhinoidis and to compare the distribution with that previously reported in Squalus acanthias (Northcutt, et al., 1988).

The fixed brains of 9 Platyrhinoidis specimens were sectioned at 30-35 um and incubated with a commercially obtained antibody to L-ENK. The antigen-antibody complex was visualized via PAP methods, using DAB as the chromogen.

The greatest concentration of L-ENK+ fibers and somata was observed in the dorsal pallium (DP). DP in Squalus is also rich in L-ENK+ cells, but in Platyrhinoidis DP has expanded greatly and extends over the rostrodorsal pole of the telencephalon. A number of discrete regions of high or low immunoreactivity were also discernible within DP.L-ENK+ somata and fibers were also observed in lesser concentration in the lateral and medial pallia, suprapeduncular nucleus, nucleus Q, the septal area, and the preoptic region. Weak staining of large somata, as well as fibers , was observed in the area superficialis basalis. L-ENK+ neurons were not observed in the APVL, a region of stong immunoreactivity in Squalus.

25.5

AN IMMUNOHISTOCHEMICAL STUDY OF THE TELENCEPHALON OF POLYPTERUS. A. Reiner and R.G. Northcutt. Dept. of Anatomy and Neurobiology, Univ. Tenn., Memphis, TN and Dept of Neurosciences, UCSD, La Jolla, CA.

In teleosts, the most advanced ray-finned fish, eversion of the pallial roof of the telencephalon together with neuronal proliferation and migration makes comparisons between cell groups of the teleost telencephalon with those of the telencephalon in other vertebrates extremely difficult. The telencephalon of polypterus, a primitive living ray-finned fish, however, is much simpler in cytoarchitecture than that of teleosts and is also everted. To aid in clarifying the evolution of the telencephalon in teleosts and aid in making comparisons between the telencephalon in ray-finned fish and other vertebrates, we have applied immunohistochemical techniques to the study of the telencephalon of polypterus.

Antisera specific for the following substances were used: 1) 5-HT; 2) tyrosine hydroxylase; 3) substance P; 4) leucine-enkephalin; 5) NPY; and 6) LANT6. The staining pattern for the various regions of the telencephalon of polypterus revealed similarities to specific regions of the telencephalon of other vertebrates: 1) the dorsal and ventral subdivisions of the area ventralis appear to include the homologues of the basal ganglia and septum; 2) the olfactory pallium (P1) appears comparable to olfactory cortex; and 3) the P2 (dorsal) and P3 (lateral) fields of the pallium appear to correspond largely to the dorsomedial and medial pallial walls, respectively, in lungfish, sharks, frogs and reptiles. These results thus suggest homologues in polypterus for some of the major parts of the telencephalon in tetrapods and non-ray-finned fish.

Supported by NIH grants NS19620 (AR) and NS11006 (RGN).

25.6

DISTRIBUTION OF GAP JUNCTION PROTEIN-IMMUNOREACTIVITY IN THE BRAIN OF THE WEAKLY ELECTRIC FISH. T. Yamamoto[1], L. Maler[2], E.L. Hertzberg*[3] and J.I. Nagy[1] [1]Dept. of Physiology, Univ. of Manitoba, Winnipeg, CANADA, [2]Dept. of Anat., Univ. of Ottawa, Ontario, CANADA, and [3]Dept. of Biochemistry, Baylor College of Med., Houston, TX.

Electrotonic coupling via gap junctions between neurons in the CNS of weakly electric fish represents an important mode of communication in certain structures associated with the electrosensory system. We have used an affinity-purified polyclonal antibody against a 27KD gap junction protein (GJP) to determine immunohistochemically the distribution of GJP-immunoreactivity (GJP-IR) in the brain of gymnotiform fish. GJP-IR consisted of individual punctate deposits of immunoreaction product and sequences of puncta which appeared to be varicosities along fibers. The density of both patterns was remarkably heterogeneous throughout the brain. Areas with very dnese GJP-IR included the nucleus electrosensorius, the inferior lobe, nucleus praeeminentialis dorsalis and the vagal sensory nucleus. Moderate to high densities were observed in the torus semicircularis dorsalis, the periglomerular nucleus and the descending trigeminal nucleus. In several areas, punctate or fibrous GJP-IR was associated with neuronal somas or initial segments of dendrites. Preliminary EM observations indicate that GJP-IR is localized, in part, to regions of cytoplasmic membranes forming gap junctions.

25.7

HEXOKINASE HISTOCHEMISTRY IN THE BRAIN OF GOLDFISH. J.G. Dulka,* T.L. Krukoff, J.I. Goldberg and N.E. Stacey*. (SPON: B. Calancie). Department of Zoology, University of Alberta, Edmonton, T6G 2E9, Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Histochemical demonstration of hexokinase (HK), the first enzyme in the glycolytic pathway, has been used as an indicator of metabolic activity in selected brain regions in rats (Krukoff et al., Am. J. Physiol. 251:R268, 1986). We are using HK histochemistry on male goldfish to ultimately identify brain regions that control the release of gonadotropin into the blood following exposure of males to a sex pheromone, 17o, 20B-dihydroxy-4-pregnen-3-one (Dulka et al., Nature, 325: 251, 1987).

In this preliminary report, the distribution of HK staining in the brains of normal, unstimulated males is described. Brain regions showing strong HK staining include the habenula, the ventral floor of the preoptic recess, periventricular hypothalamic nuclei, portions of nuclei associated with the lateral and posterior recesses, and neurohypophysial fibers in the pituitary. Distinct cells which stained strongly for HK were identified in the dorsal midbrain and throughout the medulla. The results suggest that these brain regions have high levels of glucose utilization. Interestingly, most areas staining strongly for HK overlap with regions known to contain biogenic amines. In support, HK staining in the nucleus recessus posterioris was found to overlap with immunocytochemical staining of serotonin on alternate sections. However, additional research is needed to determine if other aminergic systems also show high HK activity.

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25.8

EIGENMANNIA POSSESSES AUTAPOMORPHIC RAMI OF THE ANTERIOR LATERAL LINE NERVES. R. G. Northcutt and H. A. Vischer (SPON: M. F. Wullimann), SIO Neurobiol. Unit & Dept. Neurosci's., UCSD, La Jolla, CA 92093

Examination of histological serial sections (Bodian stained) of entire heads, and Sudan Black preparations that render the head transparent while staining the cranial nerves, indicates that the anterior lateral line nerve complex of Eigenmannia consists of anterodorsal and anteroventral nerves. The anterodorsal nerve (ADN) consists of superficial ophthalmic and buccal rami that innervate the neuromasts of the supra- and infraorbital canals, respectively. The anteroventral nerve (AVN), as in other teleosts, exhibits an external mandibular ramus that innervates neuromasts of the preoperculomandibular canal as well as three additional rami not observed in non-gymnotid teleosts: supraorbital, infraorbial and recurrent. The supraorbital and infraorbital rami anastomose, respectively, with the superficial ophthalmic and buccal rami of the ADN distally, so that without experimental studies it is impossible to establish which lateral line organs these rami innervate. However, as siluroid teleosts are the sister group of gymnotid teleosts, and are electroreceptive but do not exhibit supraorbital and infraorbital rami of the AVN, or tuberous electroreceptors, we hypothesize that one or both classes of tuberous organs may be innervated only by the ADN and may thus arise or be induced in relation to the hyoid dorsolateral placode.

25.9

MAUTHNER-LIKE RETICULOSPINAL NEURONS IN THE ADULT GOLDFISH. **R.K.K. Lee, S.J. Zottoli and R.C. Eaton. Neuroscience Group, Campus Box 334, Univ. of Colorado, Boulder, CO 80309, & Dept. of Biol., Williams College, Williamstown, MA 01267.**

The Mauthner (M-) cells are a pair of prominent reticulospinal (RS) neurons that play a major role in the C-start escape responses but are not required for it to occur. This and other evidence suggests that the C-start must be mediated by a group of RS neurons. We are presently constructing a map of RS neurons for analysis of network interactions underlying the C-start. We report two new pairs of RS neurons whose morphology suggests a role in the C-start behavior.

We analyzed 17 brains retrogradely filled with HRP injected into various positions along the spinal cord. The identified cells are located together ventromedial and caudal to the M-cell somas at the level of the M-axon decussation. The coordinates of one of the pairs are at 274 ± 14 um (± SEM) posterior to the M-soma, 296 ± 16 um ventral to the M-axons and 243 ± 7 um lateral to the midline. This pair has a single, crossed, decussating axon in the MLF and a lateral and bifurcating ventral dendrite. These seem to receive afferents similar to the M-cell. Two pairs are distinguished because only one has an axon reaching the caudal end of the spinal cord.

[Supported by NIH grants NS22621 & NS26032 and NSF grant CSI8650488].

25.10

DIENCEPHALIC NUCLEI THAT PROJECT TO THE PITUITARY IN GOLDFISH: AN IN VITRO HRP STUDY. H.E. Sloan and L.S. Demski, School of Biological Sciences, University of Kentucky. Lexington, KY 40506.

Neurons of the diencephalon were labeled following application of horseradish peroxidase (HRP) to the pituitary of the goldfish. Animals were anesthetized and perfused through the heart with teleost ringers. The pituitary was exposed and punctured with an HRP coated insect pin. The brain and pituitary were incubated in teleost ringers at 5-10°C for 1 or 2 days and then processed for HRP histochemistry. Nomenclature is from Braford and Northcutt or Peter and Fryer in Fish Neurobiology, vol. 2, 1983.

All labeled neuronal populations have bilateral projections to the pituitary. HRP-filled cells are found in the parvocellular, (PPa and PMp), magnocellular (PMm) and gigantocellular (PMg) regions of the preoptic area. HRP-labeled cells are present in the suprachiasmatic nucleus (SC), the lateral portion of the nucleus lateral tuberis (NLTl) and the lateral hypothalamic area (LH). Caudally, HRP-filled cells are found in the caudal hypothalamic area (Hc or NRP) and between Hc and the dorsal hypothalamic area (Hd or NRL). Labeled neuronal populations are similar to mammalian projections to the median eminence and pituitary. Supported in part by NIH Grant NS 19431.

25.11

BONY FISH PIT LINE NEUROMASTS ARE HOMOLOGOUS TO CANAL NEUROMASTS. J.F. Webb* and R.G. Northcutt. Neurobiol. Unit, SIO and Dep't Neurosciences, Univ. California, San Diego, La Jolla, CA 92093.

An examination of the distribution of lateral line canals and their homologues in major vertebrate taxa reveals frequent paedomorphic trends from a system composed exclusively of canals to a system characterized by the replacement of many of these canals by lines of superficial neuromasts (pit lines). If all pit lines have canal homologues in early vertebrates, then pit line neuromasts and canal neuromasts are components of a single mechanoreceptive lateral line system. As such, they should look fundamentally similar unless natural selection has resulted in their morphological differentiation. The pit line neuromasts in the lungfishes Lepidosiren and Protopterus and the bichir Polypterus were examined using scanning electron microscopy. Despite having quite long kinocilia, pit line neuromasts resemble canal neuromasts in shape, topography and hair cell orientation. This supports the hypothesis that they have a phylogenetic history as canal neuromasts and further suggests that pit line neuromast morphology is quite conservative having changed little since the late Devonian.

25.12

THE MORPHOLOGY OF THE BARBEL SENSORY SYSTEM IN A GOATFISH, PARUPENUS MULTIFASCIATUS. **M.A. Barry, C.S. Beeman*, and L.D. Savoy*, Dept. of Biostructure and Function, Univ. Conn. Health Ctr., Farmington, CT. 06032**

The paired mandibular barbels in goatfish are used for tactile and chemosensory exploration, and during intraspecific behaviors. Morphological studies of the barbel and on the central projections of the barbel nerve revealed a unique and complex organization of the brain. Numerous taste buds are located on the barbel. The barbel is innervated by the terminal and largest branch of the mandibular nerve. Barbel afferents enter the brain in the facial and trigeminal nerve roots. Trigeminal afferents course caudally in the descending tract of V. Facial afferents course in a fascicle dorsal to the IVth ventricle. Barbel afferents terminate in an enlarged region of the rhombencephalon composed of dorsal, intermediate, and ventral lobes. Facial afferents terminate primarily in the dorsal and intermediate lobes, whereas the ventral lobe receives trigeminal input. The ventral lobe contains numerous spheroidal glomeruli (100-200 μ in diameter). Each glomerulus is a cluster of small (5-8 μ) round cells surrounded by a molecular zone with scattered large and small cells. Clusters in the intermediate lobe are smaller and less well differentiated. The dorsal lobe is divided into 3-4 lobules extending rostro-caudally. Lobules consist of 4 layers: superficial cellular, molecular, cellular with multiple clusters of cells (like those in ventral lobe), and deep molecular with fiber tracts and scattered cells including 15-30 μ elongate cells. NIH-NS16993.

26.1

GABA NEURONS IN THE CORPUS STRIATUM OF THE SALAMANDER. Lorraine Dubé and Laurent Descarries. Centre de recherche en sciences neurologiques, Départements d'anatomie et de physiologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

PAP-immunohistochemistry with an antiserum against GABA-BSA conjugate (Immunonuclear) was used to identify putative GABA-containing neurons in the brain of Necturus maculosus. Within the corpus striatum, 3 concentric layers of nerve cell bodies were distinguished on the basis of their immuno-reactivity. From the lateral ventricle outward, small immunonegative neurons formed a first layer, 1 to 3 cells thick, immediately beneath the ependyma. The second layer consisted of a single row of small, strongly positive somata, interlinked by long processes which were also intensely reactive. The third layer comprised several rows of larger neurons uniformly displaying a moderate GABA immunoreactivity and surrounded, individually or in small groups, by numerous GABA-positive punctate structures. Such punctae were also found in increasing number dorso-ventrally, within the neuropil layer at the base of the brain. This peculiar GABA distribution in the corpus striatum differed from that in the proeminentia ventralis, medially, where all cell layers were thicker and the strongly positive neurons exhibited processes which were mainly oriented vertically, reaching into the neuropil. Laterally, it contrasted sharply with the amygdalar pattern showing only a few immunoreactive nerve cell bodies interspersed among negative neurons. These data reveal the existence of a major contingent of GABA-containing neurons within the corpus striatum of the salamander and suggest that GABA could be strongly implicated in the striatal function of amphibians as well as higher vertebrates. (Supported by MRC grant MA-9847).

26.2

FUNICULAR PROJECTIONS OF BULBOSPINAL NUCLEI IN NOTOPHTHALMUS VIRIDESCENS. M.C. Anderson*, B.M. Davis, S.B. Simpson, Jr. (SPON: J Leonard). Dept. of Biological Science, Univ. of Illinois at Chicago, Chicago, IL 60680.

While numerous studies have determined the cells of origin of the bulbospinal projections in anurans (e.g., Corvaja and Grofova, 1972; Shapovolov, 1972; Fuller, 1974; Soller, 1977; Ten Donkelaar, 1981) little is known about these pathways in urodeles. To investigate the origin and funicular trajectories of the bublospinal nuclei HRP was injected into various spinal tracts at cervical and lumbar levels of adult salamanders.

Following injection of HRP, labeled nuclei were observed within the rhombencephalon in the nucleus reticularus isthmi, nucleus reticularus superiorus, nucleus reticularus medius, and nucleus reticularus raphe. These nuclei project bilaterally via the ventral and ventral lateral funiculi. Nucleus reticularus inferiorus courses bilaterally in the lateral funiculus. Vestibular projections appeared to be bilateral and were located in the ventral funiculus. The apparent bilaterality of this projection may be misleading. Descrete vestibular nuclei, which might project unilaterally, could not be identified. At the level of the vestibular nuclei two large contralaterally projecting Mauthner neurons were also present. Within the mesencephalon HRP filled cells are located in the presumed homologue of the red nucleus and the interstitial nucleus of the FLM. The red nucleus projection is located in the contralateral dorsolateral tract while the interstitial nucleus is located bilaterally in the ventral funiculus. In the diencephalon labeled nuclei include the preoptic and the periventricular (PVN) nuclei. The preoptic nucleus courses ipsilaterally in the lateral funiculus as far caudal as the thoracic spinal cord. PVN also projects ipsilaterally in the lateral funiculus, but appears to descend to the lumbar spinal cord. (Supported by NS 24162 to SBS).

26.3

CONNECTIONS OF THE MAIN AND ACCESSORY OLFACTORY BULBS IN RANID FROGS. T.J. Neary Anatomy Dept., Creighton Univ., Omaha, NE 68178.

The connections of the main and accessory olfactory bulbs (MOB & AOB) were examined following applications of the tracer wheat germ agglutinin/horseradish peroxidase (WGA-HRP). Fibers left the MOB to supply the ipsilateral dorsal and lateral pallia, the post-olfactory eminence, and nucleus of the diagonal band/olfactory tubercle (NDB/OT). Labelled fibers supplied the same structures in the opposite hemisphere by crossing directly to the contralateral MOB and by coursing through the habenular commissure. Labelled neurons were found in the medial pallium, the ventral dorsal pallium, and in the intermediate and ventral parts of the lateral pallium (LPi & LPv). In the subpallium, they were present in the NDB/OT, medial amygdala, and surrounding the rostral lateral forebrain bundle. Labelled cells were also found in the anterior thalamic nucleus. Most cells were found bilaterally, but were more numerous ipsilateral to the applications. AOB applications labelled fibers which projected to the ipsilateral caudal LPi and LPv, and lateral amygdala. A small projection to the ipsilateral anterior preoptic area was also present. Fibers to the contralateral lateral amygdala, LPi, and LPv crossed in the anterior commissure.

Supported by a BRSG grant to Creighton University.

26.4

MARGINAL NEURONS AND THE DENTICULATE LIGAMENT OF THE SPINAL CORD IN URODELE AMPHIBIANS. D. Schroeder and M. W. Egar*, Ind. Sch. of Med., Med. Sci. Prog., Bloomington, IN 47405 and Dept. of Anat., Indianapolis, IN 46223.

There has been a renewed interest in the marginal neurons of the spinal cord since they may be intrinisic mechanoreceptors (Grillner *et al.*, Science 223:500, 1984). They appear to be also structurally and functionally associated with the denticulate ligaments (Schroeder and Richardson, Somatosens. Res. 4:127, 1986). Marginal neurons have been described in several vertebrates but not amphibians. We examined the spinal cord of *Ambystoma mexicanum* and *Necturus maculosus* with light and electron microscopes. The spinal cord extends the length of the vertebral column and consists of about 50 pairs of spinal nerves: approximately 20 are associated with the trunk and 30 with the tail. The denticulate ligaments are prominent on each side and vary somewhat in shape and design with the two different urodeles. The teeth of the ligaments extend laterally from the spinal cord surface and are continous through the dura to a projection of bone on each side of the vertebral wall. The marginal neurons, approximately 30 u in diameter, are located in a scattered column just medial to the ligaments and at the lateral edge of the spinal cord. They are associated with neuropil that contains many boutons containing round, polymorphic or dense-cored vesicles, dendritic processes, axons, and a variety of glial processes. A more medially located neuropil is also present. In our preliminary examination of an anuran amphibian (*Rana pipiens*) spinal cord we have not found marginal neurons. Supported by PHS SO7 RR 53711.

26.5

SEROTONIN NEURONS IN THE BRAIN OF THE REPTILE CAIMAN CROCODILUS. S. E. Brauth, Department of Psychology, University of Maryland, College Park, Maryland 20742.

Immunohistochemical methods were used to map the distribution of serotonergic neurons in the caiman brain. Many serotonergic neurons were observed in the nuclei raphe magnus, raphe pontis and superior centralis. Serotonin neurons are also present in cell groups which contain catecholamine neurons including the nuclei subcoeruleus ventralis and dorsalis, the locus coeruleus and both the pars ventralis and pars compacta of the substantia nigra. The caudal portion of the area ventralis of Tsai contains a large number of serotonin neurons surrounding the interpeduncular nucleus on its dorsal and lateral margins.

Many subependymal and supraependymal CSF-contacting serotonin neurons are present in the caiman hypothalamus at tuberal levels. The paraventricular organ contains a large number of small, intensely staining serotonin immunoreactive neurons. Serotonergic fibers are present within both the lateral and medial hypothalamus and coarse immunoreactive puncta are prominent within the lateral hypothalamus and lateral external layer of the median eminence. Fine serotonin fibers and terminals are present in the dorsal thalamus including the lateral geniculate nucleus, nucleus dorsomedialis anterior and dorsolateralis anterior. The habenular and subhabenular nuclei also contain serotonergic fibers and terminals.

Supported by NIH Grant No. NS 24992

26.6

CEREBELLAR PROJECTING AND LOCAL CIRCUIT NEURONS OF REPTILIAN DORSAL COLUMN NUCLEUS. M.B. Pritz and M.E. Stritzel*. Div. Neurol. Surg., Univ. of California Irvine Medical Center, Orange, CA 92668.

The dorsal column nucleus (DCN) in reptiles, Caiman crocodilus, like its mammilian counterpart, is a heterogeneous area. The present experiments investigated the origin of cerebellar projecting neurons of the DCN as well as the presence of local circuit neurons (LCN).

The origin of cerebellar projecting DCN neurons was determined by horseradish peroxidase (HRP) injections into the cerebellum in 10 animals. Tissue was processed by standard neurohistochemical techniques using tetramethylbenzidine as the chromogen. Rostral and central cerebellar HRP injections, primarily labeled neurons in the rostral half of the DCN. However, injections of the most posterior cerebellum resulted in retrogradely labeled DCN cells that were located caudally as well as rostrally.

The presence of LCN was investigated by standard immunocytochemical techniques using a polyclonal antibody to GABA, a polyclonal antibody to GAD, and a monoclonal antibody to GAD (epitopes GAD-1 and GAD-2) both with and without colchicine pretreatment. Although reactive neurons were found in other brain regions, no GABA (+) or GAD (+) neurons were identified in the DCN.

These present results, and those of previous studies, indicate the following. First, relay cells of the DCN of Caiman that project to the spinal cord, midbrain, and cerebellum originate from separate populations in the DCN. Second, using GABA and GAD immunoreactivity as an indicator for the presence of LCN, the DCN of Caiman lacks these presumed intrinsic inhibitory cells. These data suggest that neuronal circuits and relay cells of the DCN that project to the spinal cord, midbrain, and cerebellum are phylogenetically ancient but that the presence of LCN may be a new feature in the evolution of the DCN.

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26.7

GABA AND GLYCINE IMMUNOREACTIVITY IN THE TECTUM OF THE RATTLESNAKE. R.M. Meszler and L.B. McCleary*. Anatomy Dept., Dental School, Univ. of Maryland at Baltimore, Baltimore, Maryland 21201.

The optic tectum in the rattlesnake is known to be the site of integration of signals from the visual and infrared receptor systems. Retinal projections terminate in superficial layers including SGFS a,b,c (stratum griseum et fibrosum superficiale) and SGC (stratum griseum centrale) whereas infrared projections end in the deeper portion of SGC and SFC (stratum fibrosum centrale; Hartline et. al. Science 199:1225-1229, 1978). Golgi preparations of rattlesnake tectum reveal several morphological types of neurons with radial orientation and dendritic fields that straddle 2 or more tectal layers. Furthermore, the spectrum of morphological types of synapse varies significantly between the superficial and deeper layers. (Meszler, Anat.Rec.218(1):90A-91A,1987).

The localization of immunoreactivity to GABA and glycine in the rattlesnake tectum was investigated using antibodies kindly supplied by Dr. R. J. Wenthold (NINCDS-NIH). Vibratome sections of perfusion fixed brains (4% formaldehyde, 0.5% glutaraldehyde, 0.1M cacodylate buf. pH 7.4) were processed immunocytochemically, visualized using the Vectastain avidin-biotin-peroxidase complex method and prepared for light and electron microscopy.

Both antibodies produced strong staining of somata and dendrites. Staining for GABA reactivity appeared in: (1) occasional small somata and dendrites between the bundles of axons in SO (stratum opticum), (2) small somata in all three sublayers of SGFS including cells with tangentially running dendrites, (3) small, medium and large somata with radial dendrites in SGC and (4) scattered small somata in SFC. Glycine immunoreactivity was also present in somata in the same tectal layers, but mainly restricted to small neurons.

These observations are consistent with those of Hartline and Rockland (Soc.for Neurosci.Abstr.13(1):307,1987) for GAD reactivity and suggest that GABA and glycine, which are believed to be major inhibitory neurotransmitters, play an important role in the tectum, presumably in relation to integration of converging sensory signals. Supported by NIH Grant #1R01NS21989-01A1.

26.8

THE DISTRIBUTION OF GLUTAMATE, GABA, BENZODIAZEPINE, MUSCARINIC, AND OPIATE RECEPTORS IN THE AVIAN TECTUM R.L. Albin, E.K. Richfield, A. Reiner, A.B. Young, J.B. Penney. Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109; and Dept. of Anatomy, University of Tennessee Health Science Center, Memphis, TN 38163.

A major visual center of vertebrates is the tectum. This structure achieves its greatest complexity in birds, where it possesses fifteen distinct laminae. Prior studies have provided data about the neurotransmitters of afferents and interneurons of the avian tectum. To complement these studies, we used standard techniques of receptor autoradiography to study the distribution of several neurotransmitter receptors in three White Carneaux pigeons (Columba livia). There was differential distribution of receptors with GABA-A and benzodiazepine receptors densest in layers 1-13 and 15; GABA-B receptors densest in layers 1-2 and 4; muscarinic receptors densest in layers 1-2, and 5; delta opiate receptors densest in layers 1-13, and 15; total glutamate binding densest in layers 1-2, 5, 7, and 15, NMDA subtype in layers 1-13, Quisqualate subtype in layers 1-2, 5, 15. The laminar distribution of receptors correlates well with previous knowledge about neurotransmitters of tectal afferents and interneurons. Supported by EY05298 and NS19620 to A. Reiner, and NS 19613 to A.B. Young.

26.9

CEREBELLAR CONNECTIONS OF THE TRIGEMINAL SYSTEM IN THE PIGEON. J.J.A. Arends and H. P. Zeigler, West Laboratory, American Museum of Natural History, NY 10024, and Biopsychology Program, Hunter College (CUNY), NY 10021.

WGA-HRP tracing methods were used to delineate trigemino-cerebellar projections in the pigeon and identify cerebellar connections with jaw premotor neurons.

The mossy fiber projection originates solely from subnucleus oralis of the nucleus of the descending trigeminal tract (nTTD) and terminates bilaterally (predominantly ipsilaterally) in cerebellar cortical lobules VIII and IXa. Trigeminal input to the climbing fiber system originates in the caudal part of the subnucleus interpolaris of nTTD and is almost completely contralateral. Interpolaris injections produce anterograde label in medial portions of both the ventral and dorsal lamellae of the inferior olive (OI), overlapping the areas retrogradely labelled from injections in lobules VIII and IXa. However, OI injections also retrogradely label a portion of the medullary lateral parvocellular reticular formation (Rpc) adjacent to nTTD and this region (Plexus of Horsley) also receives a projection from nTTD. Thus the existence of a second (trigemino-reticulo-cerebellar) pathway cannot be excluded.

The medial cerebellar cortical zone of lobules VIII and IXa receiving dense trigeminal projections connects exclusively with the caudal pole of the medial cerebellar nuclear complex (CbM). CbM projects upon a portion of Rpc containing jaw premotor neurons. Our results thus define a trigemino-cerebellar circuit, putatively involved in the sensorimotor control of the pigeon jaw.
Supported by Grants BNS 85-07374, MH-008366 and RSA MH-00320.

LEARNING AND MEMORY: PHARMACOLOGY I

27.1

RECOVERY ON A REINFORCED ALTERNATION TASK WITH THA USING A SEQUENTIAL LESION MODEL FOR ALZHEIMER'S DISEASE. J.P. Kesslak, K. Kamali*, A. Korotzer*, A. Song*, & C.W. Cotman, Dept. of Psychobiol., Univ. of Calif., Irvine, CA 92717

The neuropathology of Alzheimer's disease (AD) is characterized by loss of neurons in the entorhinal cortex. This loss may contribute to memory impairment in AD. Entorhinal cell loss such as seen in AD can be simulated in rats by sequential lesions of the entorhinal cortex. The behavioral consequences of the lesions can be evaluated on a reinforced alternation task in a T-maze. Tetrahydro-aminoacridine (THA), a cholinesterase inhibitor, was administered to rats after entorhinal lesion to determine its effects on behavioral recovery. Rats were given unilateral entorhinal lesions and tested for 15 days. The lesions produced a transient deficit on the alternation task. THA (0.5 mg/kg) enhanced recovery while a higher dose of THA (5 mg/kg) impaired recovery. After this phase of testing, rats received lesions of the contralateral entorhinal cortex. Rats receiving a unilateral lesion after extensive training (i.e., undamaged in phase 1) did not show any impairment on the alternation task. Sequential bilateral lesions produced a long term deficit, making significantly more errors than undamaged controls during the 15 days of testing in phase 2. The 0.5 mg/kg THA group exhibited recovery intermediate between control and bilateral-lesion saline injected group. The group receiving 5 mg/kg THA after bilateral lesions did not differ from the bilateral lesion-saline group.

27.2

CHOLINESTERASE INHIBITION AFTER IN VIVO TACRINE IN RAT BRAIN AND BLOOD: DOSE-RESPONSE AND TIME COURSE. Kathleen A. Sherman and Erik Messamore*, Dept. Pharmacol., Southern IL Univ. Sch. Med., Springfield, IL 62794-9230

Tacrine (THA) is a cholinesterase (ChE) inhibitor which has stimulated considerable interest because of marked improvement reported in Alzheimer dementia after THA (Summers et al., NEJM 315:1241, 1986). THA has comparable potency to the carbamate inhibitor physostigmine (PHYSO) in vitro, but acts reversibly on an allosteric site rather than the catalytic site of ChE. Inhibition of acetyl-ChE (AChE) after THA in vivo, however, was modest compared to PHYSO in several studies, raising questions regarding the role of AChE inhibition in the mechanism of therapeutic concentrations. We report that AChE inhibition measured after THA in vivo decreases with increasing dilution of tissue for assay. We have characterized dose-response and time course functions for ChE inhibition after THA in various brain regions, plasma and red blood cells (RBC) using minimal tissue dilution for assay. Plasma ChE was inhibited near maximally by 5 min, but peak inhibition of brain or RBC did not occur until 30-60 min after 5 mg/kg THA, s.c. In brain and plasma, near maximal inhibition was maintained through 3 hr and then declined to control activity by 9 hr. AChE in cortex and hippocampus was inhibited 14-21% after 0.625-2.5 mg/kg THA, the dose range associated with improved retention in mice. Maximal inhibition occurred after 10-20 mg/kg THA and was 72-80% in hippocampus and cortex and 80% in plasma and RBC.

27.3

BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF GALANTHAMINE IN MICE. J.E. Sweeney and J.T. Coyle. Departments of Environmental Health Science and Neuroscience. The Johns Hopkins University, Baltimore, MD 21205.

Previously, we have shown that galanthamine (Gal), a long-acting reversible central acetylcholinesterase (AChE) inhibitor, significantly improved performance on a water maze task of nucleus basalis magnocellularis (nBM)-lesioned mice and significantly impaired performance of controls (5.0 mg/kg, i.p. given 3.5 hours before testing). We now show that Gal improves performance of nBM-lesioned mice on a passive avoidance task by increasing latency to enter the dark compartment. Gal exhibits an inverted U-shaped dose-response curve characteristic of AChE inhibitors, with 3.0 mg/kg being the most effective dose at reversing the behavioral deficit. Further studies indicate that alterations in performance are not due primarily to changes in motor activity.

Using the Ellman method, *in vitro* analyses of AChE inhibition in cortical tissue have shown that Gal exhibits an IC_{50} of 17 μM. Analysis of brain homogenates *in vitro* 4 hrs after a Gal dose of 5.0 mg/kg i.p. (17 μmoles/kg) revealed significant inhibition of AChE activity. When extrapolated for the dilution of the tissue in the assay, this inhibition is equivalent to an 85% inhibition of AChE in cortical tissue *in vivo*. These data continue to support the hypothesis that Gal is a centrally active AChE inhibitor that reverses the behavioral deficits resulting from basal forebrain cholinergic lesions. Thus, Gal may have therapeutic utility in correcting the cholinergic deficits of Alzheimer's Disease.

27.4

HEMICHOLINIUM-3 (HC-3) PRETREATMENT ATTENUATES THE MEMORY IMPAIRMENTS AND PRESYNAPTIC CHOLINERGIC DEFICITS INDUCED BY ETHYLCHOLINE AZIRIDINIUM ION (AF64A). J.J. Chrobak, M. Spates*, T. Demetriou* & T.J. Walsh. Dept. of Psychology, Rutgers Univ., New Brunswick, NJ 08903.

Ethylcholine aziridinium ion (AF64A) is a neurotoxic analog of choline whose specificity is a consequence of an interaction with high affinity choline transport (HAChT) sites located on cholinergic nerve terminals. ICV administration of doses less than 3 nmol/side produces regionally select alterations of hippocampal cholinergic indices (Leventer et. al., Neuropharmacology, 26, 36,1, 1987). The loss of cholinergic markers induced by AF64A can be attenuated in vitro and in vivo by pretreatment with inhibitors of high affinity choline transport (HAChT) (Curti et. al., J. Membrane Biol., 82, 259, 1984; Potter et. al., Soc. Neurosci. Abst., 326.7, 1987). Previously, we have shown that icv administration of AF64A produces working memory impairments in the rat (Chrobak et. al., Brain Res., 414, 15, 1987; Chrobak et. al., Brain Res., in press). The present study examined whether pretreatment with HC-3, a more potent inhibitor of HAChT than AF64A, would attenuate the memory deficits and neurochemical alterations induced by icv administration of AF64A.

Male Sprague-Dawley rats were trained to perform a eight arm radial maze (RAM) task in which a one hour delay was imposed between the fourth and fifth arm choice. Following acquisition, rats were injected with AF64A (3nmol/side/icv)(AF group) or AF64A preceded by HC-3 (20 μg/ side/icv)(HA group). Control animals received either CSF or HC-3 (20 μg).

AF64A-treated rats were significantly impaired in their performance of the RAM task. This behavioral deficit was associated with a significant decrease (34%) in HAChT in the hippocampus. In contrast, no significant decreases in HAChT or RAM performance were observed in HA group. These findings support the hypotheses that the behavioral deficits associated with icv administration of AF64A are directly related to the compound's cholinotoxic properties and in particular its interaction with the HAChT carrier and further, that a select alteration of septohippocampal cholinergic activity is sufficient to produce an impairment in working memory processes.

This work was supported by a BRSG Grant (PHS 07058-21) to TJW.

27.5

SELECTIVE CENTRAL NICOTINIC RECEPTOR BLOCKADE INHIBITS PASSIVE, BUT NOT ACTIVE, AVOIDANCE LEARNING IN RATS. K. Elrod and J.J. Buccafusco. Dept. Pharmacology and Toxicology, Med. Col. GA and Vet. Admin. Med. Ctr., Augusta, GA 30912.

The influence of s.c. administration of the centrally-acting nicotinic antagonist mecamylamine (MEC) 20 min prior to active (AA) or passive (PA) avoidance training or testing was examined in male, Wistar rats. MEC (10mg/kg) prior to AA training failed to induce performance deficits compared with saline (SAL) controls. A higher dose of MEC (25mg/kg) did produce AA impairment, however, selective blockade of peripheral nicotinic receptors with 25mg/kg hexamethonium (HEX) produced a similar degree of impairment. MEC (0.5, 5 or 25mg/kg) prior to AA testing or testing in a standard 1 trial 24 hr PA paradigm failed to alter performance in either task. MEC (25mg/kg) however, impaired PA performance when administered prior to training as indicated by significantly lower testing step-through latencies compared with pretraining injection of SAL or HEX. Furthermore, MEC (25mg/kg) prior to training in a multi-trial acquisition version of PA failed to induce deficits in acquisition. The selective effect of MEC on PA performance, then, appears to be directed at processes mediating retention. Ongoing neurochemical studies are directed at elucidating the mechanism of action by which MEC exerts its cognitive effect. Suppt. by Smokeless Tobacco Research Council and the Vet. Admin. Med. Ctr.

27.6

PHYSOSTIGMINE ENHANCES SHORT-TERM MEMORY PROCESSES IN YOUNG RATS. C.A. Castro, R. Paylor, J.W. Rudy, and T.B. Moye*. Departments of Psychology, Uninversity of Colorado, Boulder, CO 80309 and San Jose State University, San Jose, CA.

Recently, we used a conditional-spatial alternation discrimination task to demonstrate that the rats' ability to use its short-term memory for recent events begins to emerge between 21 and 24 days after birth. Rats 21 days old are able to remember events that are seperated by 30 sec delays, but are unable to remember events sepeated by 60 sec. Since the cholinergic system is thought to contribute to performance in this task, we sought to determine whether the administration of the anticholinesterase, physostigmine, could enhance the 21 day olds' short-term memory capacity.

Twenty-one day old subjects were trained to solve a conditional-spatial discrimination problem. After solving the problem, subjects received an injection (s.c.) of either physostigmine (0.2, 0.02, 0.002 mg/kg), neostigmine (0.02 mg/kg), or a saline vehicle. The subjects were then tested for their ability to remember information over either a 30 or 60 sec interval. The results were dramatic. Saline and neostigmine treated animals could bridge the 30 sec delay, but their performance fell to chance when the delay was 60 sec. In contrast, there was no drop in the performance of physostigmine treated (.02) animals at either the 30 or 60 sec delay.

These results suggest that the maturation of the central cholinergic system contributes to the emergence of short-term memory processes in the developing animal.

27.7

EFFECTS OF COMBINED NICOTINIC AND MUSCARINIC BLOCKADE ON RADIAL-ARM MAZE PERFORMANCE. E. D. Levin, S. R. McGurk, D. South* and L. L. Butcher. Dept. of Psychology, Univ. of California, Los Angeles, CA 90024-1563.

Separate blockade of either nicotinic or muscarinic acetylcholine receptors has been found to impair choice accuracy in the radial-arm maze. We investigated the effects of combined nicotinic and muscarinic blockade. After being trained to run in an 8-arm radial maze for food rewards, female albino rats (N=18) were injected ip with saline, scopolamine (0.15 mg/kg), mecamylamine (10 mg/kg) or a combination of both drugs in a counterbalanced order 20 min before testing. With saline the rats made 6.89±0.23 (Mean±SEM) arm entries before repeating an entry. All three drug treatments caused significant ($p<.01$) decreases in choice accuracy. The entries to repeat scores were: scopolamine 4.50±0.58, mecamylamine 5.39±0.36 and scopolamine + mecamylamine 3.06±0.39. The combination treatment significantly ($p<.05$) decreased accuracy relative to either of the drugs alone. The scores after combination treatment were lowered to essentially random performance (3.19). These data show that nicotinic and muscarinic blockade have at least additive effects in producing a cognitive deficit. Given that both types of receptors have been found to be reduced in Alzheimer's disease, combination blockade may provide a good model for this disorder. (This study was supported by a grant from the MacArthur Ftd. and grant NS 10928 to L.L.B)

27.8

SND-5008 (AF102B), A NOVEL MUSCALINIC AGONIST, IMPROVES EXPERIMENTAL COGNITIVE DYSFUNCTIONS IN RODENTS. Y.Iga*, N.Nakahara*, F.Mizobe, N.Ohgane*, T.Sawai*, T.Nakajima* and G.Kawanishi*. Res.Inst.of Life Sci., Snow Brand Milk Products Co.,Ltd. Tochigi 329-05, Japan

Senile dementia of Alzheimer's type (SDAT) is characterized by central cholinergic dysfunctions, whereas post-synaptic M1 receptors on cholinoceptic neurons are found to be preserved. Therefore, M1 agonist is considered as a candidate drug for SDAT. In the present study we examined the effects of a novel M1 agonist SND-5008 on learning behavior in AF64A-injected (i.c.v.) rats, basal forebrain (BF) lesioned rats and scopolamine-induced amnesia mice.

AF64A-rats showed inferior performance in a radial-arm maze task. SND-5008 (1.0 and 5.0 mg/kg, p.o.) significantly decreased the number of incorrect response. BF lesions produced significant deficiency in two-way shuttle active avoidance performance. SND-5008(1.0 mg/kg, p.o.) significantly enhanced the avoidance rate without increace of unavailable response. Scopolamine impaired the retention latency in a passive avoidance task in mice. SND-5008 (1.0 and 5.0 mg/kg, p.o.) significantly extended the retention latency. Taken together with our earlier data (Soc.Neusci.Abstr.13 837,1987), the results strongly indicate that SND-5008 improves the learning and memory impairment associated with central cholinergic dysfunctions in rodents.

27.9

ASSOCIATIVE AND NONASSOCIATIVE CONTRIBUTIONS OF THE CHOLINERGIC SYSTEM TO PERFORMANCE IN THE MORRIS WATER TASK IN DEVELOPING RATS. R. Paylor and J.W. Rudy, Psychology Dept., Colorado University, Boulder, CO 80309.

Rat pups learn to navigate to a visible platform in the Morris water task when they are 17 days old, but are unable to learn the location of a platform until they are 21 days old. In adult rats, blockade of central cholinergic muscarinic receptors impairs place learning, without effecting cued navigation. In the present study we looked at the contributions the cholinergic system makes to place and cued learning in 17, 21, and 80 day-old rats using both a gray and white Morris water maze.

Consistent with the existing literature, cholinergic receptor blockade completely disrupted place learning. However, cued navigation was also impaired. The magnitude of the cued-navigation impairment was dependent upon age, context, and drug: (a) both 24- and 80-day-old scopolamine-treated rats were impaired in a gray tank compared to a white tank, but the younger pups were more impaired. (b) Saline-treated 24 day olds, but not adults were impaired in the gray tank. (c) The performance of 17-day-old scopolamine-treated pups on the cued task did not improve with training. In addition, the 17-day olds impaired performance on the cued-navigation task in the gray tank was attenuated by the cholinergic agonist, physostigmine.

The results suggest that the developing central cholinergic systems contribute to successful performance in the Morris water task by enabling the animal to (a) acquire spatial information and (b) inhibit interfering behaviors.

27.10

EFFECTS OF AF64A ON RADIAL-ARM MAZE MEMORY TASKS IN RATS. S.E. MacNeill, D.E. Walters, and R. C. Speth. Dept. of Psychology, Wash. State Univ., Pullman, WA 99164-4830.

ICV injections of mustard aziridinium (AF64A) have been shown to permanently damage cholinergic neurons of the hippocampus, while preserving the integrity of the cerebral cortex and straitum (Hanin, 1983). Because of this apparent long term cholinergic deficit, AF64A has been suggested in the study of Alzheimer's disease. Adult male Sprague-Dawley rats were given intracerebral bilateral injections of 8nmol AF64A, followed by testing for reference memory on a modified radial-arm maze; and choline acetyltransferase (ChAT) assays. Second, dipsogenic responses to carbachol were tested, and a third experiment tested recall of working memory, and delayed response on the radial-arm maze. Results indicate faster completion of the maze and increased frequency of errors in treated rats, accompanied by 50% decreases of ChAT activity.

	Average Time on Maze*	Average Errors*
Lesioned Rats	100.5±28	18.0±14.0
Control Rats	282.5±37	7.8± 2.9

*mean ± s.d.

The carbachol studies, and ensuing behavioral studies are currently in progress. These results should help assess the value of AF64A in studies of neurological disorders such as Alzheimer's disease.

27.11

EFFECTS OF PRAMIRACETAM AND PHOSPHATIDYL CHOLINE ON SPATIAL MEMORY DEFICITS IN MICE WITH LESIONS OF THE NUCLEUS BASALIS OF MEYNERT. N. M. Qashu* and A. E. Powell* (SPON: F. Volkmann). Department of Psychology, Smith College, Northampton, MA 01063.

This study investigated the role of pramiracetam as a cognitive adjuvant in an animal model of Alzheimer's Disease. The study examined the effects of nucleus basalis of Meynert (nbM) lesions and pharmacological agents on spatial memory as measured in a milk maze task. There were four groups: a control group receiving saline only, a control group receiving a diet of (86.4mg/kg) phosphatidyl choline (PC) and pramiracetam (10mg/kg), a lesion group (nbM) receiving saline, and a lesion group receiving pramiracetam and PC. Results showed that the interaction of lesion and day was marginally significant. There was no effect of day for lesioned animals, yet unlesioned animals did show such an effect. The drug by lesion effect was also significant. The unlesioned animals receiving saline performed significantly faster than the lesioned animals receiving saline. There was no significant difference in performance between control animals receiving drug/diet and lesioned animals receiving drug and diet. There was also no significant difference in performance between the unlesioned animals that received saline and the unlesioned animals receiving drug and diet. However, among lesioned animals, the animals receiving drug and diet performed significantly faster than those receiving saline. These results confirm the hypothesis that pramiracetam administered with phosphatidyl choline can reverse spatial memory deficits caused by lesions of the nucleus basalis of Meynert. (Supported by Smith College CFCD.)

27.12

THE EFFECTS OF GALANTHAMINE, AN ACETYLCHOLINESTERASE INHIBITOR, ON LEARNING AND MEMORY IN MICE AND MONKEYS. G.P. Vincent, N. Pietrusiak*, L. Rumennik* and J. Sepinwall. Neurobiology and Obesity Research, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Galanthamine (Gal), claimed to be a long-acting acetylcholinesterase inhibitor, improved acquisition in a Morris water maze by C57BL/10 mice, i.e., it significantly decreased the latency to find the hidden platform. When given i.p. 30 min before testing, it was active from 0.1 to 1 mg/kg. When the pretreatment time (pt) was varied, Gal 0.1 mg/kg i.p. was active from 15 min to 3 hr, with peak effect occurring at 2 hr. When given i.p. with 2 hr pt, Gal was active from 0.1 to 3 mg/kg. When given p.o. 60 min before testing, Gal was active from 0.03 to 0.3 mg/kg. When the pt was varied, Gal was active 60 and 90 min after p.o. dosing. C57BL/10 mice are deficient in using distal room cues to locate the hidden platform and this deficit can be ameliorated with certain cognitive enhancing (CE) agents (Pietrusiak, et al., Soc. Neurosci. Abstr. 12:705, 1986). Gal (0.1 mg/kg, i.p., 2 hr pt.) tested using only distal room cues significantly decreased the latency to locate the hidden platform, thus reversing the behavioral deficit. In squirrel monkeys Gal improved the accuracy of retention on a delayed match-to-sample procedure by 6-21% (0.03-1.0 mg/kg, i.m.). Thus Gal has potent CE activity with a rapid onset and a long duration of action, especially after parenteral administration.

27.13

PRENATAL CHOLINE EXPOSURE PRODUCES LONG-TERM IMPROVEMENT IN TEMPORAL PROCESSING. W.H. Meck, A.G. Shapiro, and C.L. Williams. Department of Psychology Columbia University, New York, New York 10027.

Exposure to choline chloride supplementation during early development has been shown to have facilitative effects on spatial memory processes that extend well into adulthood (Meck et al., Dev. Psychobio. 21, 1988). We now report that a relatively brief period of prenatal choline supplementation is sufficient to modify temporal processing in adult male Sprague Dawley rats as indexed by the peak-interval (PI) timing procedure (Meck et al., J. Neurosci. 2:292, 1987). Using a 20-sec PI procedure with visual signals we demonstrated that in the course of 5 weeks of training prenatal choline exposure 1) alters the content of temporal in a dynamic fashion as a function of training and 2) significantly improves the differentiation between the response states involved in temporal regulation. These data support previous findings that modifications in cholinergic function alter the content and sensitivity of temporal memory by changing the speed of memory storage processes (Meck & Church, Behav. Neurosci. 101:457, 1987).

27.14

CHOLINE SUPPLEMENTATION DURING DEVELOPMENT: TIME FRAMES FOR SPATIAL MEMORY FACILITATION. A.G. Shapiro, C.L. Williams, and W.H. Meck. Department of Psychology Columbia University, New York, New York 10027.

Supplementation of choline chloride during early developmental periods has been reported to facilitate performance in spatial memory tasks such as the radial arm maze. This effect can be measured long after the initial treatments, suggesting that early doses produce organizational changes in cholinergic function. In the present study, six groups of male rats were exposed to choline at various periods of development in search of time frames where different organizational changes could occur. One pre- and 5 postnatal exposure periods were evaluated. Prenatally choline was administered through the drinking water (5 ml/L) of the pregnant dams. Postnatally choline was given by s.c. injections (300 mg/kg) on days 1-15, 16-30, 31-45, 46-60, and 61-75.

From 7-17 months of age treated and untreated rats were tested on a 12-arm radial maze with working and reference memory components. The results indicate a choline produced facilitation of spatial memory that varied as a function of exposure period in terms of susceptibility to disruption of memory processes.

27.15

EXPOSURE TO CHOLINE DURING DEVELOPMENT IMPROVES SPATIAL MEMORY CAPACITY OF MALE AND FEMALE RATS.

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Barnard Col. & Columbia Univ., NY, NY 10027.

Previous work has shown: **1)** exposure to choline during development enhances spatial memory of male rats (Meck et al., Dev. Psychobiol. 21: 1988), **2)** females are less accurate than males during the acquisition of a spatial task, perhaps due to differential cue selection (Williams et al., Behav. Neurosci., in press). In the present study, we investigated the organizational effects of choline in both males and females. Pregnant dams received 0 or 5 ml choline chloride/L water. Pups (n=40, 10/condition) were raised by untreated foster dams and injected s.c. with saline or choline chloride (300 mg/kg) for 24 days. Subjects were gonadectomized at 60 days of age; testing began 20 days later. A radial maze with 8 baited and 4 unbaited arms was used for behavioral tests.

Results indicate that while early choline treatment significantly enhances memory capacity of both male and female rats, choline appears to improve choice accuracy of males more than females, and to influence choice latency in a sexually dimorphic manner. (NS 30671 to CLW)

27.16

RECEPTOR CHANGES ASSOCIATED WITH MEMORY DEFICITS FOLLOWING REPEATED ORGANOPHOSPHATE (DFP) EXPOSURE. K.C. Raffaele and D.S. Olton. The Johns Hopkins University, Baltimore, MD 21218.

The central cholinergic nervous system is important for performance of many learning and memory tasks. Organophosphates (OPs) interfere with the normal functioning of the cholinergic nervous system by irreversibly inhibiting the enzyme, acetylcholinesterase, which breaks down acetylcholine. Repeated exposure to OPs produces a down-regulation of muscarinic receptors. To investigate the relationship between sensitivity to scopolamine (a muscarinic receptor antagonist) and changes in muscarinic receptor levels, rat were tested in two different memory tasks before, during, and after repeated exposure to DFP, an organophosphate anticholinesterase. The rats were evaluated for changes in performance and changes in sensitivity to scopolamine, and changes in receptor levels and acetylcholinesterase activity were measured. The total number of muscarinic receptors (QNB binding) was reduced in both cortex and hippocampus at two time points after DFP treatment had ended. Acetylcholinesterase activity was also reduced at both time points. These changes in acetylcholinesterase activity and the number of muscarinic receptors were associated with the changes in sensitivity to scopolamine, which lasted throughout the period during which reductions were demonstrated.

27.17

ANTAGONISTIC EFFECTS OF ATROPINE, SCOPOLAMINE, AND APROPHEN AGAINST PHYSOSTIGMINE ON A REPEATED ACQUISITION RADIAL-ARM MAZE TASK IN RATS. J. R. Leu. Department of Medical Neurosciences, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Three anticholinergic drugs, atropine, scopolamine, and aprophen, were tested for efficacy in antagonizing the performance decrements induced by the cholinergic drug physostigmine. The task used was a repeated acquisition radial-maze task described elsewhere (1,2,3). This task requires the animal to search the various arms of a multi-arm radial maze to determine which arms are reinforced during a particular test session. The animal may then repeatedly procure food by alternating between these active arms. This procedure measures long-term memory of the task, the ability to form new associations and the ability to remember immediate past activities.

The range of effective antagonism of anticholinergic drugs, against the performance altering effects of physostigmine, was determined. All drugs provided effective antagonism at some dose. Aprophen was the most potent, and had the widest range of antagonism. Atropine and scopolamine were neither as potent nor as effective. Refs: (1)Popp & Leu (March, 1985). Proceedings and Abstracts: Eastern Psychological Association, 56. (2)Leu & Johnson (April, 1987). Proceedings & Abstracts: Southwestern Psychological Association, 33. (3)Leu & Johnson (1988) (manuscript under review).

27.18

THE EFFECTS OF SCOPOLAMINE ON DELAYED RESPONSE IN RHESUS MONKEYS. R. Lalonde and T.G. Aigner. Neurology Service, Hotel-Dieu Hospital, Montreal, Canada H2W 1T8 and Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

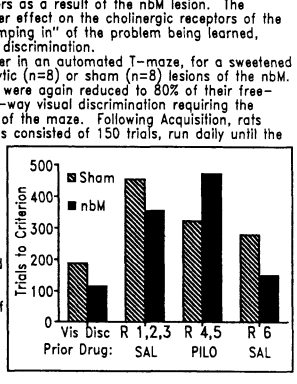
We have previously reported that the anticholinergic agent scopolamine (SCOP) impairs the performance of monkeys on a variety of object memory tests including recognition, recency, and recall. The results further indicated that SCOP acts at an early stage of memory, preventing information from entering even into an immediate store. To determine if these findings also apply to spatial memory, we administered SCOP to 2 monkeys trained in delayed response with 0 to 10-sec delay intervals. During the delays, an opaque screen was lowered to block the animal's view of the wells, except on half of the 0-sec delays when the screen was not lowered. The effects of SCOP (10.0, 17.8, 32.0, and 56.0 ug/kg, i.m. 20 min before testing) were then evaluated, first at the 10-sec interval and ending at the 0-sec intervals, with and without the screen. Although higher doses were required, SCOP had the same consequences on this task as on our previous ones, decreasing the percent correct choices in both monkeys in a dose-related manner on all conditions except the 0-sec delay without the screen, where it was ineffective. These results support and extend our previous conclusion that SCOP impairs the earliest stage of information storage.

27.19

PILOCARPINE DURING ACQUISITION DISRUPTS SUBSEQUENT REVERSAL IN N. BASALIS-LESIONED RATS: PRELIMINARY DATA. W. Jeffrey Wilson & Jennifer C. Hall*. Dept. of Psychological Sciences, Indiana-Purdue University, Fort Wayne, IN 46805

The n. basalis magnocellularis, source of cholinergic innervation of much of the cortex, has been implicated in normal memory functions, and in the impairments of memory that accompany Alzheimer's disease. Rats with lesions of the nbM have proven deficient in the acquisition and reversal of simple (i.e., learnable within a single session) discrimination problems. We report data from a preliminary study involving acquisition and reversal of a difficult visual discrimination problem, in which lesioned rats did not differ from sham-lesioned controls. However, exposure to the cholinergic agonist pilocarpine during a given reversal made the subsequent reversal more difficult. We suggest that this could have resulted from the development of supersensitive cortical cholinergic receptors as a result of the nbM lesion. The pilocarpine would thus have had a greater effect on the cholinergic receptors of the lesioned rats, resulting in a greater "stamping in" of the problem being learned, interfering with the later reversal of that discrimination.

After being shaped to press either lever in an automated T-maze, for a sweetened milk reward, rats received either electrolytic (n=8) or sham (n=8) lesions of the nbM. Following a 1 week recovery period, rats were again reduced to 80% of their free-feeding weight, then were trained in a 2-way visual discrimination requiring the choice of either the lighted or dark arm of the maze. Following Acquisition, rats received 6 consecutive Reversals. Sessions consisted of 150 trials, run daily until the criterion of 8 correct choices in a row was achieved. Reversal began at the start of the next session. 15 min prior to Reversals 3&4 rats received 3 mg/kg pilocarpine i.p., preceded by 1 mg/kg methyl scopolamine i.p. Saline was given on the other Reversals. As a result of post-op deaths and histology, n's were Sham = 7 and nbM = 3. Pilocarpine had no effect on performance during the session on which it was given; reversals of habits acquired under the influence of pilocarpine required more trials for nbM rats than for sham-lesioned controls. [IPFW Research & Instructional Development Support Program Grant to W.J.W.]



27.20

NEUROCHEMICAL STUDIES OF E-2020, A NOVEL CENTRALLY ACTING ACETYLCHOLINESTERASE INHIBITOR. Y.Yamanishi*, S.Araki*T.Kosasa*H.Ogura*and K.Yamatsu*(SPON:Y.Sugiura). Eisai Tsukuba Res.Lab.,5-1-3 Tokodai,Tsukuba-city, Ibaraki, 300-26 Japan.

Novel piperidine derivatives, newly synthesized in our laboratory, showed potent inhibitory action on cholinesterase. In this study, we performed some neurochemical experiments to test E-2020, 1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]methylpiperidine hydrochloride, one of the derivatives, compared with physostigmine(PHY) and tetrahydroaminoacridine(THA). IC50 of E-2020 in vitro on acetylcholinesterase(AChE) was 5.3nM. IC50 of THA and PHY were 52nM and 0.43nM, respectively. E-2020 inhibited AChE selectively 570 fold more than butyrylcholinesterase. The inhibition of AChE was reversible. In ex vivo experiment, E-2020 inhibited AChE in rats' brains dose-dependently at 1 to 30 mg/kg, p.o.. This inhibition lasted more than 4 hrs at 5 mg/kg. The inhibitory effect of E-2020 on AChE was more potent and longer than those of THA and PHY. E-2020 increased ACh content in rats' brains at 5 to 20 mg/kg, p.o.. At 2.5 mg/kg, p.o., it antagonized not only the decrease of ACh content in the frontal cortex induced by the lesion of nucleus basalis magnocellularis but also the depletion of ACh by scopolamine.

In conclusion, these data indicate that E-2020 is a potent centrally acting AChE inhibitor which may serve as a drug for Alzheimer's disease.

27.21

BEHAVIORAL STUDY OF E-2020, A NOVEL CENTRALLY ACTING ACETYLCHOLINESTERASE INHIBITOR. H.Ogura*,T.Kosasa*,S.Araki*, Y.Yamanishi* and K.Yamatsu*(SPON:Y.Arakawa). Eisai Tsukuba Res.Lab.,5-1-3 Tokodai,Tsukuba-city,Ibaraki,300-26 Japan.

E-2020 is a new piperidine derivative, which inhibits acetylcholinesterase potently in in vitro and ex vivo studies. We designed this study to evaluate its potency in behavioral tests compared with tetrahydroaminoacridine (THA). First, we studied effects on scopolamine-induced impairment in 8-arm radial maze. Male Wistar rats were thoroughly trained to get food chips. Scopolamine (0.5 mg/kg,i.p.) disrupted this behavior, i.e. increased errors and duration to complete the task. E-2020 antagonized these changes dose-dependently at 0.25 to 0.5 mg/kg, p.o., while THA at 1 to 2 mg/kg antagonized it. Secondly, E-2020 was tested in nucleus basalis magnocellularis (NBM) lesioned rats in a passive avoidance test. Male Wistar rats received ibotenate (5µg/0.5µl) bilaterally in NBM. Lesioned rats showed marked shortening of retention latency. E-2020 reversed the effect of the lesion at 0.125 to 1 mg/kg, p.o.. THA had a tendency to improve at 0.5 mg/kg. In the observation of general behavior after oral dosings, both E-2020 and THA produced fasciculation, a peripheral cholinergic sign, at the same dose of more than 2.5 mg/kg.

These results suggest that E-2020 has potent activity in behavioral tests and its safty margin is 4 times as wide as that of THA.

27.22

CHOLINERGIC RECEPTOR ACTIVATION AND ONE-TRIAL TASTE-AVOIDANCE LEARNING IN THE CHICK. M.R. Rosenzweig, T.A. Patterson, J. Lipton, & E.L.Bennett. Dept. of Psychology, University of California, Berkeley, CA 94720.

It has been shown that cholinergic receptor binding increases as a result of avoidance learning in the chick (Rose et al., 1980). However, involvement in a specific stage of memory formation was not determined. The present experiments were designed to examine the effects of muscarinic receptor antagonists on specific stages of memory formation (Patterson et al., 1986).

Experiment 1 determined the range of amnestic doses of scopolamine (SCOP). Chicks were given bilateral injections into the medial hyperstriatum ventrale (MHV; 10 µl per hemisphere) of either saline or different doses of SCOP, 5 min before training. Chicks were tested 24 hr after training. Results showed that 25mM - 100mM (millimolar) SCOP produced significant amnesia. In Experiment 2, the susceptibility gradient for SCOP was determined. SCOP (25mM) produced amnesia, tested 24 hr after training, when injected 10 or 5 min before training, but was not amnestic when injected earlier than 10 min before training, or at any time after training.

The time course of amnesia development following 5 min pretraining injection of SCOP was examined. SCOP (25 mM) produced amnesia that developed between 15 and 30 min posttraining; this amnesia was permanent. Further studies showed that this effect is similar to that produced by agents such as ouabain that interfere with the formation of intermediate-term memory (ITM), suggesting that cholinergic receptor activation is involved in formation of ITM.

The effects of antagonists to two cholinergic receptor-subtypes were examined. Pirenzepine (an M1 antagonist) produced amnesia in a dose-dependent manner (1.0-25 mM doses were amnestic). Gallamine (an M2 antagonist) was not amnestic, suggesting that M1 but not M2 cholinergic receptors are involved in formation of ITM in the chick.

Supported by NSF grant BNS-86-06938.

27.23

THE EFFECT OF ATROPINE ON THE INTRACORTICAL PROFILE OF AUDITORY EVOKED POTENTIALS IN CLASSICAL AVERSIVE CONDITIONING. M. Molnár[x], G. Karmos[x], V. Csépe[x] and I. Winkler[x]. (SPON: European Brain and Behavior Society). Institute for Psychology of the Hungarian Academy of Sciences, Budapest, Hungary.

In a classical aversive conditioning paradigm intracortical auditory evoked potentials (EPs) were recorded simultaneously in six depths of the AI acoustic cortex of freely moving cats. 4 kHz meaningless background clicks given at 2/s rate were randomly interrupted by 3.6 kHz signal ones followed by an unavoidable electric shock. EPs evoked by the background and signal stimuli were averaged separately. As a result of conditioning the amplitude of a middle-latency (40-70 ms) negative component increased and its latency decreased. The observed waveform changes of the EPs were restricted to the upper layers of the auditory cortex.

Atropine (2 mg/kg i.p.) had no effect on this phenomenon. It was concluded that the observed EP changes accompanying aversive classical conditioning were probably the result of EPSPs summating in the upper cortical layers evoked by unspecific non-cholinergic afferent input.

27.24

ACTIVATION OF THE IMMUNE SYSTEM FACILITATES CONDITIONED EMOTIONAL RESPONSE LEARNING. D.T. Lysle*, B.J. Kucinski*, J.E. Cunnick*, H. Fowler*, & B.S. Rabin* (SPON: H. Barry). Departments of Pathology and Psychology, University of Pittsburgh, Pittsburgh, PA 15213-3417.

Activated lymphocytes produce many of the same hormonal factors found in the central nervous system (CNS). There is also evidence that the same hormones are involved in learning and memory processes. To assess the influence of immune activity on CNS function, the present research examined the acquisition and extinction of a conditioned emotional response during lymphocyte activation. An initial experiment showed that the learning of a conditioned fear response by Lewis rats was facilitated when the immune system was activated by sheep red blood cells (SRBC), a T-dependent antigen. However, there was no facilitation when TNP-Ficoll, a T-independent antigen, was employed. Likewise, the extinction of a conditioned fear response was facilitated by the response to SRBC, but not to TNP. Since a thermal-sensitivity test failed to show any analgesic effects of immunization, the present findings indicate that the facilitated conditioning effects were due, not to differences in unconditioned reactivity, but to differences in the rate of learning. These results imply that the immune system directly influences the CNS processes.

27.25

THE EFFECTS OF EXPERIENCE ON GAP INHIBTION OF THE ACOUSTIC STARTLE RESPONSE (ASR). K. M. Crofton, L. P. Sheets*, K. F. Dean*, and D. B. Peele, NTD, US EPA, RTP, NC 27711 and Northrop Services, Inc, RTP, NC 27709

The experiments reported here were designed to study the effects of experience on gap inhibition of the ASR. Two experiments were conducted with independent, naive groups of adult Long Evans hooded rats. They were tested using 20-msec gaps in white noise (ON 80dB/OFF 35dB) as the prestimulus (ISI = 190 msec) and a 120-dB, 40-msec white noise burst as the eliciting stimulus (ES). The first experiment was a test of associative conditioning. Three groups of rats were tested daily for 7 days under one of the following conditions: the gap and ES paired, ES only, or gap only. All groups then received both the Gap and ES for a further 7 days of testing. The second experiment studied the effects of scopolamine (SCOP) on the acquisition of gap inhibition. Animals were tested for 6 days under the following regimins: vehicle, 0.5 mg/kg SCOP 20 min prior to testing, or 0.5 mg/kg SCOP after testing. On day 7, vehicle and SCOP-after-testing groups recieved SCOP prior to testing. Results of these experiments indicate that 5-6 daily sessions are required to reach an asymptotic level of inhibition. The two stimuli must be paired for the inhibition to develop. SCOP blocks the development of this inhibition and also blocks the inhibition once it is achieved. These data suggest a learning component in the gap inhibition paradigm.

27.26

A COMPARISON OF DRUG-INDUCED EFFECTS ON ACQUISITION AND RETENTION IN THE SQUIRREL MONKEY. L. Rumennik*, G. P. Vincent, E. Schwam, and J. Sepinwall Neurobiology and Obesity Research, Hoffmann-La Roche Inc., Nutley, NJ 07110

The repeated acquisition procedure (RA) has been shown to be sensitive in identifying compounds which impair learning (Thompson, D.M. and Moerschbaecher, J.M., JEAB, 32: 433, 1979). Here a variation of the procedure was used to evaluate the learning-enhancing properties of five compounds in squirrel monkeys: d-amphetamine (0.003-0.03 mg/kg), physostigmine (0.003-0.03 mg/kg), TRH (0.01-1.0 mg/kg), piracetam (200-1600 mg/kg) and aniracetam (10-300 mg/kg). For comparison, these compounds were also tested at similar doses for their memory-enhancing effects using a delayed match-to-sample procedure (DMTS) (Schwam et al, Soc. Neurosci. Abstr., 8: 823, 1982) with retention intervals ranging from 0-195 sec. During the performance component of the RA procedure, the same well-established 4-response chain was repeated across days, while during the learning component a different 5-response chain was acquired each day. On RA, only piracetam at a single dose (800 mg/kg, ig) enhanced learning. On DMTS consistent memory-enhancing effects were obtained across retention intervals with several dose levels of aniracetam (10-100 mg/kg, ig). Less consistent memory-enhancing effects were obtained with the other compounds. The contrasting results between procedures suggest their usefulness for differentiating drug-induced enhancement of learning and memory.

27.27

HABITUATION AND RETENTION OF THE HEAD-SHAKE RESPONSE: LACK OF IMPAIRMENT BY NUCLEUS BASALIS MAGNOCELLULARIS LESIONS OR SCOPOLAMINE. C.P.J. Dokla[1], S.C. Parker*[1],& L.J. Thal[2]. [1]Dept.of Psychology,Fairfield Univ.,Fairfield,CT 06430 and [2]Neurology Service,VA Medical Center, La Jolla, CA 92161.

Pharmacological and anatomical studies have strongly implicated central cholinergic systems in learning and memory but are equivocal in regard to habituation. The present study examined the effects of cholinergic manipulation on habituation and retention of the head-shake response (HSR). HSR is a rapid, stereotyped rotation of the head about a front-to-rear axis which in the rat may be elicited by a stream of mildly pressurized air directed at the ear (Askew et al., J. Comp.Physiol.Psychol. ,67:497,1969). Fischer 344 rats received either bilateral ibotenic-acid lesions of the nucleus basalis magnocellularis (NBM) or sham-operations (SHAM). HSR training consisted of 40 stimulated trials followed by a 30-min retention test of 20 trials. Stimulus duration was 15 sec with a 15-sec inter-trial-interval. One group was given scopolamine (0.32 mg/kg,ip) 30 min prior to acquisition trials, while the NBM and SHAM groups received vehicle only. NBM lesions did not affect either habituation or retention of HSR. Although scopolamine decreased frequency and increased latency of HSR (ps $<.05$), habituation rate and retention were not significantly affected. The present results indicate that the cholinergic system may not be important in elicited types of habituation, such as HSR.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: POSTURE AND MOVEMENT I

28.1

PERCEPTION OF STIFFNESS: SENSORY THRESHOLDS L. A. Jones and I. W. Hunter. Faculty of Medicine, McGill University, Montreal, Canada H3G 1Y5

Classical psychophysical techniques have been used to determine sensory thresholds for a number of aspects of proprioception. With the exception of passively imposed movements, the stimuli detected in these experiments are generated by the subjects themselves and so it is only possible to measure difference thresholds. The sensitivity of subjects to changes in stiffness has not been systematically investigated; the objective of this experiment was to determine the difference thresholds for stiffness and to compare these values to those reported for force.

Subjects sat in an experimental rig with a linear motor connected to the wrist of each arm. Force and displacement were recorded on-line from transducers mounted on each motor and these signals were used for real-time servo-control of the stiffness of the motors. The procedure used to calculate the difference thresholds was the method of limits. On successive trials the stiffness of one motor (the comparison stimulus) was decreased or increased incrementally in the direction of the stiffness of the other motor (the standard stimulus).

The results indicate that the difference thresholds for stiffness are greater than those found for force (0.07), and that subjects can accurately detect changes in the stiffness of an external mechanical system.

(Supported by MRC)

28.2

A PSYCHOPHYSICAL MODEL OF KINESTHESIS: PERCEPTION OF FORCE, DISPLACEMENT AND EFFORT. C.L. Van Doren* and L.M. Javorsky* (SPON: M.W. Keith). Depts. of Biomedical Engineering and Orthopaedics, Case Western Reserve Univ., Cleveland, OH 44106.

To assist in the development of kinesthetic feedback for users of upper limb prostheses, a quantitative, psychophysical model of normal kinesthetic perception is developed. In the model, the total kinesthetic sensation is the weighted sum of three component sensations: force, displacement, and effort. The latter is proportional to the efferent command sent to the active muscles. The force and displacement sensations are power functions of their respective stimuli. The weighting given to each component depends on the instructions for a given task, such as "match a target force," or "match a target displacement." The model accounts for the interactions of force, displacement, and effort observed in previous investigations. In addition, by using a simple physiological model of motor unit recruitment, the efferent command can be expressed in terms of the fraction of MUs active in a muscle, and the probability density function for MU twitch strengths can be derived. The theoretical density function agrees well with existing measurements of MU twitch strengths in humans.

The results of a preliminary experiment are also compared to predictions of the model. Subjects matched the displacement or the force generated by MP flexion of their left and right index fingers, which were loaded by springs of different stiffnesses. The effects of spring stiffness and instructions on the matching errors are correctly predicted by the model.

28.3

FLEXION-EXTENSION DIFFERENCES IN SIMPLE REACTION TIME J.G. Anson and H.M. O'Connor*. Faculty of Physical Education and Neuroscience Research Centre, University of Otago, Dunedin, New Zealand.

Previous results (Anson & Wilson, Int. J. Neuroscience, 40, 1988) indicated no significant difference in simple reaction time (SRT) between flexion and extension for rapid initiation of movement of the index finger. However, significant ($p<.05$) differences for premotor time (PMT) were reported. Regardless of starting position, extensor PMT was shorter than flexor PMT. The present study sought to replicate the effect. In addition EMG was recorded from First Dorsal Interosseous (FDI), a possible flexor synergist though not, anatomically, an agonistic flexor of the index finger. Also subjects were asked to choose flexion and extension starting positions separately. (We had assumed in the previous study that the initially chosen starting position was equally appropriate for rapid iniation of either flexion or extension). In a second condition the agonist was put on stretch (index finger 10^{o} from self-selected start). Data from the self-selected start position support the previous result, PMT for FDI exaggerated the effect. Flexion took longer to initiate than extension but initiation from stretch reduced flexor PMT more than extensor PMT. While response conditions - limb segment, inertia and position are similar - the mechanisms underlying SRT seem different for flexion and extension.

28.4

IS 'YIELD TASK' SUPPRESSION OF PERTURBATION EVOKED M2-3 LATENCY EMG ACTIVITY DUE TO STRETCH TRIGGERED INHIBITION? J. S. Thomas, Dept. of Physiology, Meharry Medical College, Nashville, TN 37208

EMG response envelopes recorded from arm muscles of volunteer human subjects for both 'Resist' and 'Yield' TASK conditions reveal several features consistent with Yield TASK enabling of an anti-myotatically signed M3 (75-100 ms) latency response to agonist stretch. 1) Yield TASK EMG responses to muscle stretch drop below the pre-stimulus level of EMG activity (associated with resisting the pre-stimulus load offset) at 75 ms, and this suppression of agonist activity typically passes smoothly into a burst of antagonist activity. 2) The pattern of EMG inhibition evoked to an unloading torque, during a Resist TASK, is interrupted in the Yield TASK by a burst of agonist response which exceeds the pre-stimulus level of activity beginning at 75 ms. 3) A small 'neutral pulse' (+/- torque pulses) perturbation, which results in no net limb displacement and evokes no M2-3 latency activity when given alone, markedly enhances Yield TASK suppression of M2 (50-75 ms) latency activity evoked to a torque step when delivered 20-50 ms prior to a sustained loading torque stimulus. These results cannot be explained by Yield TASK 'gating' of myotatically signed M2-3 activity, but are consistent with a Yield/Assist TASK enabled, stimulus triggered, anti-myotatically signed response to load/stretch whose direct peripheral expression occurs at M3 (75 ms) latency. Stimulus triggered Yield/Assist inhibition of centrally conducted (i.e., trans-cortical?) myotatically signed transmission could occur earlier, accounting for Yield/Assist inhibition of M2 (50-75 ms) activity.

28.5

CHARACTERIZATION OF AN ARRAY OF SPINAL REFLEXES FROM THE COMMON PERONEAL NERVE TO THE VASTUS MEDIALIS MUSCLE IN HUMANS. P. Yoon, J.D. Brooke and W.E. McIlroy. Human Biology/Biophysics, Univ. of Guelph, Guelph, Ontario, CANADA N1G 2W1

To investigate translimb links, we evoked an array of spinal reflexes in tonically contracted vastus medialis muscle, from transcutaneous activation of common peroneal afferents at caput fibula in humans. Afferents were recruited by graded stimulation, and higher threshold interneurons were investigated with a pulse train (four pulses, interpulse interval = 3 ms). Four responses occurred: 1) early excitation at 25.8 ms, with one pulse at 50% of the stimulus for Mmax in tibialis anterior; 2) early inhibition at 39.5 ms, with four pulses at 50% Mmax; 3) second excitation at 58.5 ms with, four pulses at 50% Mmax; 4) later inhibition at 78.9 ms, with one pulse of 150% Mmax. Responses were powerful, statistically significant ($p < 0.05$) and attributed to afferent groups I through III. The findings show that movement interpretation of any single spinal reflex pathway in healthy humans should be constrained by the fact that the expression of spinal reflexes varies, dependent upon methodology.

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28.6

MECHANISMS UNDERLYING MOTOR OUTPUT VARIABILITY STUDIED USING THE H-REFLEX TECHNIQUE. W.G. Darling and D. Rotella*, Dept. of Exercise Science, U. of Iowa, Iowa City, IA, 52242.

Studies of variability in motor output have shown that trial-to-trial variations in movement kinematics and muscle EMGs increase with increasing speed of movements. Such greater variability suggests that there are greater variations in centrally generated commands to muscles for fast movements. This hypothesis was studied using the H-reflex technique to examine variations in motor neuron excitability prior to initiation of muscle activity related to production of a controlled torque.

Volunteer human subjects produced increases in plantarflexion torque under two instructions: (1) increase torque smoothly and accurately to the target and (2) increase torque rapidly to the target. Subjects were cued with a verbal "ready" followed, after a variable time interval, by an auditory tone which served as the signal to initiate the contraction. H-reflexes (20% of maximum for soleus) were elicited in the triceps surae muscle group in about 1/2 of the trials by delivering rectangular pulses at various times after the ready and reaction signals. The results indicate that rapid increases in torque were associated with greater variability in the muscle EMG bursts, peak torques, peak rate of rise of torques and in the H-reflex amplitudes.

supported by a NIH Biomedical Research Support Grant

28.7

OPERANT CONDITIONING OF H-REFLEX IN UNRESTRAINED MONKEYS. P.A. Herchenroder* and J.R. Wolpaw (SPON: E.W. Wolpaw). Wadsworth Labs, NYS Dpt Hlth, Albany, NY 12201.

Monkeys can gradually increase or decrease the wholly spinal, largely monosynaptic stretch reflex or its electrical analog, the H-reflex, when reward depends on reflex amplitude (J Neurophysiol 50:1296-1311, 1983 & 57:443-458, 1987). Conditioning produces persistent spinal cord modifications, potentially accessible memory substrates (Wolpaw & Lee, this vol). The original experiment required animal restraint. This report describes a new design that achieves H-reflex conditioning in unrestrained animals.

Monkeys (Macaca nemestrina) are implanted under general anesthesia with chronic triceps surae (TS) fine-wire EMG electrodes and tibial nerve stimulating cuffs. Wires exit in mid-back into a pocket in a cloth harness. A 1 m flexible stainless steel cable anchored in the pocket conducts the wires through the cage top to a 24-lead electronic swivel. The swivel connects to amplifiers and stimulators that in turn connect to a minicomputer that interfaces with 6 animals 24 h/day. Animals move freely about their cages and readily adapt to the harnesses.

Each monkey learns a simple task. It places its mouth in front of the cage's water-delivery port and provides a given level of TS EMG in both legs for 1.2-1.8 s. Then, stimuli just above M response threshold elicit H-reflexes in both legs, and 200 ms later a solenoid-powered syringe delivers liquid reward to the animal's mouth. In control mode, reward always occurs. In HR↑ or HR↓ mode, reward occurs only if the H-reflex in one leg (the conditioned leg) is above (HR↑) or below (HR↓) a set criterion. Monkeys perform 3,000-6,000 trials/day.

Under HR↑ or HR↓ mode, H-reflex amplitude changes appropriately over weeks, just as it did with the original design. The new design provides precise reflex data and capacity for reflex modification in unrestrained animals.

(Supported by NIH NS22189 and United Cerebral Palsy.)

28.8

ROLE OF SMALL DIAMETER AFFERENTS IN REFLEX INHIBITION DURING FATIGUE OF HUMAN SOLEUS MUSCLE. SJ Garland* and AJ McComas. Dept of Neurosciences, McMaster University, Hamilton, ON, Canada L8N 3Z5.

We have previously shown that H-reflex excitability of human soleus motoneurones is reduced during fatigue and is accompanied by an equivalent decrease in EMG activity during maximal voluntary contractions (MVC). These findings provide suggestive evidence for reflex inhibition of the alpha motoneuron pool during fatigue. To elucidate the contribution of different sized afferents in the reflex inhibition, compression of the sciatic nerve was used in an attempt to block large myelinated afferents. Fatigue of soleus muscle was then induced under ischemic conditions by intermittent electrical stimulation at 15 Hz in 5 healthy subjects. Subjects also participated in a control test in which the compression block was followed by ischemia without fatigue. Both mean EMG and MVC declined following nerve compression alone (by 10.9 $\pm$ 21.8% and 16.5 $\pm$ 12.3%, $p < .01$, respectively). Following fatigue, the mean MVC decreased by 43.8 $\pm$ 16.5% ($p < .01$) from the postblock value compared to a decrease of 6.4 $\pm$ 8.4% in the ischemia control. The mean EMG decreased from postblock values by 48.6 $\pm$ 12% ($p < .01$) following fatigue and by only 7.8 $\pm$ 6.6% following ischemia alone. The findings implicate smaller diameter muscle afferents in reflex inhibition of the alpha motoneuron pool during fatigue.

28.9

INTERACTIONS IN THE MODIFICATIONS INDUCED BY PROLONGED MUSCLE STRETCH ON REFLEX, VOLUNTARY AND SEMI-AUTOMATIC MUSCLE ACTIVATIONS. F. Malouin, C.L. Richards, F. Dumas* and F. Tremblay*. Physiotherapy Dept. and Neurobiology Lab., Fac. of Med., Laval Univ., Quebec, Canada.

Interactions in the effects induced by prolonged muscle stretch (PMS) were studied by comparing significant changes on ankle muscle activations during: reflex (passive movements), voluntary (isometric contractions) and semi-automatic (gait) types of activities. Ten spastic children (4-11 yrs) were tested before and after 30 min of stretch imposed on plantarflexor muscles (PF) by standing the child on a tilt-table. PMS-induced inhibitory effects in the triceps surae (TS) and the tibialis anterior (TA) during passive ankle movements were associated with increased TS ($p<0.08$) but not TA agonist activation during isometric contractions and higher TS activation ($p<0.05$) in early stance (0-15%) of the gait cycle. The higher TS activation during gait which was associated with a concomitant decrease in TA activation (0-15%) and an increased cadence is thus not likely due to hyperactive stretch reflexes. It is proposed that PMS-induced reflex inhibition resulted in improved supraspinal drive to the TS but not the TA during gait and isometric contractions and promoted reciprocal inhibition patterns in the TS and TA during gait.

This work was supported by Health and Welfare Canada.

28.10

PATTERNS OF IRRADIATION OF MOTOR UNIT ACTIVITY IN FATIGUE. M. R. Dimitrijevic, W. J. Eaton*, L. Emerich*, G. Vrbova* and W. B. McKay*. Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030

When a primary muscle fails to generate the intended force, progressive activation occurs in ipsilateral and contralateral muscles not directly involved in the force production. Increased levels of activity in response to demands for more force result in recruitment of more and more motor units within the motor pool. The activity spreads beyond the single motor pool when efforts to maintain the desired force begin to fail. In healthy subjects this recruitment pattern is stereotyped. Using surface EMG recordings over the lower extremities and trunk muscles, together with a force transducer to record ankle dorsiflexion force, healthy subjects were asked to make 3 repetitions of maximum voluntary contraction (MVC) levels, sustained until the subject could no longer maintain at least 50% of the initial effort, with an equal rest period between. Subjects then repeated a series of contractions at 20%, 40%, 60%, 80% and 100% of MVC for 6 seconds with 4 seconds rest between. Results of studies of these responses while seated and standing will be reported. Consistency of these patterns under varying conditions and across subjects suggest that these are indicative of the basic neurocontrol mechanisms operative under excessive loading conditions.

28.11

FUNCTIONAL ORGANIZATION OF MOTOR UNIT POOLS IN HUMAN DELTOID MUSCLE. S. Kane*, J. Hsieh* and K.C. Hayes. Dept. of Physical Medicine and Rehabilitation, The University of Western Ontario, London, Ontario, Canada.

We sought to characterize the activation of separate motor unit pools within the human deltoid muscle. Muscle fibers in the anterior head have force vector components 180° opposed to those of the posterior fibers. Different motor units within the same muscle therefore have the potential to serve agonistic or antagonistic functions within a given movement. Surface and intramuscular EMG recordings were made from the anterior and posterior fibers of the deltoid muscle in 5 healthy adult subjects while they performed rapid arm flexion or extension movements. EMG recordings consistently revealed bi- and tri- phasic patterns of motor unit activation within the deltoid muscle in the same manner as typically recorded from anatomically distinct agonist and antagonist muscles during ballistic movements. Suppression of background activity in the antagonist fibers often preceded activation of the agonist fibers (Hufschmidt phenomenon) by 30-50 milliseconds. This implies the presence of central descending influences; there also exists the possibility of peripheral inputs contributing to the later stages of inhibition. These results suggest that motor units within the anterior and posterior heads of deltoid are controlled similarly to motor units in anatomically defined antagonistic muscle pairs.

28.12

EMG COACTIVATION: THE REGULATORY ACTION OF THE MUSCLE ANTAGONIST. M. Solomonow, R. Baratta* and R. D'Ambrosia*. Bioengineering Laboratory, Louisiana State University Medical Center, New Orleans, LA 70112.

The EMG of the knee antagonist muscle group during maximal effort isometric and isokinetic contractions in flexion and extension over the full range of motion were studied. The simultaneously recorded EMG from the hamstrings and quadriceps, when acting as antagonists, were normalized with respect to their EMG during maximal effort when acting as agonist at each joint angle. The pooled data of 56 subjects was analyzed statistically to yield the following conclusions: athletes (volleyball and basketball) showed depressed activity probably due to increased joint efficiency resulting from frequent use of quadriceps; the antagonist compensated for the impact of gravity on the limb mass, increasing and decreasing activity during motion along and against the gravity vector, respectively; during contraction on the plane parallel to ground, the antagonist compensated for variations in its moment arm about the joint's center of rotation to generate nearly constant opposing torque, the antagonist allowed acceleration in the initial phase of motion, but increased opposition (dynamic braking) towards the terminal phase of the motion as velocity increased. The data points out that the role of the antagonist is to regulate the movement about the joint by compensating for various internal and external disturbances.

28.13

LOCAL CIRCUIT CONTROL OF LOAD COMPENSATORY REACTIONS IN LOCUSTS. S. N. Zill. Dept. of Anatomy, Marshall Univ. School of Medicine, Huntington, WV 25704.

In order to maintain an upright posture, many animals react to environmental perturbations with appropriate contractions of limb muscles. We have applied the paradigm of Nashner (Exp. Brain Res. 26:59, 1976) to the locust and have shown that these animals can respond with two discrete motor strategies when the substrate upon which they are standing is repetitively displaced: a resistance strategy, in which bursting occurs in motoneurons to tibial muscles to oppose imposed forces and a flexion strategy, in which the tibia is moved to full flexion and held in a locked position. We here report that resistance bursting is directional and occurs in a fixed range of phase (mean .49±.10) during the period when the animal is forced away from the substrate. In contrast, flexion responses show little rhythmicity and are non-directional. Simultaneous recordings of the activities of the tibial muscles of both hindlegs show that each leg can be used independently in either strategy. These findings imply that local circuit processing probably determines activities of tibial muscles in load compensation. We are presently examining whether the same or different interneurons mediate each of these discrete load compensatory reactions.

Supported by NIH-NINCDS grant 22682 and a grant from the Whitehall Foundation.

28.14

ACTIVITY OF LATERAL GASTROCNEMIUS COMPARTMENTS IN THE CAT DURING POSTURAL CORRECTIONS IN THE HORIZONTAL PLANE. D.C. Dunbar*, J.M. Macpherson, and J.T. Inglis* (SPON: H. Dinsdale). Depts. of Anatomy, Univ. of Puerto Rico Med. School, San Juan, PR 00936 (USA) and Queen's Univ., Kingston, Ontario K7L 3N6 (Canada)

This study investigated the activity patterns of the lateral gastrocnemius (LG) compartments in cats during postural corrections to support surface translations in the horizontal plane. Wire electrodes were surgically implanted into the four LG compartments (LG1, LG2, LG3, and LGm), plantaris, and soleus in the left hindlimb. The cats were trained to stand freely on a moveable platform. Quiet stance was disturbed by suddenly translating the cat in one of 16 different horizontal directions. Ground reaction forces and EMG activity were recorded on-line.

Each LG compartment and muscle responded over a range of directions and the amplitude of response varied with the direction platform movement. Maximum EMG responses for all four LG compartments, plantaris, and soleus occurred in the same region of translation where loading of the left hindlimb was maximum. Variation in LG compartment activity occurred at the boundaries of the response regions. Thus, LG compartmental activity patterns reflected the mechanical requirements, perhaps knee joint rotation, for postural correction. Supported by MRC of Canada.

28.15

THE ROLE OF THE CAT VESTIBULAR SYSTEM IN THE RESPONSE TO LINEAR ACCELERATION OF THE SUPPORT SURFACE J.T. Inglis* and J.M. Macpherson. Dept. of Anatomy, Queen's Univ., Kingston, Ont. Canada. K7L 3N6.

Linear acceleration (ramp displacement) of the supporting surface evokes fast, automatic postural responses in the cat. The latency of EMG activation is 30-40 ms following onset of platform movement. It is not clear for these perturbations when the acceleration of the support surface is transmitted to the head. In order for the vestibular apparatus to provide the triggering input for the postural response, there must be a change in the velocity of the head before the onset of the EMG response.

Quietly standing cats were subjected to horizontal plane, linear translation of the supporting surface. Acceleration of the cat's head was measured using an accelerometer mounted on the skull. The timing of head acceleration from onset of platform movement was variable, and often followed the onset of the evoked EMG. Only occasionally did head acceleration occur within 20 ms of the onset of platform movement. These data suggest that vestibular afferents are not the primary sensory inputs that trigger the postural responses. Preliminary results from a bilaterally labyrinthectomized cat support this conclusion since postural responses remain intact in the absence of the vestibular organs. Supported by the MRC of Canada.

28.16

GEOMETRICAL DETERMINANTS OF LIMB POSTURE IN CATS. C. Maioli*, F. Lacquaniti and L. Lopiano*. Istituto di Fisiologia dei Centri Nervosi, C.N.R., 20131 Milano, Italy.

We previously showed that the length and the orientation of the main axis of the limbs are actively controlled in cats standing on a platform statically tilted in the sagittal plane. We here address the question whether the redundant degree of freedom (which is inherent to this postural control of 3-jointed limbs) is constrained by geometrical factors or by cost criteria related to a preferred torque distribution. The former hypothesis is supported by the analysis of the overall pool of the data obtained at different inclinations (±20 deg) and interfeet distances, which reveals a strict planar covariation of the joint angles at both fore- and hindlimbs. The internal representation of postural geometry can thus be construed in terms of a simple linear compound of 2 independent parameters which establishes a unique mapping between the extrinsic space and the intrinsic space of 3 joint angles.

On the other hand, a weighted torque distribution between fore- and hindlimbs is effected via independent control of the contact shear forces.

28.17

MOTOR OUTPUT CAPABILITIES OF ADULT SPINAL CATS FOLLOWING POSTURAL TRAINING. R. J. Gregor, E. G. Fowler and R. R. Roy. Brain Research Institute and Department of Kinesiology, UCLA, LA, CA 90024

It has been demonstrated that the hindlimbs of cats spinalized (Sp) as adults are capable of full weight-bearing locomotion. In addition, Sp cats receiving regular treadmill training attain higher walking speeds than untrained Sp cats (*Exp. Neurol.*, 92:421–435, 1986). Weight support capability is requisite to locomotion and since postural training is often used in clinical settings, this study was designed to investigate the effect of postural training on the motor output of Sp cats. Three adult cats underwent spinal cord transection (T12–T13) and received daily care including range of motion therapy for six months. A postural training program (30 minutes daily, 5 days per week) for the hindlimbs was initiated one month post-transection. Medial gastrocnemius (MG) and soleus (Sol) were instrumented for force, EMG and muscle length measurements six months post-transection (*J. Biomech.* 17:685–694, 1984).

Despite postural training and daily range of motion exercises, none of the Sp cats were able to walk after the training period with the most notable decrement occurring during the flexion phase. Limited analysis during posture yielded forces ranging from 3.8 to 6.3N in the Sol and from 1.0 to 3.9N in the MG. When compared to locomotor performance by cats in untrained and treadmill exercise groups (*Exp. Neurol.* 92:421–435, 1986), postural training was detrimental. These findings have clinical relevance to rehabilitation programs following spinal cord trauma.

Supported by NIH Grant NS–16333

28.18

REAL-TIME PROCESSING OF CUTANEOUS NERVE ACTIVITY TO OBTAIN CONTACT FORCE INFORMATION. J.A. Hoffer and Thaddeus Li*. Depts. Clin. Neurosci. & Med. Physiol. and Electr. Engng., University of Calgary, Calgary, Alberta T2N 4N1, Canada.

Nerve cuff electrodes implanted on skin nerves may record signals suitable for closed-loop control of FES (functional electrical stimulation) of paralyzed muscles in tetra- or paraplegics (Hoffer & Sinkjaer, Soc NS Abst 12:1306, 1986). To test this hypothesis we implemented two real-time analog processors on tibial nerve data recorded from anesthetized cats. One signal processor was based on PID control theory, where proportional, integral and differential versions of the rectified electroneurogram were linearly combined. The other version involved also an exponential transformation.

With PID processing alone, the resultant signal revealed the basic shape and risetime of the vertical contact force applied to the footpads, but the fit appeared logarithmic (presumably reflecting the force/frequency relation typical of mechanoreceptors) and the off-response was sluggish when the force suddenly declined. The second processor included transistors placed in feedback loops to give an exponential transformation. With this approach the fit was effectively linearized over most of the physiological range of contact forces. The speed of the off-response was also improved by the exponential version. Thus, real-time analog processing of chronically recorded nerve activity can provide a useful and accurate replica of the contact force on glabrous skin.

(Funded by the U.S. Spinal Cord Research Foundation)

28.19

USE OF LINEAR QUADRATIC CONTROL THEORY TO PREDICT OPTIMAL FEEDBACK FROM PROPRIOCEPTORS ONTO MOTONEURON POOLS OF THE CAT HINDLIMB. Jiping He*, W.S. Levine* and G.E. Loeb. Dept. Elect. Engng., Univ. Maryland, College Park, MD 20742, and Lab. Neural Control, NIH, Bethesda, MD 20892.

Spinal cord circuits from the various proprioceptors to the various muscles of the limbs are now known to be highly convergent and divergent, in a manner that seems inconsistent with servocontrol of individual muscles. A linear quadratic regulator is an alternative scheme (for small perturbations) that seems more suitable for such complex mechanical linkages having multiple, partially redundant actuators. Implementation has three main requirements: 1) Model of system dynamics - simplified musculoskeletal system with 3 segments (thigh, shank, and foot) moving parasaggitally and driven by 10 muscle groups, each with output dynamics dependent on activation kinetics, length & velocity. 2) Set of sensory signals - We have assumed receptors for muscle force (GTO), length & velocity (spindle primaries), and joint angle and velocity (optional; we are working on reconstructing kinesthesia from other sensory modalities). 3) Performance criteria to be optimized - We have examined the relative effects of control strategies that minimize muscle activation following a perturbation versus those that minimize deviations of a) muscle lengths, b) joint angles, and c) combined joint angles and muscle force levels (limb "stiffness").

So far, we have modeled regulators for stable standing and their responses to small horizontal perturbations of the feet. As is expected for this type of controller, feedback gain matrices tend to show a high degree of coupling, even among muscles acting on different joints. Homonymous feedback showed consistent inhibition from GTO's and excitation from spindles, but heteronymous feedback gains often switched sign depending on the control strategy employed and the relative importance of maintaining state vs. conserving activation. Another general pattern was that the sign of feedback from muscles crossing the adjacent joint was usually reversed from that of homonymous receptors. The most physiological responses to perturbation resulted from the limb stiffness controller operated fairly compliantly (emphasis on conserving activation).

We believe that these feedback matrices and their postural dependencies will improve our understanding of real motor control problems. They may also provide guidance for the design and interpretation of studies of reflexes, their gating during locomotion and their underlying spinal cord circuitry.

28.20

ANKLE PERFORMANCE PRE AND POST LATERAL COLUMN LESION IN THE BEHAVING MONKEY. M.H. Clare, S.A. Sahrmann, E.B. Montgomery, W.M. Landau, Dept of Neruology, Washington University School of Medicine, St. Louis, MO 63110

A macaque was trained to perform 4 mirror image isometric ankle tasks triggered and guided by light signals; 1. rest to large (L) plantarflexion (PL), hold for a random period, rapidly reverse into L dorsiflexion (DO) and return to rest; 2. the mirror image tasks; 3. small (S) PL with random hold, relax to rest; and 4. its mirror image S DO. After recording normative data daily for 3 months a complete right lateral column lesion was made at T10. Two days post lesion recording was resumed and continued daily for 3 more months. Force and EMG from tibialis anterior and gastrocnemius trial by trial and averaged records were used for data analysis. For all tasks the latency from the go signal in rest to onset of force change ranged from 210 - 250 msec. All DO related tasks were slower than PL tasks except for the return to rest following reversal. The most rapidly performed task, as measured from onset of force change to acceptable criterion was LPL (156 msec). LDO was 266 msec while the S tasks were 300 msec. Following the lesion the only timing deficits were slowed LPL reversal (pre 172, post 247) and prolonged return to rest following L DO reversal (pre 195, post 275) during the first 20 days. Prolonged TA activity was evident in both motions. In addition to slight weakness and difficulty maintaining force, there were increased errors for the S tasks (70 per day) not found in the L tasks (12 per day).

28.21

THE IMPORTANCE OF FOOT POSITION IN THE DEVELOPMENT OF WHOLE LIMB STRATEGIES FOR LOAD BEARING. W.E. McIlroy, P. Yoon and J.D. Brooke, Interdepartmental Biophysics and Human Biology, University of Guelph, Guelph, Ontario, CANADA. N1G 2W1.

Is the initial foot position a significant determinant of the pattern of muscular activity adopted for lower limb extensions? Subjects performed ballistic leg extensions, while seated, against a load which contacted the plantar surface of the foot. The ankle joint, which could be loaded independently, was set at different positions prior to the triggering of leg extensions. The initial activity in both tibialis anterior and soleus was directed towards re-aligning the foot. This re-alignment, which usually involved moving the foot to a dorsiflexed position, appeared to be a necessary precursor to peak knee and hip extension. The results confirm the importance of the distal segment in the organization of whole limb strategies for load bearing movements. It is also significant to note that this relationship appears to hold true for prevolitional responses when evoked by interruption of pedalling. Supported by NSERC Grant A0025.

29.1

PELVIC AND AXIAL MUSCLES. D. Filipini* & B. Dubrovsky. Neurophysiol. Lab, Royal Vic. Hosp., Montreal QC H3A 1A1.

EMG activity of external ani sphincter (EAS) and diaphragma pelvic (DP) muscles, both pelvic floor muscles, was studied in chloralose anesthetized cats using copper electrodes. Quantitative evaluation was carried out with the method of zero crossing. We will present evidence that the threshold for exteroceptive stimuli is lower for the EAS than for the DP muscles, in turn DP responds swiftly to interoceptive stimuli. EAS shares with epaxial muscles such as dorsal neck, lumbar dorsal and tail muscles, 1) absence of monosynaptic ventral root potentials in the presence of a muscle spindle apparatus, 2) strong dependence on suprasegmental stimuli and 3) large cutaneous input. Together with other evidence (Dubrovsky, Am. J. Physiol. 254: G100-107) the data suggests that the EAS is involved in continence by responding to threatening situations produced by distant events, e.g. coughing, postural changes, while DP muscles are involved both in continence and defecation by controlling expulsion of fecal mass. EAS and DP show different spinal connectivities, responsiveness to stimuli and segmental dependency. (Supported by NIH Grant IR01AM.34877-01.)

29.2

EFFECTS OF INDUCED SACCADIC FREQUENCIES ON POSTURAL SWAY IN CHILDREN AND ADULTS. J.L. Starkes and C.L. Riach. School of Physical Education, McMaster University, Hamilton, Ont., Canada, L8S 4K1.

Young children do not use visual information as an efficient means of reducing sway. In fact children 5-7 years are unable to fixate a target without spontaneous saccadic shifts. This study examined the effects of induced saccadic frequencies on postural sway in children and adults. Four groups of subjects (N=37) aged: 5-7, 8-9, 10-12 years and adults performed a series of 10 sec force platform (AMTI-OR6) trials. They stood in either a normal or Romberg stance and visually tracked two lights alternating in central vision at 0.5, 1, or 2 Hz. Eye movements were simultaneously recorded using corneal reflection. Dependent measures included: number of saccades made vs. required, and sd of centre of pressure in both AP and LAT planes. Children under 9 years made more saccadic shifts than adults at all frequencies. For AP sway, the Romberg condition was most difficult for children under 9 years regardless of induced saccadic frequency. Lateral sway during the Romberg stance was increased for young children only when saccadic frequency was low.

29.3

THE EFFECT OF PRACTICE ON THE STABILITY OF ANTICIPATORY POSTURAL PATTERNS DURING A BILATERAL ARM RAISING TASK. C.S. Layne. Dept. of Physical Education and Dept. of Physical Therapy Rehabilitation Research Laboratory, The University of Wisconsin-La Crosse, WI 54601

A number of investigators have assessed the effects of skill acquisition on a variety of electromyographic (EMG) variables. Efforts in this area have focused primarily on agonist and antagonist activity with the literature reflecting much inconsistency (Moore & Marteniuk), J. Mtr. Beh., 18: 4, 1986). However, there is a scarcity of studies designed to evaluate possible changes resulting from practice in the anticipatory postural patterns (APPs) observed during voluntary arm movement.

In order to assess the stability of APPs, five male subjects performed a total of 390 bilateral arm raises over three days. The subjects were instructed to move at a time of their choosing following activation of a light stimulus. Surface EMG was used to monitor the activity of the following muscles of the right side: gastrocnemius (G), biceps femoris (BF), erector spinae (ES) and anterior deltoid (AD). Trials 1-10 and 381-390 were analyzed in the following manner. EMG onset latencies were determined relative to movement onset. Movement onset was determined relative to the stimulus signal. With-in subject mean latencies were calculated and ratios were developed between the various muscle onsets and between muscle onsets and movement onset. With practice there were decreases in movement onset latencies and decreases in the muscle onset latencies. However, the variability of the latencies did not change with practice. In general, the ratios between the various muscle onset latencies did not vary despite the decreases in the latencies.

29.4

ABNORMAL POSTURAL COORDINATION IN PATIENTS WITH DISTORTED VESTIBULAR FUNCTION. Shupert, C., Horak, F., Black, F.O. Robert Dow Neurological Sciences Inst. and Dept. of Neurootology, Good Samaritan Hospital, Portland, OR 97210.

Normal subjects standing on narrow beams use a hip strategy to control posture. Some patients with peripheral vestibular disorders with abnormal ("distorted"), rather than absent, vestibular function also use hip sway even when standing on normal surfaces. We studied stance posture in 8 normals and 10 patients with vestibular pathology secondary to infection or head trauma to show whether hip sway is normally coordinated in these patients. All patients showed excessive hip sway and had positional nystagmus and/or vertigo, normal horizontal VOR gains, and no signs of CNS injury. Automatic postural responses to backward translation of the suport surface with eyes open and closed were compared. Ankle, knee, hip and neck angles; leg, trunk and neck EMGs; surface reactive forces; and head angular velocity were measured. Normals using hip strategy on a narrow beam activate trunk and neck flexors to coordinate trunk motion with good head stabilization. Patients showed two patterns of trunk muscle activation: 1) passive trunk collapse due to late or absent trunk muscle bursts, and 2) normal trunk flexor activation. However, all patients had angular head velocities about twice as large as normals, and none showed normal neck activation patterns. Thus, patients with vestibular distortions and excessive hip sway do not use a normal hip strategy to control postural sway. Supported by grants NS19222 and NS01094.

29.5

THE RELATIONSHIP OF AGE AND OTHER VARIABLES WITH UPRIGHT STANCE S. Bandinelli*, V.P. Panzer*, S.L. Thomas* and M. Hallett
NINCDS, NIH, Bethesda, MD USA 20892

The purpose of this study was to investigate the relationship of age, sex and visual condition with whole body and segmental measures of postural sway when standing. Thirteen normal volunteers (age 29-68; 7 female, 6 male) were evaluated. Three-dimensional kinematic data were collected (50 Hz) with a video based system (VICON) and kinetic data were obtained (200 Hz) from a force platform (AMTI) while subjects stood upright for 30 s with eyes open and eyes closed. The total excursion (TE) of the center-of-foot-pressure (COP), center-of-gravity (COG) and body segments (head-HD, shoulder-SH, hip-HP & knee-KN) and the variability (VAR) of these parameters about the mean, linear and quadratic fits to the respective displacement data sets were calculated in the vertical (V), anterior-posterior (A/P) and medial/lateral (M/L) planes. The relationship of subject variables with these measures was assessed using a stepwise regression procedure. Age was strongly related ($p < .05$) to increasing vertical VAR measures (COGV, HDV, SHV & HPV) and to a concurrent decrease in TE of HDA/P and HPV. These changes may characterize a strategy wherein the upper body segments are relatively fixed with gross postural adjustments. Sex was significantly ($p<.05$) related to M/L VAR and TE of COG & COP, which were greater in females, and accompanied by increased VAR of HPV and KNV. These differences are due to M/L weight shift apparently initiated by lower body segments. Vision was significantly related to TE COPA/P, probably reflecting an increase in ankle joint adjustments. In conclusion, there are identifiable differences in balance control associated with aging, and these are clearly distinct from those that are related to sex and vision.

29.6

AGE DEPENDENT DIFFERENCES IN PROGRAMMING LANDING FROM A JUMP IN HUMANS. L. Pelland* and P.A. McKinley. Sch Physical, Occupational Therapy, McGill, Montreal,Que., H3G 1Y5

Landing from a jump (45cm) was studied in children < 7 yrs, & 11-12 yrs under 4 conditions: Normal vision (*NV*), occluded vision (*OV*), stable visual field (*SV*), and disrupted vision (*DV*) using a strobe light at 3,5 and 8Hz. Surface EMG of the soleus (SOL), lateral gastrocnemious (LG), tibialis anterior (TA), rectus femorus (RF), vastus lateralis (VL) and biceps femorus (BF) was differentially amplified (1000x),bandpass filtered (10-1000Hz), and digitally stored on video tape. Take off and landing was monitored by use of footswitches. In children <7, for *NV*, EMG throughout flight was tonic in VL,BF,RF; SOL and LG were quiescent, while a burst of activity in the TA occurred 115-140ms before landing. *SV* increased the gain of the EMG but did not alter the pattern. *OV* resulted in pattern alteration but no gain change: TA was tonic while the BF,VL,RF became more burst-like. *DV* had little effect on the EMG activity. In contrast, all EMG was consistently burst-like in 11-12 yr-olds. With *NV*, prior to landing, co-contraction of the ankle muscles was followed by knee (VL) activity, but minimal activity at the hip (BF,RF). *SV* increased the gain only in the SOL, while *OV* and *DV* delayed the onset of activity, until post-landing, except in the SOL. It is concluded that visual perturbation responses varied in the 2 age groups and that children <7 used proximal joint control, while 11-12 yr-olds exercised distal to proximal joint control. Supported by NSERC.

29.7

EFFECT OF VISUAL PERTURBATIONS IN PROGRAMMING LANDING FROM A JUMP IN HUMANS. H.W.Thompson*, and P.A. McKinley. (SPON: D.. Rushmer) School of Physical & Occupational Therapy, McGill, Montreal, Que., H3G 1Y5

Surface EMG activity was analyzed during landing from a jump (45cm) in 6 normal adult subjects (Ss) with and without normal vision (*NV*). Visual perturbations included stable vision (*SV*), occluded vision (*OV*) & disrupted vision (*DV*) using the same methodology as described in an accompanying report. With *NV*, activity occurred prior to landing and was typically burst-like in all muscles, although 1 plantarflexor was often tonically active. There was usually co-contraction of the ankle musculature (TA,LG,SOL), followed by activity at the knee (VL) while activity at the hip (RF,BF) was minimal until post-landing. Strategies used during visual perturbation trials varied among Ss. Some Ss were largely unaffected by visual perturbations. In others, visual perturbations caused a delay in EMG onset and a decrease in peak pre-landing activity although the pattern of activation was unchanged. In some of these Ss the changes occurred only during *DV*, while in others, effects were seen under all conditions. In the latter group, when jumping with *DV*, the EMG was delayed until post-landing. It is concluded that musculature is activated in a distal to proximal sequence, and this pattern is not disrupted by visual perturbations. Onset and amplitude of EMG may be altered to varying degrees depending on the individual, and is most consistently affected during *DV*. Supported by NSERC.

29.8

EFFECT OF COMPLIANCE ON ANKLE, KNEE & HIP MOTION IN LANDING FROM A JUMP IN HUMANS. M.D. Bastien* and P.A. McKinley. (Spon: P. Gardiner). School of Physical & Occupational Therapy, McGill, Montreal, QUE. H3G 1Y5

Adaptive strategies for surface compliance were studied from kinematic analysis of human subjects landing from a jump (45cm) under 3 conditions: jumping from and landing onto a stiff surface(S-S); jumping from a stiff and landing onto a compliant surface (S-C); jumping from a compliant and landing onto a stiff surface (C-S). Typically, velocity and range of motion (ROM) for a given joint during take-off, flight and landing phases were similar within each condition. Across conditions, both ankle and knee adjustments were minimal and flexion & extension velocities were invariant. For ankle, ROM was unchanged except for increased oscillation post-landing during C-S. At the knee, ROM was invariant, except for the landing phase of S-C, where flexion was decreased. In contrast, hip-trunk motion was greatly affected by surface conditions: flexion & extension velocities were similar for C-S and S-C, and reduced for S-S. Most affected was ROM, which was minimal (30°) during S-S, and increased during S-C (65°) and C-S (85°). Hip and knee flexion was tightly coupled as were knee flexion/ankle dorsiflexion and knee extension/ankle plantar-flexion. It is concluded that adjustments for surface differences occur primarily at the hip-trunk, strategies differ in S-S when compared to S-C or C-S, but adjustments for incongruence in surfaces are minimal. Supported by NSERC.

29.9

BIOMECHANICAL ASSESSMENT OF THERAPEUTIC STRATEGIES IN PARKINSON'S DISEASE V.P. Panzer*, R.J. Plunkett*, S.L. Thomas* and M. Hallett; (SPON: M. Goldstein). NINCDS, NIH, BETHESDA, MD USA 20892

Upright posture is a major problem in patients with Parkinson's disease (PD), but no reliable assessment method has been established. There was some improvement in the ability to maintain upright stance in a 54 yo patient with PD after adrenal implant surgery. The patient stood upright for 30 s with eyes open & closed in the ON & OFF condition on 13 occasions, while three-dimensional kinematic (VICON-50 Hz) and kinetic data (AMTI force platform-200 Hz) were obtained. The total excursion (TE) of the center-of-foot-pressure (COP), center-of-gravity (COG) and body segments (head-HD, shoulder-SH, hip-HP, & knee-KN) and the variability (VAR) of these parameters about the mean, linear and quadratic fits to the respective displacement data sets were calculated in the vertical (V), anterior-posterior (A/P) and medial-lateral (M/L) planes. Individual contributions of vision, condition (COND), dosage, time since last dose (T/DO) and weeks post surgery (WPS) were evaluated with stepwise and multiple regression procedures. Highly significant ($p<.01$) relationships were obtained for these measurements with WPS, COND and T/DO. Differences related to COND generally accompanied changes due to T/DO; however, different parameters were associated with WPS. Sway measurements (A/P-COG, COP, KN, HP, SH, HD VAR and SH TE) were strongly related to COND and T/DO. Changes associated with WPS were primarily related to vertical displacement (V-HD, SH, KN VAR and COG, HD, HP, KN TE and A/P and M/L COP). These were characterized by reduction in VAR and TE such that mean values approached those of a normal population (age 29-68 yo). These measures are sensitive to subtle differences in condition and clinical change and may provide reliable measurements useful for clinical assessment of therapeutic strategies in the treatment of PD.

29.10

NEUROMUSCULAR RESPONSES UNDERLYING BALANCE IN THE CLUMSY CHILD. H Williams* and M.H. Woollacott. College of Human Development & Perf., U. of Oregon, Eugene, Oregon 97403

The purpose of the study was to examine the neural mechanisms underlying balance control in clumsy children and to explicate differences between clumsy and normal children in speed, consistency & organization of postural muscle responses activated by external threats to balance.

Twenty-six children of 2 ages and 2 motor development levels [6-7 yr normal (n=7); 9-10 yr normal (n=7); 6-7 yr clumsy (n=6); 9-10 yr clumsy (n=6)] were exposed to 18 randomly ordered trials of anterior and posterior movements of a hydraulically driven platform. Eight muscle groups were monitored (TA, Gas, Hams, Quads, Para, Abdms, Next, Nflx) using surface electrodes and muscle onset latencies were recorded. Latencies were analysed using a 2 (age) X 2 (motor dev. level) X 8 (muscle) MANOVA repeated measures. For anterior perturbations the main effect of age and interaction between motor dev. level and muscle group were significant. Onset latencies were shorter for older than younger children. For anterior perturbations Abdms were activated at shorter latencies in clumsy than normal children; no other latency differences were significant. For posterior perturbations, muscle responses of clumsy children were significantly more variable than those of normal children. In addition, normal children showed only the classic distal-proximal muscle response organization while clumsy children had a greater tendency to activate leg muscles in a proximal-distal direction.

PRESYNAPTIC MECHANISMS: TOXINS

30.1

OMEGA-CONOTOXIN GVIA (ωCgTx) REDUCES STIMULATED RELEASE OF ACETYLCHOLINE (ACh) FROM HUMAN AND MOUSE BRAIN SYNAPTOSOMES. C.L. Williams*, H.J. De Aizpurua*, T. Kryzer*, V.A. Lennon. Depts Neurol & Immunol, Mayo Clinic, Rochester, MN 55905.

It has been suggested that neuronal voltage-gated Ca^{2+} channels (VGCC) that are highly sensitive to blockade by ωCgTx are located presynaptically and mediate neurotransmitter release (Miller, Science 235:46, 1987). To investigate the role of ωCgTx-sensitive VGCC in regulating ACh release in the central nervous system (CNS), we isolated synaptosomes from biopsied human cerebral cortex and mouse cerebrum by Percoll density gradient centrifugation.

Synaptosomes were loaded with ^{3}H-choline, and thin layer chromatography was used to determine 3 min stimulated release (SR) of ^{3}H-ACh; SR = ([^{3}H-ACh released by 90 mM K^+] - [^{3}H-ACh released by 5 mM K^+]). In the absence of ωCgTx, SR from mouse synaptosomes was 198 ± 5.2 fmol ACh/min/mg protein, and from human synaptosomes was 41.7 ± 14.1 fmol ACh/min/mg. The lower human value may reflect hypoxia due to a 2-3 hr delay in processing surgical tissue. Incubation with 1, 5 and 10 μM ω-CgTx respectively reduced mouse SR by 0, 18 and 57% and human SR by 22, 60 and 58%; 10 μM control peptide had no effect. Cerebral cortical tissues from both species (solubilized in 2% CHAPS, w/v) specifically bound ^{125}I-ωCgTx. These data support the hypothesis that ωCgTx-sensitive VGCC mediate ACh release from nerve terminals in the CNS. Supported by NIH grant CA 37343.

30.2

ω-CONOTOXIN INHIBITION OF Ca^{2+}-EVOKED RELEASE OF [^{3}H]-DOPAMINE FROM SYNAPTOSOMES. J.F. Bowyer*, and N. Weiner. Dept. of Pharm. Univ. of Colo. Hlth Sci. Ctr 4200 E. 9th Ave. Denver CO 80262.

Unlike the K^+ depolarized release of ^{3}H-dopamine ([^{3}H]DA) from synaptosomes, [^{3}H]DA release by 1.25 mM Ca^{2+} (from synaptosomes previously superfused in Ca^{2+}-free media) can be modulated by DA(D2) agonists and antagonists. This "Ca^{2+}-evoked" release is dependent on tetrodotoxin sensitive Na^+ channels and regulated by K^+ channels (sensitive to tetraethylammonium and 4-aminopyridine). We now report that Ca^{2+}-evoked [^{3}H]DA release from synaptosomes is inhibited by ω-conotoxin GVIA (CON), an antagonist of brain Ca^{2+} channels. The maximally effective concentration (10 nM) of CON inhibited the Ca^{2+}-evoked [^{3}H]DA release from striatal synaptosomes to 49.8±5.2%, n=11, of control while release from olfactory tubercle (OT) was reduced to 53.0±7.5%, n=9, of control. Concentrations of 30 nM were no more effective than 10 nM CON at blocking release. The IC_{50} for CON inhibition of Ca^{2+}-evoked release was approximately 1.5 nM in both striatal and OT synaptosomes. The remaining Ca^{2+}-evoked release insensitive to CON could still be inhibited approximately 45% in either striatal or OT synaptosomes by the DA(D2) agonist LY-171555. Cobalt (1mM) also inhibited Ca^{2+}-evoked release to 21.1±2.9%, n=8 of control in striatal and 28.2±4.1%, n=6, in OT synaptosomes. However, the voltage-dependent "L" Ca^{2+} channel blockers verapamil and nifedipine did not affect Ca^{2+}-evoked [^{3}H]DA release at 10^{-7} to 10^{-6}M concentrations. The results of these experiments indicate that much of the Ca^{2+}-evoked release of [^{3}H]DA from synaptosomes is dependent on Ca^{2+} channels (CON sensitive) that have been previously characterized and associated with neurotransmitter release in brain. Supported by USPHS grants NS07927 and NS09199.

30.3

ω-CONOTOXIN GVIA BLOCKS SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS IN VITRO. H. Kamiya* (SPON: N. Miki). Dept. Physiol., Facul. Med., Kanazawa Univ., Kanazawa 920, Japan.

The effects of some subtype-selective Ca^{2+} channel blockers on synaptic transmission were examined in hippocampal slices from the guinea pig.

Mossy fibers were stimulated at 0.1 Hz and field potentials were recorded from pyramidal cell layer in the CA3 region, which reflect synaptic activation of CA3 neurons.

ω-Conotoxin GVIA (ω-CgTX), a novel peptide toxin which blocks N type and L type calcium channels, suppressed the evoked field potential at very low concentration (ED_{50} was about 30 nM). This effect was almost irreversible. On the other hand, 100 μM verapamil and 10 μM nifedipine (L type blockers) had little effect on the evoked field potential. Phenytoin (100 μM, T type blocker) was also ineffective to suppress the evoked field potential.

These results suggest that presynaptic calcium channels which cause transmitter release in the mammalian central nervous system have pharmacological sensitivities close to those of N type calcium channel.

30.4

TETANUS TOXIN INHIBITS VASOPRESSIN and OXYTOCIN SECRETION FROM ISOLATED NERVE ENDINGS OF THE POSTERIOR PITUITARY IN CULTURE. J. T. Russell*, W. H. Habig*, and A.B. Lynn* (SPON: S. Pocotte) LDN, NICHD, NIH, and CBER, FDA, Bethesda, MD 20892.

Nerve endings (neurosecretosomes) isolated from rat posterior pituitaries were maintained overnight under tissue culture conditions in media containing 10% fetal calf serum. These neurosecretosomes secrete vasopressin and oxytocin in response to a 10 min depolarization using veratridine (60 μM) in the perfusion medium. This secretion is strictly dependent on the presence of extracellular calcium.

It has been suggested that tetanus toxin, like botulinum toxin, blocks transmitter release at nerve terminals. When neurosecretosomes were exposed to tetanus toxin, the depolarization-induced secretion of both hormones was inhibited by more than 95%. Half-maximal inhibition of secretion was observed on incubations for 18 hrs with 2.7 nM tetanus holotoxin. The blockade of secretion was also dependent on time of exposure to the toxin. At a concentration of 67 nM, half-maximal inhibition was observed when the neurosecretosomes were incubated in toxin for 2.5 hrs. These experiments suggest that the neurohypophysial nerve terminals possess receptors for tetanus toxin to gain entry into the terminals, and block depolarization-induced secretion of hormones. This isolated nerve terminal preparation provides an unique model to study toxin-induced reactions that uncouple stimulus from secretion.

30.5

CALCIUM - DEPENDENT INACTIVATION OF VERATRIDINE-INDUCED VASOPRESSIN SECRETION IN NERVE ENDINGS OF THE RAT POSTERIOR PITUITARY. K. Payza and J. T. Russell*, LDN, NICHD, Building 36, Room 4A-18, NIH, Bethesda, MD 20892.

Nerve endings (neurosecretosomes), isolated from rat posterior pituitaries and maintained overnight in culture, respond to a depolarizing stimulus of 60 μM veratridine with a strictly calcium-dependent secretion of vasopressin. During the exposure to veratridine, the rate of vasopressin secretion rapidly peaks within two minutes, but then decreases to basal level even in the presence of depolarization, with a half-time of 5 minutes. Simple depletion of the hormone does not account for this decline, since the total secretion is less than 10% of the total amount in the neurosecretosomes.

We tested the hypothesis that the inactivation of secretion was dependent upon membrane depolarization, but independent of external calcium. We perfused a preparation of neurosecretosomes for 25 minutes with 60 μM veratridine and 100 μM EGTA in the absence of calcium. During this pretreatment, vasopressin release remained at basal level. If depolarization were solely responsible for inactivation, then, even in the absence of calcium, the prolonged pretreatment with veratridine should render the neurosecretosomes incapable of secretion. On the contrary, when 2 mM calcium was returned to the veratridine perfusate, the resulting secretion of vasopressin was the same as in the control preparation. Thus, in the absence of calcium, prolonged depolarization with veratridine did not cause inactivation of secretion.

30.6

BOMBESIN INHIBITION OF ^{3}H-SEROTONIN RELEASE: EFFECTS OF PHOSPHORAMIDON AND ENALAPRILAT. M. S. Saporito and R. O. Warwick, Jr. Philadelphia College of Pharmacy and Science, Phila. PA. 19104.

We have reported that bombesin (BN) inhibits K^+ evoked ^{3}H-serotonin (^{3}H-5-HT) release from hypothalamic (HYP) slices. The present study demonstrates the effects of the endopeptidase 24.11 inhibitor phosphoramidon (PAN) and the peptidyl dipeptidase A inhibitor enalaprilat (ENP) on this BN activity. The effects of neuromedin-C (NM-C) and neuromedin-B (NM-B) on ^{3}H-5-HT release were also examined. HYP slices, preloaded with ^{3}H-5-HT, were superfused with Krebs-Henseleit buffer and release stimulated twice with 25 mM KCl. Peptides (1 uM) and/or peptidase inhibitors (10 uM) were included for the second stimulation period. Release is expressed as the ratio of the second to the first peak (S2/S1 ratio). Control S2/S1 ratios were 0.86 ± 0.02. BN and NM-C decreased ($p < 0.05$) S2/S1 ratios by 27% and 28%, respectively. NM-B had no effect. PAN abolished the inhibitory activity of BN while ENP ($p < 0.05$) enhanced the inhibitory activity of BN by 50%. These data support the hypothesis that BN-like peptides function as presynaptic modulators of 5-HT neurotransmission in the HYP and suggest that BN is enzymatically hydrolyzed prior to eliciting this effect.

30.7

BINDING TO BOVINE CHROMAFFIN CELLS IN CULTURE AND POTENCY OF BOTULINUM A NEUROTOXIN ARE INCREASED BY LOW IONIC STRENGTH MEDIUM. H.Bigalke*, P.Marxen* (SPON: European Neuroscience Association). Department of Pharmacology and Toxicology, Medical School of Hannover, 3000 Hannover 61, West Germany.

Botulinum A neurotoxin (BoNtx) produced in cultured chromaffin cells a concentration dependent blockade of ^{3}H-noradrenaline secretion. The inhibition could be enhanced when the cells were exposed to the toxin after a preincubation with a mixture of gangliosides including GD1a, the putative receptor for BoNtx. A further increase in efficacy could be achieved when the toxin was offered to the cells in a solution in which sodiumchloride was replaced by sucrose. Digestion of the cell attached gangliosides with neuraminidase abolished the inhibitory action of BoNtx. To check whether the efficacy of BoNtx correlates with its binding we measured the fixation and distribution of ^{125}I-BoNtx. The radioactivity bound to the cells was increased by gangliosides and low ionic strength medium. Neuraminidase prevented the binding of ^{125}I-BoNtx to chromaffin cells. In a further series of experiments we determined the pattern of gangliosides synthetized by the cells. The quantity of polysialogangliosides extracted from chromaffine cells in culture and determined by chromatography correlated well with the binding and efficacy of BoNtx. Offered externally, polysialogangliosides were incorporated into the cell membrane and enhanced the susceptibility of chromaffin cells to BoNtx. In contrast, neuraminidase treatment decreased the amount of polysialogangliosides, the binding and the efficacy of BoNtx. Our results indicate that polysialogangliosides may be important for the action of this Clostridial toxin.

30.8

AUTOANTIBODIES OF LAMBERT-EATON SYNDROME BLOCK PRESYNAPTIC CALCIUM CURRENTS IN THE MOUSE MOTOR NERVE TERMINALS. M.P. Viglione*, R. Penner* and Yong I. Kim. (SPON: F.E. Dreifuss). Depts. of Neurology & Biomed. Eng., U.Va. Sch. of Med., Charlottesville, VA 22908 and Max-Planck-Institut für biophysik. Chemie, Göttingen, FRG.

Lambert-Eaton syndrome (LES) IgG blocks voltage-dependent calcium channels in bovine adrenal chromaffin cells (Kim and Neher, Science 239:405, 1988). We used subendothelial recording technique (Penner and Dreyer, Pflügers Arch. 406:190, 1986) to evaluate the integrity of presynaptic Ca^{2+} channels in the mouse passive transfer model of LES. Mice were injected with IgG from three patients with LES and healthy control subjects. In the majority of LES IgG recipients, there was a distinctive alteration in the time course of the presynaptic Ca^{2+} currents, recorded from the motor nerve endings in M. triangularis sterni muscles. Quantitative analysis indicated that the Ca^{2+} currents in six mice receiving patient 1's IgG were reduced by 21% to 72% (mean 47%) compared to the control. Of four mice receiving IgG of patients 2 and 3, two exhibited 35% and 47% reductions, respectively. We conclude that LES IgG blocks voltage-dependent Ca^{2+} channels in the motor nerve terminal, which accounts for the pathophysiology of this disease characterized by a deficiency in the neurally-evoked release of acetylcholine (Supported by NIH grant NS18607 and an MDA research grant).

30.9

EFFECTS OF CLOSTRIDIAL NEUROTOXINS ON CATECHOLAMINE RELEASE FROM DIGITONIN-PERMEABILIZED CHROMAFFIN CELLS. M.A. Bittner*, R.W. Holz and B.R. DasGupta* Dept. of Pharmacol., Univ. of Mich. Med. Sch., Ann Arbor, MI 48109; Food Res. Inst., Univ. of Wis., Madison WI 53706

Clostridial neurotoxins are 150 kDa proteins which consist of heavy (100 kDa) and light (50 kDa) chains joined by a disulfide bond. Tetanus exotoxin inhibited Ca^{2+}-dependent catecholamine secretion in a dose-dependent manner in digitonin-permeabilized bovine chromaffin cells. The inhibition was specific for tetanus exotoxin and the B proteolytic fragment; the C fragment had no effect. The light chain of tetanus toxin was fully active. Inhibition was not seen when intact cells were preincubated with the toxin or toxin fragments. Botulinum toxins A, B, and E also inhibited secretion in a dose-dependent manner. Again, the light chain of the molecule possesed activity, while the heavy chain had no effect. There appeared to be differences in the mechanism of action among the different toxins. In summary, clostridial neurotoxins can enter digitonin-permeabilized cells to interact with one or more components of the Ca^{2+}-dependent exocytotic pathway to inhibit secretion.

30.10

PERTUSSIS TOXIN-SENSITIVE G-PROTEINS ARE ASSOCIATED WITH *APLYSIA* SYNAPTIC PLASMA MEMBRANES AND VESICLES. S. S. Vogel, G. J. Chin and J. H. Schwartz, H.H.M.I., Columbia University, New York, NY 10032

We previously identified a Mr 41,000 pertussis toxin (PTX) substrate as *Aplysia* Gαo. Synaptosomes are enriched in this protein (42 pmol ^{32}P-ADP-ribose/mg protein) 3-fold over the homogenate. When synaptosomes are fractionated into plasma membranes and vesicles (for characterization see Chin *et al.*, These Abstracts) both fractions have the same concentration (77 and 72 pmol ^{32}P-ADP-ribose/mg) which is 5 times more than in homogenate. The G-protein labeled in the vesicle fraction represents 5.8% of total PTX-substrate in synaptosomes while that found in the plasma membrane fraction accounts for 84%. Although it is clear from these results that PTX-sensitive G-proteins are more plentiful in plasma membrane, both the high specific activity and the substantial percent recovered in the vesicle fraction argue that a sizable portion of Go in synaptic terminals actually is in synaptic vesicles. Two plausible explanations for the presence of Go in synaptic vesicles are that vesicles carry G-proteins to terminals by axonal transport and that G-proteins play a direct role in vesicular release. Consistent with transport, we find a 6-h ligature of *Aplysia* pleuro-abdominal connectives at 22°C results in a 2.5-fold increase in PTX substrate in the 1 mm segment containing the knot as compared to untied connectives, versus a 3-fold increase in adjoining segments. In squid axolemma we find Mr 41,000 and Mr 42,000 PTX substrates. In extruded axoplasm, however, we detect only the smaller protein, which is associated with internal membranes. These results suggest that a specific PTX-sensitive G-protein is transported to terminals by fast transport. Recently we reported that a PTX-sensitive G-protein mediates presynaptic inhibition by modulating ionic currents; at present we are investigating if G-proteins are directly involved in synaptic release.

S.S.V. is a trainee on NIH grant MH15174 and conducted some of this work in the Neurobiology Course at Woods Hole MBL in 1987.

PRESYNAPTIC MECHANISMS: IONS

31.1

OSCILLATION PERIOD OF mEPP FREQUENCY AT FROG NEUROMUSCULAR JUNCTIONS IS INVERSELY CORRELATED WITH RELEASE EFFICACY AND INDEPENDENT OF ACUTE CA^{++}-LOADING. A.D. Grinnell and P.A. Pawson, Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA 90024.

We and others (Rahamimoff et al, Ann. N.Y. Acad. Sci., 307: 583-599, 1978) have noted periodic oscillations in mEPP frequency which presumably reflect corresponding changes in intraterminal free Ca^{++}. In an analysis of data related to post-tetanic changes in mEPP frequency, we find that most junctions show such oscillations, and the differences in the period of oscillation are consistently correlated with differences in synaptic release efficacy (quanta released/unit terminal length). The period is inversely proportional to release efficacy. Moreover, the oscillation period for any given junction is independent of short-term changes in Ca^{++} loading. It is essentially the same during resting mEPP release, or following tetani of different intensity in either zero Ca^{++}/EGTA or Ca^{++}-containing Ringer.

These data suggest that different junctions, as they develop differing mean mEPP frequency, release efficacy, and activity levels, acquire Ca^{++}-handling enzyme systems of a capacity and cycling rate that are appropriate to the long-term Ca^{++} handling needs applied to each, but independent of acute changes in Ca^{++} loading.
Supported by grants from NIH and the MDA.

31.2

INTRACELLULAR Ca^{2+} CHANGES IN PRE- AND POSTSYNAPTIC REGIONS OF APLYSIA NEURONS IN CULTURE MONITORED WITH FURA-2. E. Shapiro, S. Schacher and J. Connor. Howard Hughes Med. Inst., Columbia Univ. Col. of Phys. & Surg., New York, NY 10032 and AT&T Bell Laboratories, Murray Hill, NJ 07974.

Identified Aplysia neurons L10 and RB form synaptic connections in vitro. Stimulation of presynaptic cell L10 evokes a dual-action synaptic potential in RB. When L10 is fired from hyperpolarized resting potentials (-70 to -60 mV), PSP amplitude in RB is small. As L10 resting potential is depolarized (to -50 to -40 mV) the resting $[Ca^{2+}]_i$ as monitored by fura is increased and with stimulation the size of the PSP is increased in RB by 50-100%. During tetanic stimulation (5-10 Hz; 5 sec) the RB PSP was facilitated. Post-tetanic PSPs recovered to control levels very rapidly, usually within 10 sec. $[Ca^{2+}]_i$ in RB cells is also sensitive to resting potential. When RB cells were filled with fura (n=9), we sometimes could observe postsynaptic changes in $[Ca^{2+}]_i$ caused by L10 stimulation (n=4). In RB, depolarizing PSPs increased $[Ca^{2+}]_i$ and hyperpolarizing PSP trains decreased $[Ca^{2+}]_i$. These changes were greatest at localized areas of RB--good candidate regions for release sites. We are now examining changes in presynaptic fura signals in these localized regions during synaptic release and modulation.

31.3

RECORDINGS OF SINGLE CALCIUM CHANNELS FROM PRESYNAPTIC MOSSY FIBER TERMINALS IN ADULT GUINEA PIG HIPPOCAMPUS. *R. Gray and D. Johnston.* Program in Neuroscience, Baylor College of Medicine, Houston, TX 77030.

There is considerable interest in the identification and characterization of the type(s) of calcium channels responsible for neurotransmitter release at presynaptic terminals. We have used a fluorescent dye and Timm stain to identify presynaptic mossy fiber terminals (MFTs) in adult hippocampus and to record single calcium channel activity from cell-attached patches on these MFTs. A variety of fluorescent dyes (Magrassi et al., *J. Neurosci.* 7:1207, 1987) were tested for their ability to stain MFTs in slices of guinea pig hippocampus. Most of the dyes were relatively nonselective with considerable staining of putative smooth muscle cell nuclei, which are of similar size to MFTs (4-8 μm). One of the dyes (3,3'-diethyloxadicarbocyanine iodide), however, produced intense fluorescence of the mossy fiber synaptic fields with little or no staining of other, similarly-sized, structures. A modification of the acutely-exposed neuron preparation (Gray and Johnston, *Nature* 327:620, 1987) was used to dissociate the CA3 subfield from stained slices. Putative MFTs isolated from such slices were stained for zinc using the Timm method. We found that the intensely fluorescent objects 3-5 μm in diameter also stained positively for zinc, suggesting that they were indeed isolated MFTs. Cell-attached patch recordings were made from these isolated MFTs using a normal saline bath and pipettes containing isotonic $BaCl_2$, 1 μM TTX, and 0.1 mM 3,4-diaminopyridine. Channel openings were recorded in only about 25% of the patches, suggesting a relatively low channel density, although more than one channel was usually observed in these patches. Channel activity was also very transient in that it disappeared from most of the patches within several minutes. Channels with at least 3 distinct slope conductances were observed, however. The conductance levels averaged 9, 13, and 23 pS with multiple levels often being observed in the same patch. Ensemble averages from several patches suggested that there was little inactivation during 50-100 ms commands. This preparation should prove useful for investigating the function of presynaptic ion channels. (Supported by NIH grants NS11535 & HL31164 and AFOSR 85-0178).

31.4

CALCIUM CURRENTS IN FROG MOTOR NERVE TERMINALS. A. Arnon*, G. David*, E. Hevron* and Y. Yaari. (SPON: R. Rahamimoff). Dept. of Physiol., Hebrew Univ., Jerusalem 91010, Israel.

Local circuit currents flowing between motor nerve terminals and their parent axons were recorded from the perineurium of single motor axons or small nerve bundles in the frog (Rana ridibunda) cutaneous pectoris nerve-muscle preparation. Suppression of presynaptic K conductances, unmasked a current component during spike depolarization which was (i) abolished by cutting the motor axons distal to the recording site; (ii) positively related in amplitude to extracelular [Ca]; (iii) reversibly reduced by Mn (5 mM) and Cd (100 uM); and (iv) maintained when Ca was replaced by Sr. Therefore, we identified this current as an inward Ca current (ICa) at the motor nerve terminals.

ICa was markedly reduced by omega-conotoxin (5 uM) but not affected by nifedipine (1-100 uM). Omega-conotoxin, but not nifedipine, also blocked presynaptic Ca-activated K current and the endplate potential. Phenytoin and nickel (100 uM) did not affect ICa.

The pharmacological profile of ICa in frog motor nerve terminals is consistent with a high voltage-activated ICa, which mediates neurally evoked transmitter release.

Supported by the Israel Center for Psychobiology.

31.5

MODULATION OF NEUROTRANSMITTER RELEASE BY INTRACELLULAR CALCIUM CHELATORS AT THE SQUID GIANT SYNAPSE. E.M. Adler*, G.J. Augustine, S.N. Duffy*, M.P. Charlton M.B.L., Woods Hole, MA 02543.

To further characterize the calcium milieu of the presynaptic calcium-receptor/transmitter release trigger, we injected calcium chelators into the presynaptic terminal of the squid giant synapse. Release was assayed by measuring the postsynaptic response to presynaptic stimulation.

BAPTA-family buffers with estimated calcium Kds ranging from 160 to 6250 nM in squid cytoplasm, caused similar reductions in transmitter release at similar intracellular concentrations. Dinitro-BAPTA, a derivative with a Kd in the millimolar range (Payne and Jaffe) was far less effective. Arsenazo III was also effective.

Chelators did not affect presynaptic action potentials. Prolonged stimulation did not reverse buffer effects. Transmission did recover as buffer diffused out of the terminal.

EGTA, which has a similar Kd but slower binding kinetics than BAPTA, was ineffective at reducing transmitter release at concentrations several times the effective concentration of BAPTA. The results suggest that the efficacy of calcium buffers in modulating transmitter release depends on both calcium binding kinetics and Kd.

31.6

SYNAPTOSOMAL CYTOSOLIC FREE CALCIUM DURING HYPOXIA WITH VARYING EXTERNAL CALCIUM

G. E. Gibson and L. Toral-Barza*, Cornell Univ. Med. Coll., Burke Rehab. Ctr. White Plains, NY 10605

Hypoxia selectively alters neurotransmitter release and this appears to be related to changes in nerve terminal calcium homeostasis. Cytosolic free calcium (Ca_i) was estimated in purified synaptosomes (Dunkley) with fura-2 and corrected for non-hydrolyzed dye. K^+ (31 mM) depolarization increased Ca_i from 101 $\pm$ 3 nM to 202 $\pm$ 9 nM. Histotoxic hypoxia (i.e. KCN addition), in the presence of 1.3 mM external Ca, increased Ca_i in resting (100%) and depolarized (58%) synaptosomes. If the Na/Ca exchanger was blocked by replacing most of the sodium in the media with choline, basal values increased by 74% and depolarized levels were elevated by 95%. In the absence of the Na/Ca exchanger, the hypoxic-induced elevation was still apparent under resting conditions but not during depolarization. Without external calcium, hypoxia did not alter Ca_i in the presence or absence of the Na/Ca exchanger. These data suggest that KCN treatment of synaptosomes does not release calcium from internal stores but reduces the ability to buffer the influx of Ca^{2+}. We hypothesis that this impairment elevates Ca_i, reduces further calcium uptake and alters the dependence of neurotransmitter release on external calcium.

31.7

MODULATION OF CALCIUM CURRENTS IN MAMMALIAN MOTOR NERVE TERMINALS. B.R. Hamilton, Z. Lu* and D.O. Smith. Department of Physiology, University of Wisconsin, Madison, WI 53706.

Nerve terminal currents were recorded from the rat EDL nerve-muscle preparation using a loose-patch technique. Initial recordings are characterized by an outward Na^+ capacitative current followed by an inward K^+ ionic current. Blockage of this terminal K^+ current with 10 mM TEA exposes the underlying terminal Ca^{2+} current. High-frequency (20 Hz) stimulation of the nerve decreased the magnitude of the inward Ca^{2+} current. This does not appear to be explained completely by either submembrane Ca^{2+} accumulation or channel inactivation as demonstrated in twin-pulse experiments. Instead, a major component in the frequency induced reduction in Ca^{2+} inward current may come from feedback modulation at the nerve terminal. ATP released during stimulation is quickly broken down to adenosine, which has been found to reduce the inward Ca^{2+} current. Intracellular recordings corroborate these findings. End-plate potentials are reduced by ATP and adenosine, but this effect is reversed by the adenosine-receptor blocker theophylline. Furthermore, ATP's effect is antagonized by α,β-methylene ADP, which prevents its breakdown to adenosine. Presynaptic muscarinic ACh autoreceptors may also be involved. Supported by NIH (NS13600) and M.D.A.

31.8

POTASSIUM-EVOKED $[Ca^{2+}]_i$ CHANGES IN A HIGHLY PURIFIED PREPARATION OF MOUSE BRAIN SYNAPTOSOMES LOADED WITH FURA 2. J. A. Bitran*, H. B. Pollard and E. Rojas*. LCB & G, NIDDK, NIH. Bethesda, MD 20892

Secretion from presynaptic terminals occurs by a very fast process. To determine the temporal relationship between secretion and $[Ca^{2+}]_i$ we measured high K^+-evoked ATP secretion and $[Ca^{2+}]_i$ transients. $[Ca^{2+}]_i$ changes were measured using Fura 2. The dye was incorporated into the synaptosomes either (1) by exposing the synaptosomes to Fura 2 AM or (2) by loading the salt form of the dye during the preparation of the synaptosomes ([Fura-2] = 100 uM). High $[K^+]_o$ causes the synaptosomes to secrete ATP. The secretion time course was measured using a luciferin-luciferase mixture. Depending on whether depolarization occurred in the presence or absence of external calcium two different modes of secretion were observed. In the presence of Ca^{2+}, secretion was fast and the signal could be fit to a single exponential (Tau ~ 1 sec). In the absence of Ca^{2+} secretion was much slower (Tau ~ 8 sec). In the presence of $[Ca^{2+}]_o$, synaptosomes loaded by protocol 1 showed that increasing $[K^+]_o$ induced a rapid rise in $[Ca^{2+}]_i$ and the size of the change depended on $[Ca^{2+}]_o$. The increase in $[Ca^{2+}]_i$ was blocked by La^{3+}. Synaptosomes loaded by protocol (2), showed clear increases in $[Ca^{2+}]_i$ upon application of high $[K^+]_o$ in the absence of Ca^{2+}. The data show that the $[Ca^{2+}]_i$ signal preceeds the ATP release and suggests that there is another mechanism of secretion that mobilizes calcium from intracellular stores.

31.9

PHYSIOLOGICAL EVIDENCE THAT EXCITATORY AMINO ACID RELEASE FROM HIPPOCAMPAL NEURONS IS MODULATED BY CALCIUM CHANNELS SENSITIVE TO BAY K 8644. D.M. Finch and M.B. Jackson. Departments of Neurology and Biology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Whole cell patch clamp recordings from cultured embryonic mouse hippocampus show small, fast spontaneous inward currents (SICs). The currents are blocked by the broad spectrum glutamate antagonist gamma-D-Glutamylglycine, but not by 5-APV, a specific blocker of the NMDA glutamate receptor subtype. Therefore, SICs appear to be miniature excitatory synaptic currents that reflect vesicular release of excitatory amino acids and subsequent binding to a non-NMDA glutamate receptor. Increasing the bath concentration of K^+ from 4 mM to 16 mM produced a sustained, reversible, Ca^{++}-dependent increase in SIC frequency, coincident with a depolarization from about -60 mV at rest to -36 mV. The increased SIC frequency with high K^+ was blocked or substantially reduced by 50 µM Cd^{++}. At this concentration Cd^{++} has been shown to block almost completely Ca^{++} currents carried by "N" and "L" type channels, but to block the "T" type channel to a much lesser extent [Nowycky et al., *Nature*, 316 (1985) 440-443]. Further, both N and T type channels have been shown to rapidly inactivate upon depolarization (Nowycky et al., op. cit.), a property inconsistent with their mediation of a sustained effect. Addition of 1 µM of the L type Ca^{++} channel agonist BAY K 8644 to the high K^+ bath solution produced a significant further increase in SIC frequency. This suggests that L type Ca^{++} channels are present in glutamatergic synaptic terminals of hippocampal neurons, and are important in regulating transmitter release. Supported by NIH Grant NS 21908.

31.10

A CHARYBDOTOXIN-SENSITIVE CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE AT THE CRAYFISH NEUROMUSCULAR JUNCTION: IMPLICATIONS FOR TRANSMITTER RELEASE AND FACILITATION. S. Sivaramakrishnan†, G. D. Bittner† & M. S. Brodwick*††. †Dept. of Zoology & Institute for Neurological Sciences, University of Texas, Austin, Texas 78712 & †† Dept. of Physiology & Biophysics, UTMB, Galveston, Texas 77550.

We have evidence for a calcium-activated potassium conductance [gK(Ca)] in the presynaptic terminal of the excitor axon that innervates opener muscle fibers in the crayfish walking leg. We record from the presynaptic terminal with two intracellular electrodes and from a muscle fiber with one intracellular electrode. Depolarization of the terminal by 60 mV or greater causes the membrane to begin repolarizing 0.5 ms after the start of a 5-10 ms current pulse. This repolarization is both calcium and voltage dependent. It is not blocked by 3,4-diaminopyridine, but is blocked by 0.5 mM cadmium, 13.5 mM barium, 20 mM tetraethylammonium and 50 nM charybdotoxin. This suggests that the repolarization is due to the turning on of high-conductance calcium-activated potassium channels in the presynaptic terminal membrane during the depolarizing pulse. The steady state presynaptic current-voltage relationship is linear only when gK(Ca) is blocked. The gK(Ca) has no significant effect on transmitter release though release begins at lower depolarizations when gK(Ca) is blocked. The gK(Ca) does have a dramatic effect on the growth and decay of twin pulse facilitation, primarily because of its effect on the membrane potential of the presynaptic terminal. gK(Ca) accounts for the first component of facilitation and the 'hump' in the decay curve of facilitation. gK(Ca) also accounts for the apparent decrease in facilitation at higher membrane depolarizations first reported by I. Parnas et al (Pflugers Arch., 1983, 399: 1-10). Supported by NIAAA grant #AA07746.

31.11

LIZARD MOTOR NERVE TERMINALS HAVE TWO CALCIUM-DEPENDENT POTASSIUM CONDUCTANCES. K. Morita and E.F. Barrett (SPON: K.L. Magleby). Dept. of Physiology & Biophysics, Univ. of Miami Sch. of Med., Miami, FL 33101.

Intra-axonal recordings were made from lizard motor axons impaled within a few mm of their motor terminals. In the presence of 4-aminopyridine (4-AP, 1 mM) the falling phase of the action potential is prolonged by these treatments: Ca-free solutions, 1 mM Mn, 10 nM charybdotoxin (CTx), or intra-axonal application of the Ca buffer BAPTA. In the presence of tetraethylammonium (TEA, 10 mM) and 1 mM 4-AP axons show prominent depolarizing afterpotentials followed by a small, slow hyperpolarizing afterpotential (h.a.p.) lasting several sec. These afterpotentials appear to originate in the motor terminals, and disappear in Ca-free solutions or following addition of 1 μM Cd. The slow h.a.p. is selectively blocked by 100 nM apamin, but not by CTx.

These data suggest that lizard motor nerve terminals express two kinetically and pharmacologically distinct Ca-dependent K conductances. The faster conductance, previously noted in extracellular recordings from lizard and mouse motor terminals, is sensitive to TEA and blocked by charybdotoxin. The slow, apamin-sensitive h.a.p. appears to be produced by a Ca-sensitive K conductance similar to that which underlies the afterhyperpolarization recorded in motoneuron cell bodies. Supported by NIH grant NS 12404.

31.12

EFFECTS OF DEPOLARIZATION AND Ca^{2+} INFLUX ON PROSTAGLANDIN PRODUCTION IN ISOLATED, CEREBELLAR GLOMERULI. R.V. DORMAN*. (SPON: D.M. Terrian). Dept. Biological Sciences, Kent State Univ., Kent, OH 44242.

Exogenous prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been shown to stimulate the release of preloaded D-[^{3}H]aspartate from isolated mossy fiber terminals. Cerebellar glomeruli, including mossy fiber terminals, were isolated and used to assess the effects of membrane depolarization and Ca^{2+} influx on the production of $PGF_{2\alpha}$ and prostaglandin E_2 (PGE_2). Glomerular particles were incubated in Krebs-Ringer buffer for 0 and 5 min, in the presence and absence of Ca^{2+}, prior to quantitation of the prostanoids by RIA. Depolarization with 45 mM K^+ caused a 32% increase in $PGF_{2\alpha}$ during 5 min of incubation, when compared to unstimulated controls. This increase was not observed in the absence of external Ca^{2+}. The presence of 25 μM diltiazem (Ca^{2+} channel blocker) or 1 mM EGTA prevented the depolarization-induced increase in $PGF_{2\alpha}$. The Ca^{2+} ionophore A23187 (5 μM) induced a 30% increase in $PGF_{2\alpha}$ in the absence of 45 mM K^+. There was no apparent effect of 45 mM K^+ on PGE_2 concentrations, but A23187 did induce a 47% increase during 5 min of incubation and diltiazem did inhibit incubation-dependent synthesis. It appears that depolarization-induced Ca^{2+} influx stimulated $PGF_{2\alpha}$, but not PGE_2 production in isolated cerebellar glomeruli.

This work supported by AFOSR 86-0045.

SPINAL CORD AND BRAINSTEM: LESION STUDIES

32.1

RECIPROCAL INHIBITION FOLLOWING SPINAL LESIONS IN MAN. E. Ashby and M. Wiens*. Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, Ont. M5T 2S8

Reciprocal inhibition was studied in normal subjects and patients with spinal cord lesions by stimulating the posterior tibial nerve below the threshold of the soleus alpha motoneuron axons and recording the changes in firing probability of tibialis anterior motor units activated by voluntary contraction. The strength of this inhibition was estimated from the number of displaced counts in post-stimulus time histograms (PSTH) of voluntarily activated motor units. For a given stimulus intensity this inhibition was greater in patients with spinal lesions than in normal subjects. In the patients with spinal lesions the Ia inhibitory interneurons had a lower threshold than soleus motoneurons, whereas in normal subjects the thresholds were approximately equal. These findings indicate that reciprocal inhibition of flexor muscles is enhanced following spinal lesions in man. A second period of inhibition was observed with a latency of approximately 50 ms. This was less prominent in the patients with spinal cord lesions.

32.2

THE ROLE OF INHIBITION IN RECOVERY FROM SUBTOTAL SPINAL CORD LESIONS IN A RAT MODEL. R. M. Harris and J. W. Little. Depts. of Biological Structure and Rehabilitation Medicine, University of Washington Sch. of Medicine, Seattle, WA 98195.

Spinal cord injury, in both humans and experimental animals, results in loss of muscle strength, but if the lesion is incomplete there is often considerable return of locomotor function. The neuronal mechanisms underlying this improvement, in particular the role played by inhibition in the cord, is unclear. On the one hand, inhibition is beneficial in damping hyperactive reflexes such as spasticity which act to hinder voluntary function. On the other hand, inhibition may act to block mechanisms which could be responsible for recovery of function. In order to study the role of inhibition in recovery from spinal cord injury, we injected spinal cord injured rats with either an agonist, baclofen, or an antagonist, bicuculline, to GABA, a major inhibitory neurotransmitter in the cord. Each rat had received a three-quarter cord lesion in the midthoracic region, sparing only the left lateral funiculus. For the next 4 weeks, daily intraperitoneal injections were given, and observations were made of the locomotor function of the hind legs. Our observations so far have not shown any significant differences in the rate or final level of locomotor recovery, between either baclofen- or bicuculline-treated animals and saline treated controls. We are thus unable to assign a role in the recovery process to GABAergic inhibitory effects.

32.3

OBSERVATIONS ON THE FIBER TYPE TRANSFORMATION IN SOLEUS MUSCLE AFTER SPINAL CORD TRANSECTION. E.Eidelberg, L.C. Maxwell*, R.Polich*, M.Moody*, and L.H. Nguyen*. Veterans Administration Hospital and the University of Texas Health Sciences Center, San Antonio, TX 78284.

In rats, complete transection of the spinal cord results in transformation of the slow twitch-oxidative (I) fibers that make up most of soleus muscle, to fibers with the histochemical and contractile properties of type 2A (fast-twitch, oxidative).This change takes place gradually beginning around the 10th day post transection, reaches maximum at 4-6 weeks post transection, and does not reverse afterwards.

Alpha-motoneurones innervating slow-twitch muscles, such as soleus, tend to be smaller than those innervating fast-twitch muscle.We asked whether the histochemical changes in muscle were paralled by changes in the size of the motoneurones.We compared the area of the soma, and the calculated dendritic surface area, of alpha motoneurones labeled by injection of WGA-HRP into the soleus muscles. The control rats were intact, and the test group were spinalized at L1 6 weeks prior to labeling.The soleus motoneurones from the spinal rats (N=29) had significantly (p=0.001) larger somata(mean:1230μm^2±352S.D.)than the controls(N=31)(mean:962μm^2 ±143S.D.).Perhaps this difference arises from increased motor unit innervation ratios due to collateral sprouting. (Supported by grants from the Veterans Administration and the National Institute of Health.)

32.4

PHOTOCHEMICALLY INDUCED CYSTIC CAVITY FORMATION IN RAT SPINAL CORD T. Cameron, V.R. Holets, B.D. Watson, R. Prado, P. Tarjan*. Departments of Biomedical Engineering, Neurological Surgery and Neurology, University of Miami, Miami, FL 33136.

Cystic cavity (syrinx) formation in injured spinal cord is a well-known complication of spinal cord injury. Determining the origins of cyst formation and possible ameliorative strategies have been difficult owing to its unpredictable appearance following experimental impact injury in laminectomized animals. Cystic cavities can be created reproducibly in the rat spinal cord by modification of the recently developed photochemical method of spinal cord injury in non-laminectomized rats (Prado et al., J. Neurosurg. 67:745-753).

Sprague Dawley rats (85-120g) were anesthetized and their spinal vertebra exposed by blunt dissection. The 514.5 nm beam of an argon ion laser was focused as a sharp transverse line onto the spinal column at the level of T8 vertebra. The photosensitive dye, erythrosin B (FD & C Red No. 3) was injected intravenously to an initial blood concentration of 650 uM, and the translucent spinal column irradiated through a beam chopper operating at 10% duty cycle for 10 min, with a peak intensity of 60 W/cm^2. Animals evaluated for functional deficit by the modified system of Gale et al. (Exp. Neurol. 88:123-134, 1985) exhibited 35.3 ± 2% paralysis after 11 days which remained constant.

Immunohistochemical analysis of the resultant lesion, revealed cyst formation in the gray and white matter, but some fiber tracts in the dorsal and ventral columns were spared. At 3 weeks post-lesion the cyst had expanded to a length of 7 mm. This model is ideal for studying cyst formation and functional recovery following spinal cord injury.

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32.5

BEHAVIORAL EFFECTS OF CAT SACROCAUDAL SPINAL CORD HEMISECTION OR TRANSECTION. E. L. Rhoton*, C. J. Vierck Jr., G. W. Sypert and L. A. Ritz. (SPON: H. Hirata). Univ of Florida Coll of Med, Depts. of Neurosurgery and Neuroscience, Gainesville, FL 32610.

In the cat, spinal cord segments caudal to the lumbosacral enlargement innervate the tail. These segments are designated the sacrocaudal spinal cord and extend from S3 to Ca8 (Ca = caudal). Hemisection or transection was carried out in the sacrocaudal spinal cord to study the behavioral effects of such lesions on hindlimb and tail function. Our long term goal is to develop a unique model for spinal cord injury. Under combined Ketamine and Halothane general anesthesia, cats were transected (N = 4) or hemisected (N = 6) at Ca1-Ca2. Behavior and reflexes of tail and hindlimb were observed from 3 to 7 months postoperatively.

None of the cats had evidence of bowel or bladder dysfunction. Hindlimb reflexes and gait were normal. In animals with hemisections the tail assumed a curved configuration towards the lesioned side, and tail tone increased during the first two weeks postoperatively. The duration of these effects extended at least 105 to 200 days. All animals with transections developed, after a period of hypotonia, persisting hypertonia. An exaggerated cutaneous flexion reflex was seen in the distal tail. Spontaneous side to side distal tail movements, termed spinal wagging, were also observed in 3 of 4 cats with transections.

Following sacrocaudal spinal cord hemisection or transection, no hindlimb or parasympathetic dysfunction was seen. With hemisection, tail hypertonia developed and with transection tail hypertonia and spinal wagging were seen. This model introduces an alternative location for studying spinal cord injury. (Research supported by NS23683 and the Veterans Administration Medical Research service.)

32.6

CONTRALATERAL INFLUENCES ON CAT SACROCAUDAL MOTONEURONS. L.A. Ritz, E.L. Rhoton* and G.W. Sypert, Univ of Florida Coll of Medicine, Depts of Neurosurgery and Neuroscience, Gainesville, FL 32610.

Anatomical studies in cat sacrocaudal spinal cord, from our laboratory, have shown that: 1) Ia primary afferent fibers can project into the contralateral ventral horn; 2) motoneuron dendritic trees extend into the contralateral ventral horn; and 3) commissural motoneurons are associated with tail musculature. We recorded ventral root potentials (VRPs) from sacrocaudal ventral roots to ascertain the physiological effects of contralateral dorsal root stimulation.

Adult cats, while deeply anesthetized with Halothane, were decerebrated by precollicular transection of the brainstem. The sacrocaudal spinal cord was exposed. For a given segment (S3 - Ca4) the dorsal roots (DRs) were placed on stimulating electrodes. Ventral roots potentials (VRPs) were recorded from the ipsilateral ventral roots. The efficacy and strength of dorsal root stimulation was assessed by cord dorsum responses.

Ipsilateral DR stimulation alone evoked monosynaptic and polysynaptic components of the VRPs. Contralateral DR stimulation alone produced a late polysynaptic response. When the ipsilateral and contralateral DRs were simultaneously stimulated, there was an enhancement or a diminution of the monosynaptic response. When the contralateral DR stimulation preceded the ipsilateral by 0.4 - 5 msec, the monosynaptic component was usually enhanced. When the contralateral DR stimulation preceded the ipsilateral by 6 - 30 msec, the monosynaptic and polysynaptic components were strongly inhibited and delayed.

These data indicate that, within cat sacrocaudal spinal cord, contralateral primary afferent input can have considerable influence on the output of ipsilateral ventral roots. The decreased monosynaptic response, sometimes seen, may be due to presynaptic inhibition. Of particular interest are the observations of enhancement of the monosynaptic response with simultaneous bilateral DR stimulation. This enhancement appears to be the physiological correlate of the crossed circuitry demonstrated by our anatomical studies.

(Research supported by NS23683 and the Veterans Administration Medical Research Service).

32.7

ALTERATION OF MOTONEURON PROPERTIES BY PARTIAL SPINAL LESION IN THE DECEREBRATE CAT. J.S. Carp, R.K. Powers and W.Z. Rymer. Wadsworth Labs, NYSDOH, Albany, NY 12201, Dept. Physiol. & Biophys., U. Wash. Med. Sch., Seattle, WA 98195 and Dept. Physiol., Northwestern U., Chicago, IL 60611.

Interruption of pathways descending in the dorsolateral funiculi of the spinal cord of the decerebrate cat reduces the minimum repetitive discharge rate of motoneurons during physiological activation. The present study was designed to assess if the lesion-induced reduction in minimum discharge rate could be attributed to a change in intrinsic motoneuron properties or in synaptic input to the motoneuron.

Intracellular recordings from lumbar spinal motoneurons were performed in 14 paralyzed decerebrate cats before (n=48) or after (n=43) midthoracic dorsal hemisection. The two populations had similar distributions of resting membrane potentials, axonal conduction velocities and action potential characteristics. Repetitive discharge was elicited by 2 sec depolarizing current pulses in 37 cells. Minimum rate was estimated by adjusting the current magnitude until a sustained train of at least 6 spikes was elicited. Minimum rates were higher in the post- than in the pre-lesion group. The duration of the afterhyperpolarization (AHP) following a single spike elicited by a brief current pulse was shorter in the post- than in the pre-lesion group.

These data have two implications. First, the increase in minimum rate after lesion seen with current injection is probably due to the decrease in AHP duration. This suggests that an intrinsic motoneuron property which is an important determinant of repetitive discharge behavior may be under the influence of descending pathways. Second, the lesion-induced reduction in minimum rate seen during physiological activation is not due to changes in intrinsic motoneuron properties, but rather to changes in the pattern of afferent input or the efficacy of synaptic transmission. (Supported by VA Merit Review (WZR) and NIH grants NS07820 (JSC) and NS19331)

32.8

CONTRIBUTIONS OF REGENERATION AND MECHANOSENSORY INPUTS TO BEHAVIORAL RECOVERY IN SPINAL-TRANSECTED LAMPREYS. A.D. McClellan, Dept. of Physiol. & Biophysics, Univ. of Iowa, Iowa City, IA 52242

Larval lampreys recover locomotor function 3-6 wks after receiving complete spinal transections. Descending brainstem command neurons functionally regenerate and can directly activate spinal locomotor networks below a healed transection site (McClellan, **Brain Res. 448**: 339, 1988). In the present study, the extent of this regeneration was examined using *in vitro* brain/spinal cord preparations. A short section of the spinal cord just below the healed lesion was bathed in a low-calcium Ringer's solution to block synaptic transmission at the terminals of descending axons that had possibly regenerated for short distances. Under these conditions, brainstem-evoked locomotor activity was reduced or sometimes abolished in the spinal cord below the blockade. Thus, many of the descending brainstem command axons appear to regenerate for short distances (less than 15 segments) before making synaptic connections. However, stimulation of the spinal cord above a healed transection site in low-calcium Ringer's solution could evoke action potentials more than 15 segments below the lesion site. Since locomotor activity can be initiated many segments below a healed transection site, propriospinal systems and perhaps mechanosensory inputs presumably aid in relaying the descending drive to more caudal parts of the spinal motor networks below the lesion area.

Mechanosensory inputs by themselves generally do not initiate locomotor activity below a spinal lesion but can couple motor activity across a transection site, since i.p. injection of NMDA (50 mg/kg) in animals with acute, mid-body transections evoked locomotor activity above and below the lesion that was often coupled 1:1. Since regenerated propriospinal neurons by themselves result in coupling of locomotor activity across the lesion site that is more variable than observed in recovered whole animals (McClellan, **Brain Res. 448**: 339, 1988), mechanosensory inputs appear to contribute significantly to the normal coupling of locomotor patterns across a healed transection.

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32.9

THE EFFECT OF HIGH PRESSURE ON SYNAPTIC POTENTIATION IN ISOLATED BRAINSTEM-SPINAL CORD OF NEWBORN RATS. A. Tarasiuk and Y. Grossman. Unit of Physiology, Faculty of Health Sciences, Ben-Gurion Univ. of the Negev, Beer Sheva 84105, Israel.

High pressure (HP) induces hyperexcitability and convulsions in intact animals by mechanisms that are not understood. In the present experiments we examined the effects of HP on cranial reflexes and synaptic interactions in isolated brainstem-spinal cord of newborn rats. The preparation was isolated, placed in a pressure chamber and constantly superfused with oxygenated Krebbs solution (pH 7.3) at 27°C. Reflex activity was recorded extracellulary from the cut ventral roots of the 1st cervical nerves; the 5th (V) and 10th (X) cranial nerves were stimulated. Stimuli were delivered to one cranial nerve at 0.5 Hz before and after a short train of 100 stimuli at 10 Hz delivered to the same nerve to test post tetanic potentiation (PTP), or to the other nerve to test heterosynaptic potentiation (HSP). After control measurements under normal conditions, the pressure was raised to 10.1 MPa with compressed helium. Exposure to HP 1) increased the amplitude (26±10%, Mean±SE), duration (13.7±6%) and half rise time of individual reflex responses, 2) inhibited PTP from 165±25% to 138±18% with V stimulation and from 155±35% to 130±22% with X stimulation and reduced recovery time from PTP by 50-70%, and 3) decreased the marked HSP caused by V stimulation on the X reflex from 242±25% to 156±36%. X stimulation caused less HSP of the V reflex (122±15%) and was not affected by HP. In contrast to crustacean neuromuscular junction, HP in the mammalian CNS enhanced single polysynaptic responses but depressed frequency-dependent potentiation of cranial nerve reflexes.

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33.1

REGULATION OF SOMATOSTATIN GENE EXPRESSION IN THE PERIVENTRICULAR NUCLEUS OF THE RAT HYPOTHALAMUS. J.A. Breed*, R.A. Steiner, and D.K. Clifton*. Ob & Gyn and Physiol & Biophys, Univ of Wash, Seattle, WA 98195.

Somatostatin (SS) has an important inhibitory role in the control of GH secretion. Sex steroids influence GH secretory patterns. This effect could be mediated by hypothalamic factors (e.g. SS) involved in the regulation of GH secretion. Castration of male rats results in an increase in baseline GH levels, and testosterone (T) replacement reverses this effect. These observations suggest that T stimulates hypothalamic SS synthesis. We tested the hypothesis that testosterone (T) stimulates SS gene expression in neurons of the periventricular nucleus (PeN) by measuring cellular SS messenger RNA (mRNA) using *in situ* hybridization. Male rats were castrated and implanted with either a Silastic capsule (30mm) containing T (n=3) or a sham implant (n=3). Animals were sacrificed 4 days after castration with a control group of intact animals (n=3). Coronal sections (20um) were prepared for *in situ* hybridization with an ^{35}S-labeled RNA probe complementary to the pre-pro-SS message. Sections were anatomically matched (10/animal) and read blindly. Signal levels for pre-pro-SS mRNA were measured in individual cells (approx. 100 cells/animal) in the PeN with a computerized image processing system. The results are shown with grains/cell as an index of SS mRNA content. The signal level of SS mRNA in cells of the PeN was significantly reduced

Group	Mean ± SE (grains/cell)	n
Intact	195 ± 3	3
Castrate	159 ± 6	3
T-replaced	182 ± 4	3

($p<0.006$) in castrate-sham implanted males compared with intact males. T replacement returned SS mRNA content to a level not significantly different from that of the intact animals. We conclude that T stimulates SS gene expression in neurons of the PeN and that this may, in part, account for the effect of T on GH secretory patterns.

33.2

REGULATION OF IGF-1 AND IGF-2 mRNAs IN THE MALE RAT HYPOTHALAMUS AFTER HYPOPHYSECTOMY. T.L.Wood, M.Berelowitz and J.F.McKelvy, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, N.Y., 11794

Insulin-like growth factors (IGF-1, IGF-2) and their receptors are found throughout the brain, including the hypothalamus. Although peripheral IGFs may function as mediators of growth hormone (GH) action, their role in the CNS is unknown. IGF-1 has been shown to directly inhibit pituitary GH synthesis and secretion and to stimulate hypothalamic somatostatin (SRIF) secretion. Thus, hypothalamic IGFs may be involved in GH regulation in addition to the classical GH modulators SRIF and GH- releasing factor, GRF. If these peptides are involved in GH regulation then they should be influenced by GH removal.

We isolated total RNA from individual sham and hypox rat hypothalami 8, 15, and 27 days post op. IGF-1 and GRF RNA levels were determined by nuclease protection assays; IGF-2 and SRIF RNA levels were determined by Northern blot hybridizations. Hypothalamic IGF-1 and IGF-2 mRNAs decreased markedly 8 days after hypox and progressively declined over the course of the study. Confirming previous results, GRF mRNA increased over the same time course. Hypothalamic SRIF mRNA did not change significantly.

The novel demonstration of decreases in hypothalamic IGF-1 and IGF-2 mRNAs following hypox suggests a role for these peptides in hypothalamic-pituitary GH regulation. Studies using pituitary hormone replacement therapy in hypox rats are currently underway to determine GH specificity of the IGF-1 and IGF-2 mRNA regulation.

33.3

PERINATAL CHANGES IN THE GROWTH HORMONE (GH) RESPONSE OF CULTURED PITUITARY CELLS TO ISOBUTYLMETHYLXANTHINE (IBMX) AND FORSKOLIN (FSK). L. Cuttler, B. Collins* and M. Szabo*
Depts. Pediatrics/Medicine, Univ. of Chicago, Chicago IL

We have demonstrated that pituitary GH release in response to GH-releasing factor (GRF) and $(Bu)_2$cAMP in vitro is greater in newborn rats than in adults (Endocrinology 118:69). To determine the mechanism(s) underlying this developmental pattern, we tested the effects of 0.001-1.0 mM IBMX and 0.01-10.0 uM FSK, agents that increase intracellular cAMP content independent of the GRF receptor, on GH release from cultured pituitary cells of 2-day(d)-old, 15-d-old, and young adult male rats (n=3/group). The magnitude of GH release in response to IBMX and FSK was highly age-dependent, the fractional increase in GH release being greatest in newborn pituitaries and least in those of adults (ANCOVA: F=19.3 and 9.9 for age effects of IBMX and FSK, respectively; P <0.001 for both). GH release in response to a maximally stimulatory dose of IBMX (1 mM), was 376+45, 238+38, and 220+26% (mean ± SE) of controls by pituitary cultures of 2-d, 15-d, and adult rats respectively (ANOVA: F=4.5, P <0.05). Similarly, GH release with 10 uM FSK was 557+78, 264+25, and 226+51% of controls in 2-d, 15-d, and adults respectively (ANOVA: F=10.6, P<0.005). Conclusions: In the rat, pituitary GH responses to IBMX and FSK are highest in the newborn and decline with advancing age after birth. The findings suggest the existence of maturational changes within the somatotroph distal to both the GRF receptor and cAMP formation.

33.4

FORSKOLIN-STIMULATED GROWTH HORMONE (GH) SECRETION BY INDIVIDUAL SOMATOTROPHS FROM MALE, FEMALE AND TESTICULAR FEMINIZED (TFM) RATS. P. Martha, Jr.*, R. Krieg, Jr., A. Rogol* and W. Evans*. Univ. of VA, Charlottesville, VA 22908 and Medical College of VA, Richmond, VA 23298.

The quantity of GH released by individual somatotrophs from normal female and Tfm rats in response to GH-releasing hormone (GHRH) is similar, although significantly diminished compared to normal males. To define further the mechanisms subserving these differences we used a reverse hemolytic plaque assay to evaluate GH secretion by somatotrophs from male, female and Tfm rats following 90 min exposure to the adenylate cyclase activator, forskolin (3μM). The number of recovered somatotrophs ($x10^6$) responding to forskolin (as the percent plaque-forming x total dispersed pituitary cells) was greater from Tfm rats (1.05 ± 0.19) than males (0.51 ± 0.07; p=0.01), but no different than females (0.67 ± 0.11) although a strong trend was present (p=0.06). GH secretion, as assessed by the logarithmic mean GH plaque areas (μm^2 x 10^3; ± SE), was greater by somatotrophs from male rats both basally [5.8 ± 1.0 vs 2.7 ± 0.4 (female; p=0.006) and 3.3 ± 0.6 (Tfm; p=0.021)] and following forskolin stimulation [47.1 ± 8.9 vs 14.8 ± 1.9 (female; p=0.0008) and 16.2 ± 2.7 (Tfm; p=0.001)]. Plaque areas were not different between female and Tfm cells. The similarity between these and our previous results using GHRH as the GH secretagogue indicates that, compared to males, the reduced GH secretory capacity of female and Tfm somatotrophs is primarily determined by differences that occur distal to the GHRH receptor.

33.5

ONTOGENIC STUDY OF rGRF (1-29) NH2-INDUCED GH RESPONSE IN MALE OBESE ZUCKER RATS. P. Gaudreau, G. Renier*, N. Deslauriers* and P. Brazeau*. Neuroendocrinology Laboratory, Notre-Dame Hospital Research Center, Montreal, Canada, H2L 4M1.

To determine the onset of GH alterations in obese Zucker rats, we evaluated the in vitro and in vivo rGRF(1-29)NH_2-induced secretion (net area under the curve), at 60 and 90 days of age. The basal GH level in perifused dispersed pituitary cells of lean animals was significantly higher than that of their obese littermates. No significant deficit in stimulated GH secretion of 60-day-old rats was seen with increasing concentrations of rGRF (1.56 to 50 pM), although a trend was observed; however, in 90-day-old rats, a marked reduction of GH secretion was seen at all rGRF concentrations tested. The in vivo basal GH levels were similar in all animals, GH responsiveness was not altered after i.v. administration of 0.8 µg/kg b.w. rGRF, in 60-day-old obese rats, but was significantly diminished in 90-day-old rats. Moreover, a dose of 4.0 µg/kg b.w. rGRF significantly lowered GH secretion in obese animals of both ages. Altogether, these results indicate that the reduced sensitivity of somatotroph cells, observed in obese Zucker rats, is not primary in the development of the GH hyporesponse associated with obesity. Specific factors that influence in vivo somatotroph secretion and that represent important markers in the development of obesity, still remain to be found.

33.6

THREE DIMENSIONAL ORGANIZATION OF MICROTUBULES IN LACTOTROPHS.

A. Pollack*, C. Padula*, P.C. Goldsmith and R. Weiner. Reproductive Endocrinology Center, Univ. of Calif., San Francisco, CA 94143.

Recent evidence supports a role for cytoskeletal elements in intracellular transport of organelles and vesicles. To study the role of microtubules (MTs) in regulated prolactin (PRL) secretion, we established optimal conditions to stabilize MTs and retain cell ultrastructure during immunostaining of cultured lactotrophs. To characterize the inhibited state, we studied cells maintained under tonic dopamine (DA) control. Anterior pituitaries from ovex rats treated with estradiol-17B for 2 weeks were enzymatically dispersed. Cells were cultured on ECM-coated plastic coverslips in the presence of 500nM DA. After 2 days in culture, the cells were incubated in a stabilization + extraction buffer containing 500nM DA, fixed, and double immunolabeled for PRL and tubulin using 5 nm and 15 nm colloidal gold. Sections were viewed with bright field microscopy (LM) and both transmission (TEM, 60kV) and high voltage (HVEM, 300 kV) electron microscopy. At the LM level, the configuration of MTs in secretory cells was easily distinguished from stress fibers in fibroblasts. Polarized arrays of MTs radiated from a dense area around the nucleus. Patterns of MTs appeared as an intertwined cytoplasmic web or as a dense network embracing the Golgi apparatus and extending towards the plasma membrane (PM). In the TEM, the presence of long MTs subjacent to and following the contours of the PM was confirmed. Close associations between PRL granules and MTs were observed, and often seemed to involve an unlabeled connecting substance. In the HVEM, thick sections viewed at incremental angles gave stereoscopic images of 3-dimensional arrangements, displaying MT nucleating sites and granule-MT associations. These results demonstrate a highly organized MT network in lactotrophs which is closely associated with the secretory apparatus, and suggest a role for these cytoskeletal elements in PRL secretion. Supported by NIH grants HD08924 and HD11979.

33.7

DOPAMINE EFFECTS ON POTASSIUM CURRENTS OF IDENTIFIED RAT LACTOTROPH CELLS. PM LLEDO*, P LEGENDRE*, J ZHANG*, JM ISRAEL*, JD VINCENT (SPON : B. Bioulac). INSERM U.176, rue Camille Saint-Saëns, 33077 Bordeaux Cédex, France.

We have previously proposed that dopamine (DA) could inhibit prolactin release by changing the membrane potential of rat lactotrophs in primary culture (ISRAEL et al,1987). The present work was undertaken to study the effects of DA on identified K^+ currents using the "whole cell" patch clamp technique applied to enriched population of rat lactotrophs in primary culture. Each cell under recording was characterized by the reverse hemolytic plaque assay. In presence of Co^{2+} (2.5 mM), two K^+ currents were observed: a fast activated and inactivated current (IKt) and a sustained current (IKs). IKt had a range of activation from -40mV to +10mV and an inactivation range from -90mV to -30mV. It was slightly inhibited by 4AP (2mM). IKs had a range of activation from -20 mV to +40 mV, was not inactivated during long voltage step (up to 25 seconds), and was blocked by TEA (10 mM). DA added in bath (10nM) increased both potassium currents, with a major action on IKt without changing its steady state activation. These effects were mimicked by bromocriptine and blocked by the D2 antagonist sulpiride. We conclude that DA may control PRL release by increasing mainly transient outward K^+ current, thus decreasing excitability of the lactotrophs.

33.8

D_2 DOPAMINERGIC ATTENUATION OF PHOSPHOINOSITIDE PHOSPHORYLTRANSFERASES I AND II (PPT-I, PPT-II). W.D. Jarvis, A.M. Judd*, and R.M. MacLeod. Depts. of Medicine and Neuroscience, University of Virginia Medical Ctr., Charlottesville, VA 22908.

We have previously demonstrated that D_2 dopamine receptor activity attenuates the activities of PPT-I and PPT-II, thereby reducing the basal levels of PtdIns(4)P and PtdIns(4,5)P_2, but not of PtdIns, in the normal rat anterior pituitary gland. The concentration-response characteristics of this effect were recently examined as was the possibility that the somatostatin receptor may exert a similar influence.

Primary cultures of normal female rat anterior pituitary cells were incubated for 48 hrs with [^{3}H]Ins in the absence or presence of bromocriptine or SMS 201-995 (BRC, SMS), respective agonists of D_2 dopamine and somatostatin receptors. Following organic extraction, radiolabeled phosphoinositides were deacylated under alkaline conditions and assayed by conventional anion-exchange resin chromatography and liquid scintillation counting.

BRC (.001-10 μM) exerted profound, concentration-dependent reductions in the formation of [^{3}H]PtdIns(4)P (EC_{50}=.725 μM) and [^{3}H]PtdIns(4,5)P_2 (EC_{50}=.5 μM), but had no effect on the formation of [^{3}H]PtdIns; inhibition of prolactin release was also concentration-dependent (EC_{50}=.01 μM). In contrast, SMS produced dramatic inhibitions of both prolactin and growth hormone release, but failed to modify any aspect of phosphoinositide metabolism.

These data provide further evidence that PPT-I and PPT-II are the components of phosphoinositide metabolism that are sensitive to dopaminergic tone. We suggest that this capacity is unique to the D_2 dopamine receptor. (Supported by NIH grant CA-07535-24.)

33.9

ROLE OF VIP IMMUNOREACTIVE (VIP-IR) NEURONS WITHIN THE INFUNDIBULAR NUCLEAR COMPLEX (INF) IN REGULATION OF PROLACTIN (PRL) RELEASE IN THE DOMESTIC TURKEY L. J. Mauro , M. E. El Halawani*, and R. P. Elde. U of Minnesota, Dept. of Animal Science, St. Paul, MN 55108 and Dept. of Cell Biology and Neuroanatomy, Minneapolis, MN 55414.

During the reproductive cycle of the turkey, an initial elevation in plasma PRL occurs after photostimulation followed by a second rise during egg laying, culminating in the hyperprolactinemic state of incubation. Changes in PRL releasing factors such as VIP may account for these alterations. Using immunocytochemical techniques, we examined changes in hypothalamic VIP-IR expression in hens during different reproductive states. Birds were transcardially perfused with fixative,10μm cryostat sections of hypothalamus were incubated with rabbit VIP antisera followed by FITC-labeled goat anti-rabbit antisera. Marked differences in VIP-IR in the INF were noted in comparisons of non-photostimulated hypoprolactinemic (NP) and incubating hyperprolactinemic (HYP) hens. Numerous VIP-IR cells are present in the anterior (aINF=28±2 cells/section) and posterior (pINF=56±3) INF of HYP bird whereas 0-2 cells appear in the INF of the NP bird. The transition from the NP state to photostimulated (2 wks., 16L:8D) results in the appearance of VIP-IR cells (aINF=13±1; pINF=11±1) as well as increases in fibers in INF and median eminence. A second, greater increase in VIP-IR expression occurs in laying hens (aINF=30±5; pINF=39±3). These changes in VIP-IR in the INF coincide with alterations in plasma PRL and suggest that fluctuation in VIP expression by INF neurons may regulate PRL release during reproduction in this species.

33.10

CSF PROLACTIN CONCENTRATIONS IN FEMALE RATS CHANGE WITH ENDOCRINE STATE. B.S. Rubin, P.M. Ronsheim* and R.S. Bridges. LHRRB, Harvard Medical School, Boston, MA 02115.

Push pull-perfusion was used to estimate prolactin (PRL) levels in the CSF of female rats in various endocrine conditions. Cannulae were implanted in the lateral ventricle, and 10 minute perfusates were collected on ice and stored at -20°C until PRL content was measured by RIA. PRL was not detectable in perfusates collected from ovariectomized females. However, 4 days after sc implantation of a 5 mm estradiol-filled Silastic capsule, measurable levels of PRL were present in CSF samples from these same animals. Another group of animals was sampled on days 18 and 22 of pregnancy and again on day 8 of lactation. Levels of PRL in perfusates from pregnant animals were below the detectability of the RIA. On day 8 of lactation, pups were removed at 1000 h, CSF sampling began 2 hours later, and pups were returned to their mothers 4 hours after separation. Four of the 7 mothers showed a dramatic rise in CSF PRL levels after pups were returned and nursing resumed. These data suggest that when serum PRL levels are high, significant levels of PRL are present in the CSF and may therefore gain access to neural tissues that influence neuroendocrine and behavioral processes. Given that the lateral ventricles were sampled, it seems likely, that the major source of CSF PRL in the present study is the pituitary gland rather than the recently identified brain PRL system localized primarily in the basal hypothalamus. (HD 19789)

33.11

CHRONIC COCAINE (C) ALTERS THE RELEASE OF PROLACTIN (PRL) IN MALE RATS. N.S. Pilotte & L.G. Sharpe. Addiction Res. Ctr., Nat'l. Inst. on Drug Abuse, Baltimore, MD 21224.

A single exposure to C can alter the release of dopamine (DA; Pharmacol Biochem Beh 27:533). Such an alteration may be apparent in vivo as a change in the DA-controlled release of adenohypophysial PRL. We used the release of PRL as an index of hypothalamic DA activity in male Lewis rats that were passively injected with C for the first time or after 4 consecutive days of short-term exposure to the drug or its vehicle (V). A catheter was inserted into the right jugular vein of each rat. Three days later, blood was removed at 30, 15 and 0 min before and at 5, 10, 30, 45 and 60 min after the administration of C (1 mg/kg, iv) or V (0.15 M NaCl). Similar stable basal levels of PRL were observed both prior to and after the first administration of C or V on that day. However, when [PRL] was assessed similarly in the same rats after 4 consecutive days of short-term exposure to C, the basal [PRL] was 50% higher in rats exposed to C than to either their own previous levels or that of the V-injected rats. Furthermore, exposure to C, but not to V, resulted in a rapid and marked decrease (66%) in the peripheral PRL which was sustained throughout the sampling period. These results suggest that chronic exposure to C may decrease the amount of DA available tonically without necessarily interfering with the mechanisms of its release.

33.12

SEROTONIN (5HT) INFLUENCE ON ANTERIOR PITUITARY PROLACTIN (PRL) SECRETION: NOVEL SITES AND MECHANISMS OF ACTION. C.A. Johnston, F. Lopez*, G. Wisniewski* and A. Negro-Vilar. Lab of Molecular and Integrative Neuroscience, NIEHS, NIH, Research Triangle Park, NC 27709.

The site(s) and mechanisms by which 5HT stimulates PRL secretion from the anterior pituitary (AP) are poorly understood. We examined the effects of 5HT synthesis inhibition [using p-chlorophenylalanine (PCPA)] on basal, TRH or 5-hydroxytryptophan (5HTP) stimulated PRL release in vivo. We also examined the effects of in vivo 5HT synthesis inhibition or stimulation [tryptophan (TRYPT)] on basal or TRH-stimulated PRL secretion from dispersed AP cells as well as on the ability of neurointermediate pituitary (NIL) extracts obtained from the three treatment groups to stimulate PRL release in vitro.

PCPA treatment decreased basal PRL secretion in vitro and in vivo but did not diminish the PRL response to TRH either in vivo or in vitro, or to 5HTP administration in vivo. AP cells from PCPA-treated animals released less PRL than those from vehicle- or TRYPT-treated rats. NIL extracts from TRYPT-treated rats elicited a PRL response from AP cells at a concentration which did not affect PRL secretion when NIL extracts from control or PCPA-treated animals were used. In conclusion, changes in 5HT synthesis seemed to affect the ability of lactotrophs to release PRL under basal conditions, without impairing the response to known PRL secretagogues.

33.13

CORRELATIVE EVIDENCE FOR A ROLE OF HYPOTHALAMIC OXYTOCIN (OT) AND B-ENDORPHIN (B-EP) IN SUCKLING-INDUCED PROLACTIN (PRL) RELEASE. S.A. Frautschy* and D.K. Sarkar. (SPON: K. Heidenreich). Repro. Med. Univ. Calif. San Diego. La Jolla, CA. 92093. The role of OT and β-EP in the suckling-induced PRL release was investigated in urethane-anesthetized lactating rats. Unlike in control animals, PRL rose in response to suckling by 45 min. By 135 minutes levels reached 16-fold higher than controls. Suckled mothers displayed 1.6-fold higher pituitary portal levels of OT and 4.5-fold higher levels of β-EP between 0 and 45 minutes than control mothers. β-EP but not OT levels were 2.6-fold higher between 46-135 min during suckling than during control periods. Suckled mothers with intact pituitaries exhibited hypothalamic concentrations of OT and β-EP 24.5- and 2.7-fold lower respectively than in controls, and hypophysectomized-suckled mothers showed hypothalamic concentrations of OT and β-EP 1.4- and 1.6-fold lower respectively than in unsuckled/hypophysectomized mothers. In suckling mothers, the prolonged increase in β-EP in pituitary portal blood which coincided with a release of PRL and a decrease in hypothalamic concentrations of β-EP suggests that β-EP may modulate PRL secretion during lactation. The brief increase in pituitary portal plasma levels of OT coinciding with the suckling-induced PRL release and a decrease in hypothalamic OT concentrations would also support a role for OT in PRL secretion during lactation. The decrease in hypothalamic concentration of OT and β-EP, which was more accentuated in animals with intact pituitaries, is perhaps indicative that the pituitary plays a facilitory role in the suckling-induced surge of PRL. (supported by NIH grants # AG05453, HD20498).

33.14

SMALL DOSE OF DOMPERIDONE (DOMP) POTENTIATED THE EFFECTS OF TRH AND SEROTONIN (5-HT) ON PROLACTIN (PRL) SECRETION IN ESTROGEN-TREATED, OVARIECTOMIZED RATS. J.-T. Pan, K.-L. Teo* and C.-W. Chen*. Inst. Physiol., Nat. Yang-Ming Med. Coll., Taipei, Taiwan, Republic of China

A pharmacological regimen using Domp, a peripheral dopamine (DA) antagonist, plus CB154, a DA agonist, was developed by Haisenleder et al. and has been shown to enhance the effect of TRH on PRL release. However, the dose of Domp used (10 µg/rat) seems to be non-physiological since it induced a much higher PRL release and in order to reverse its effect, the use of CB154 which is dissolved in 50% ethanol solution further complicates the system. We found in this study that a smaller dose of Domp (1 µg/rat) without the accompanying CB154 induced a small but significant PRL release which lasted for over an hour. Giving TRH (1 µg/rat, iv) or 5-HT (0.3 mg/rat, iv) an hour later resulted in a significant rise of PRL which was much higher than that induced by TRH or 5-HT alone. The potentiating effect of Domp was even larger in the 5-HT- than in the TRH-stimulated PRL release. Pretreatment of vasoactive intestinal polypeptide (VIP, 10 µg/rat) or 5-HT, however, did not have any potentiating effect on the action of TRH. In conclusion, antagonizing the DA action on the anterior pituitary appears to potentiate the stimulatory effects of TRH and 5-HT on PRL secretion. (Supported in part by NSC77-0412-B010-11)

33.15

ROLE OF DOPAMINE AND VASOACTIVE INTESTINAL PEPTIDE (VIP) ON PULSATILE PROLACTIN SECRETION IN THE MALE RAT. F. López*, J.R. Dominguez*, F. Sanchez-Franco*, and A. Negro-Vilar. Reprod. Neuroend. Sect., LMIN, NIEHS, NIH, Research Triangle Park, NC 27709.

Pulsatile prolactin (PRL) secretion was analyzed by taking frequent blood samples (every 3 minutes for 2.5 hours) in undisturbed male rats. Animals were treated with a dopaminergic antagonist, domperidone (DOM, 0.1 mg/kg i.v.), a VIP antiserum (VIP-AS), the combination of both treatments or their respective vehicles. Samples were assayed for PRL by RIA, and data were submitted to the program "Detect" to analyze PRL pulsatile patterns. DOM treatment increased PRL pulse frequency as well as all quantitative parameters of pulsatile PRL secretion (peak and trough values, pulse amplitude, area under the pulses and mean secretion). Treatment with VIP-AS partially blocked the domperidone-induced increase in the quantitative parameters of pulsatile PRL secretion. By itself, however, VIP-AS did not alter pulsatile PRL secretion. The results suggest that in the presence of the tonic dopaminergic inhibition of PRL secretion, VIP is not able to elicit its stimulatory effects. On the other hand, in the absence of dopaminergic inhibition, VIP seems to participate in the genesis of dopamine-independent PRL pulses.

33.16

EFFECT OF HYPERPROLACTINEMIA ON 3H-NALOXONE BINDING IN SPECIFIC HYPOTHALAMIC NUCLEI OF OVARIECTOMIZED RATS. P.M. Wise and N.G. Weiland. Department of Physiology, University of Maryland, Baltimore, MD 21201

Hyperprolactinemia (hyperPRL) produced by administration of ovine prolactin (oPRL), suppresses plasma LH and PRL levels in ovariectomized rats. To test the hypothesis that hyperPRL influences LH or PRL through an opiatergic mechanism, we quantitated naloxone binding sites in the arcuate nucleus (AN), median eminence (ME), medial preoptic nucleus (MPN) and suprachiasmatic nucleus (SCN).

Young female rats were ovariectomized (day 0). Rats were treated with oPRL (4 mg/kg, sc, every 8h) or vehicle on days 4-6. Rats were decapitated on day 6; brains were removed and 20µ frozen sections were mounted onto slides and incubated in the presence of 2.5nM [3H]-naloxone in 50mM Tris buffer containing 100mM NaCl. Opiate receptor densities were quantitated in the rostral, middle and caudal AN and ME and rostral and caudal MPN and SCN. The densities of opiate receptors were significantly lower (17-25%) in all aspects of the AN of hyperPRL compared to control rats. In contrast, receptor densities were unaffected by hyperPRL in any other brain area examined. We speculate that the decrease in opiate receptor densities in the AN suppresses responsiveness to opiates and thereby mediates the PRL-induced suppression of PRL secretion. (NIH HD15955 & AG02224 [PMW]; AG05357 [NGW])

BASAL GANGLIA AND THALAMUS: MOTOR SYSTEMS I

34.1

CORTICAL INPUTS IN THE RAT STRIATO-STRIATAL GRAFTS: ELECTRON MICROSCOPIC ANALYSIS WITH THE PHA-L METHOD. Z. C. Xu, C. J. Wilson, P. C. Emson. Department of Anatomy and Neurobiology, University of Tennessee, Memphis. Memphis, TN 38163, and Institute of Animal Physiology, Babraham, Cambridge, U.K.

We have previously shown that stimulation of the cerebral cortex evokes monosynaptic EPSP's in neurons located within grafts of fetal striatal tissue placed in the striatum of an adult host. We now report the direct anatomical observation of restored corticostriatal connection in neostriatal grafts.

Three to five days after creation of unilateral kainic acid lesions in the neostriata of Sprague-Dawley rats, a cell suspension prepared from striatal primodia collected from embryonic rats of 15-18 day gestation was implanted into the lesioned site. After a survival period of 2-4 months, PHA-L was iontophoretically injected unilaterally into the medial agranular region of the frontal cortex of the host rats. One to two weeks later, the brains of these rats were fixed and sections through the forebrain were prepared for light and electron microscopic studies using PHA-L immunohistochemistry.

Corticostriatal fibers labeled by medial agranular cortex injections arborized densely in the host striatum surrounding the grafts. Although the density of labeled fibers within the grafts was much lower than in the surrounding host tissue, many fibers could be seen to enter the graft and form terminal arboriztions within it. Electron microscopic examination of labeled terminals within the grafts revealed that they contained small round synaptic vesicles and formed asymmetric synaptic contacts with somata, dendritic shafts and dendritic spines. These synapses were similar in morphology to contacts formed by labeled fibers in the host neostriatum. The identity of the postsynaptic elements was different in the grafts from that of the hosts, however. In the host neostriatum, 90% of the labeled fibers contacted dendritic spines. In the grafts, only 47% of the corticostriatal contacts were axospinous. Most of the remaining synapses were on dendritic shafts. Supported by NIH grant NS20743.

34.2

INTRACELLULAR RECORDING OF PATCH AND MATRIX CELLS IN RAT STRIATAL SLICE PRESERVING CORTICAL INPUTS. Y. Kawaguchi, C.J. Wilson, P.C. Emson, Department of Anatomy and Neurobiology, University of Tennessee, Memphis, TN 38163 and Institute of Animal Physiology, Babraham, Cambridge, CB2 4AT, UK.

Spiny neostriatal projection neurons of the patch and matrix compartments are known to have somewhat different cytochemical staining characteristics, to have different efferent connections and to receive afferent fibers from topographically different regions of the cerebral cortex, but they are often supposed to be identical in their synaptic organization and electrical properties. The aim of this work is to compare their electrophysiological properties and responses to afferent input in vitro. We prepared rat brain slices which contained striatum and neocortex and preserved corticostriatal pathways. Extracellular stimuli were delivered through bipolar electrodes situated in both the medial agranular and prelimbic cortex. After physiological investigation, biocytin was injected intracellularly. Striatum slices with biocytin-injected cells were immersion-fixed, cut and incubated in the avidin-biotin-HRP complex and reacted with DAB. To identify whether the recorded cells belonged to the patch or matrix, calbindin immunohistochemistry was used to label the matrix in the same sections.

Calbindin staining clearly revealed patch and matrix in the immersion-fixed striatal slices and could be used for patch-matrix identification of recorded cells. Both patch and matrix cells extended their dendrites and local axonal collaterals only in their respective compartments. Both patch and matrix cells showed ramp depolarizing responses to depolarizing current steps, prominent anomalous rectification, and similar repetetive firing characteristics. Medial agranular cortex stimulation evoked three types of EPSP differing in latency and threshold. These EPSPs could elicit a depolarizing plateau potential, which was terminated by a resulting of action potential. Supported by NIH grant NS20743 (to CJW) and by a grant from the Japanese Ministry of Education (to YK).

34.3

MORPHOLOGICAL DIFFERENCES BETWEEN CORTICOSTRIATAL FIBERS TERMINATING IN THE PATCH AND MATRIX COMPARTMENTS OF RAT NEOSTRIATUM. C.J. Wilson and Z.C. Xu. Department of Anatomy and Neurobiology, University of Tennessee, Memphis, Memphis, TN 38163.

We have examined the corticostriatal connections formed by fibers arising from the medial agranular frontal cortical field and the medial prelimbic cortical field in the rat using orthograde labelling with PHA-L. These two cortical areas are known to project into the same general area in the neostriatum, but to different tissue compartments within that area. The medial agranular cortex projects to the matrix, while the medial prelimbic area has connections with the neostriatal patches. The PHA-L labelled fibers were examined using (1) light microscopy to determine the overall patterns of axonal arborization, (2) high voltage electron microscopy using thick (3-5μm) sections to quantitatively analyse the distances between boutons and to measure axonal diameters, and (3) conventional electron microscopy to determine the nature of the postsynaptic targets of each fiber type.

In both compartments, corticostriatal synaptic contacts are made primarily upon dendritic spines. Their convergence patterns are very different, however. Corticostriatal fibers terminating in the matrix have very extended arborizations that cover large areas in the neostriatum. Individual fibers can be followed for several millimeters. They make synapses almost exclusively *en passant*, and synaptic boutons from individual fibers are spaced so far apart that no fiber could possibly make more than a few contacts on each spiny neuron it encounters. Fibers terminating in the patches show a very different pattern. Individual fibers branch extensively and arborize within a region comparable to the dendritic field of a single spiny neuron. This pattern makes it possible for a single fiber to make many contacts with an individual neuron.

These observations suggest that the convergence ratio of corticostriatal connections may differ dramatically in the patch and matrix compartments. Supported by NIH grant NS20743.

34.4

REGULATION OF SODIUM AND CALCIUM SPIKES IN IMMUNOCYTOCHEMICALLY IDENTIFIED RAT DOPAMINE NEURONS RECORDED IN VITRO. AA Grace & SP Onn, Dept. Behav. Neurosci. & Psychiat., CNUP, Univ. Pittsburgh, Pittsburgh, PA 15260.

In vitro intracellular recordings were made from dopamine (DA) neurons identified by combined intracellular staining and tyrosine hydroxylase immunocytochemistry. DA neurons could be reliably identified by the slow depolarizing current (I_{SD}) and pacemaker firing pattern. DA neuron action potentials were long in duration (>2 msec) and exhibited comparatively depolarized spike thresholds (approx. -35 to -45 mV) which varied with membrane potential. The action potential was composed of two distinct components, a fast (1-1.5 msec duration) initial segment (IS) spike triggering a slow (1.5-3 msec duration) somatodendritic (SD) component. Cobalt application (4 mM:2 mM calcium) blocked the calcium-dependent SD spike component without blocking the IS spike, causing subsequent depolarizations to trigger a series of IS spikes at short (15-20 msec) interspike intervals. The IS spike could also be triggered alone in untreated slices by the rebound of strong hyperpolarizing pulses, with the threshold and amplitude of the IS spike strongly dependent on the initial membrane potential. The threshold for activating the rebound IS spike decreased with 4-aminopyridine (4-AP) application, but was unaltered by tetraethylammonium (TEA). When the IS spike is blocked by TTX, depolarization triggered high threshold calcium spikes after TEA but not after 4-AP. This evidence suggests that dendritic and axonal spiking regions exist in different functional subcompartments of the DA neuron, and can be independently modulated by pharmacologically distinct potassium currents. (Supported by USPHS MH42217 & MH30915)

34.5

ANALYSIS OF FUNCTIONALLY DEFINED STRIATAL NEURONS BY COMBINED IN VIVO INTRACELLULAR RECORDING AND IMMUNOCHEMISTRY. SP Onn, TW Berger & AA Grace, Dept. Behav. Neurosci. & Psychiat., Univ. Pittsburgh, Pittsburgh, PA 15260.

In previous studies, we have identified two subtypes of striatal neurons based on their differential response to paired pulse stimulation of frontal cortex. However, it is unclear whether these neurons represent different histological types of striatal neurons. Using in vivo intracellular recording techniques, we have begun to examine the synaptic activity underlying these physiological responses, in addition to characterizing the cells recorded in terms of their morphology and neurotransmitter content.

Responses to the second pulse of paired cortical stimulation was characterized as facilitation, inhibition, or no change with respect to the amplitude of the response to the first stimulus. Using interstimulus intervals ranging from 10 to 100 msec, three different response patterns were observed: 1) inhibition followed by facilitation, 2) facilitation followed by inhibition, or 3) facilitation at all intervals. In each case examined, neurons showing the first response pattern, after labelling with Lucifer yellow, were shown to be of the medium or large spiny neuron type. We are currently classifying these neurons further by combining this with immunocytochemical localization of GABA, substance P and enkephalin. Another type of neuron showing primarily burst-type phasic firing patterns was found, after labelling, to resemble the large aspiny neuron of the striatum. Through this type of analysis, we hope to provide further information concerning the structural and functional organization of the basal ganglia. (Supported by USPHS NS19608 and the Tourette's Foundation Postdoc Fellowship.

34.6

RESPONSES OF STRIATAL NEURONS TO PAIRED IMPULSE STIMULATION IN A CORTICOSTRIATAL SLICE PREPARATION. ES Nisenbaum, AA Grace and TW Berger. Depts. Behavioral Neuroscience & Psychiatry, U. of Pittsburgh, Pittsburgh, PA 15260.

Striatal neurons recorded in vivo can be segregated into two functionally distinct subpopulations, Type I and Type II, based on their inversely related probabilities of spike discharge to paired impulse stimulation of the corticostriatal pathway. We investigated whether or not the same two cell classes could be identified using an in vitro corticostriatal slice preparation.

Paired impulse stimulation of cortical fibers in vitro produced a facilitation of striatal cell discharge to the second impulse at interstimulus intervals (ISIs) of 10-400 ms. This paralleled a similar facilitation displayed by Type II neurons in vivo. Type II neurons also exhibit inhibition at ISIs of 10-30 ms, which was not observed in vitro. Consistent with previous reports of a GABA-mediated inhibition of striatal cells, addition of GABAergic agonists, pentobarbitol (50-100 uM) or pregnanolone (5-10 uM), produced a dose-dependent increase in short ISI inhibition, but did not affect the facilitation at longer ISIs. Direct stimulation of adjacent striatal tissue in vitro produced a similar selective enhancement of short ISI inhibition. Both drug- and stimulation-induced inhibitory effects could be blocked with bicuculline (20 uM). These results define conditions under which Type II-like neurons can be identified in vitro, and indicate that the magnitude of short ISI inhibition induced by selective activation of cortical afferents is reduced in vitro, but can be enhanced by GABAergic agonists or direct stimulation of striatal tissue. (NS19608, MH00343, MHCRC1225).

34.7

NONLINEAR RESPONSE CHARACTERISTICS OF STRIATAL NEURONS TO RANDOM IMPULSE TRAIN STIMULATION OF CORTICAL AFFERENTS. T.W. Berger, J.P. Sidney, E.S. Nisenbaum, and R.J. Sclabassi. Depts. of Behavioral Neuroscience, Psychiatry & Neurological Surgery, U. of Pittsburgh, Pittsburgh, PA 15260

Striatal neurons modulate excitatory input from the cortex through a substantial feedback network consisting of voltage-dependent membrane conductances, recurrent axon collaterals, and reciprocal connections with the substantia nigra. We quantitatively characterized the transformational properties of this network using nonlinear systems analytic procedures.

Unit activity of Type II striatal neurons was recorded in response to a train of 4064 impulses with a Poisson interval distribution (mean: 500 ms; range: 1-5000 ms) applied to the corticostriatal pathway. The relationship between inter-impulse intervals of the train and probability of spike output was represented as the first four kernels of a functional power series; the kernels were computed using cross correlation methods. Results showed that the probability of striatal cell discharge varied by as much as 40-50% depending on the interval since a prior impulse, with as many as four different ranges of intervals resulting in such nonlinearites. These second order nonlinear response properties were significantly different from those observed using paired impulse stimulation. Spike output also varied markedly depending on the intervals since two preceding impulses, with spike probability varying by 10-40% in response to several discrete input patterns. Supported by NS19608 and MH00343.

34.8

SINGLE UNIT ACTIVITY IN LATERAL STRIATUM OF FREELY MOVING RATS CORRELATES WITH SPECIFIC LIMB MOVEMENTS DURING LEVER PRESSING. R.M. Carelli* and M.O. West. (SPON: A. Tomie) Dept. of Psychology, Rutgers University, New Brunswick, NJ 08903.

We have previously demonstrated that units in the lateral striatum exhibit robust, short duration (50-150 msec) bursts of activity highly correlated with discrete limb movements (Neurosci. Abstr. 13:979, 1987). Units increased activity during the swing phase of forward treadmill locomotion, passive manipulation and cutaneous tapping of a particular limb. The objective of the present study was to further evaluate striatal involvement in sensorimotor integration via a more stringent behavioral measure, the force lever task. Long-Evans rats, aged 90-120 days, were prepared for chronic recording via a detachable microdrive positioned above the lateral striatum. Stereotaxic coordinates were referenced to bregma, using a level skull (A-P 0.8 to 1.7, M-L 3.2 to 4.0, D-V 3.0 to 6.0 mm). A mechanically operated force lever was electronically controlled by a computer which simultaneously recorded neural events. Rats were water deprived to 82% body weight and trained to depress the lever with the contralateral forepaw using a specified amount of force (10-15 grams) for durations ranging from 0-1.5 sec. This response produced an audio feedback cue and resulted in water reinforcement. In preliminary studies, striatal units correlated with locomotor movements and passive manipulation of the forelimb showed a pattern of activity consisting of maximal firing during extension of the forelimb as the subject reached for the lever. This was followed by a marked decrease in firing rate during actual contact with and depression of the lever. The significance of these studies is that they allow for an analysis of striatal function 1) in terms of quantifiable variables such as force, trajectory and timing of movement 2) in the context of unrestrained movement previously confined to studies in primates during head restraint, and 3) with respect to more global variables such as contextual cues. Supported by NSF BNS-8708523, DA 04551, PHS RR 07058-21.

34.9

SOMATIC REPRESENTATIONS IN SINGLE UNIT RECORDINGS FROM THE DORSOLATERAL STRIATUM OF FREELY MOVING RATS. S.M. Cohen*, M. Pomerantz*, R.M. Carelli*, and M.O. West. Dept. Psychology, Rutgers Univ., New Brunswick, NJ.

A long-standing issue that is beginning to be resolved is whether the striatum is functionally compartmentalized. We have shown that robust single unit discharges correlated with specific locomotor limb movements were observed only in the most lateral and dorsal portion of the striatum (Neurosci. Abstr. 13:979, 1987). We now report that other neurons in the same subregion exhibit short-latency (<20 msec) responses to sensory stimuli applied contralaterally to circumscribed parts of the body. Long-Evans male rats (300g) were chronically implanted with a detachable microdrive positioned above the lateral striatum (0.0-1.7mm A-P, 3.5-4.5mm M-L and 3.0-6.0mm D-V from Bregma). Videotape recordings of the animal were synchronized by a computer, which simultaneously recorded each neural discharge. Frame by frame analysis (60 frames/sec) allowed the construction of peri-event histograms showing correlations between neural and behavioral events. As previously reported, over 70% of the units related to active movement of a particular limb also responded to passive manipulation and/or cutaneous tapping of the same limb. Further studies have revealed that, although individual forelimb and hindlimb representations could be found throughout the entire A-P range studied, the mean A-P coordinate for forelimb representations (0.92mm±.07 SEM) was significantly anterior to that for the hindlimb (0.61mm±.09) ($t = 2.73$; $p.01$). Specific sensory responses involving no apparent motor component were demonstrated consistently across animals during light stroking of the vibrissae, or light tapping of the neck, chin, and snout, throughout the entire A-P range studied. Sensory responses to light tapping of a restricted area of the trunk were observed in one animal at 1.0mm A-P. None of these responses could be obtained using a variety of anesthetics. It appears that all body parts are represented diffusely throughout a longitudinal strip in the dorsolateral striatum, the distribution of forelimb representations, on the average, somewhat anterior to that of the hindlimb. Supported by NSF BNS-8708523, DA 04551 and PHS RR 07058-21.

34.10

SINGLE DOPAMINERGIC NIGROSTRIATAL CELLS FORM TWO SYNAPTIC TYPES. T. Hattori, M. Takada and D. van der Kooy. Dept. of Anatomy, University of Toronto, Toronto, Ontario M5S 1A8, Canada

The characterization of the dopaminergic nigrostriatal neuron of the mammalian brain remains controversial, both in terms of structure (does it make symmetrical or asymmetrical striatal synapses) and of function (are its post-synaptic effects excitatory or inhibitory). Our data suggest that all of these seemingly contradictory characteristics may be possessed by single dopaminergic nigrostriatal cells. In single ultrathin sections through the rat striatum, ^{3}H-proteins anterogradely transported from the substantia nigra over short survival times (1 day) labeled primarily asymmetrical synapses "en terminaux", whereas antibodies to tyrosine hydrxylase(TH) marked almost exclusively symmetrical synapses "en passant". However, radiolabeled unmyelinated axons were usually double-labeled with TH. Employing longer survival times (10 days after the nigral isotope injections) in order to enhance the ratio of "en passant" to "en terminaux" labeling resulted in a large increase in the radiolabeling of striatal axonal varicosities and especially of symmetrical synapses "en passant" double-labeled with both the autoradiographic and immunohistochemical markers. We conclude that both the asymmetrical "en terminaux" and symmetrical "en passant" synapses take origin from nigrostriatal neurons.

34.11

EXTENSIVE CO-OCCURRENCE OF SUBSTANCE P AND DYNORPHIN IN STRIATAL PROJECTION NEURONS IN RATS. K.D. Anderson and A. Reiner. Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, TN

Recent immunohistochemical studies have shown that substance P (SP) and dynorphin (DYN) co-occur extensively in striatal projection neurons in two non-mammalian amniote species and one mammalian species. Whether this is a typical feature of basal ganglia organization among other mammalian and non-mammalian amniotes is unknown. Since much work on the regulation of SP and DYN levels in striatal neurons has been done in rats, it is important to know the extent of SP/DYN co-occurrence in striatal projection neurons in this species. We therefore used the simultaneous immunofluorescence technique to examine the co-occurrence of SP and DYN in striatal projection neurons in rats (pretreated with colchicine). The studies were performed using antisera against different DYN peptides, each blocked by leu-enkephalin (LENK), to rule out possible spurious crossreactivity of anti-DYN antisera with LENK. Results consistently showed that the majority of SP+ or DYN+ neurons observed in the striatum were positive for both peptides. In contrast, SP and ENK co-occurrence was rare. Similarly, SP/DYN double-labeling of fibers and terminals of striatal projection neurons, both in the striatum itself and in striatal projection targets (i.e. substantia nigra and entopeduncular nucleus), was extensive, whereas SP/ENK double-labeling was rare. These results support the view that SP/DYN co-occurrence in specific populations of striatonigral and striatopallidal projection neurons is a widespread feature among amniotes and thus of fundamental importance to basal ganglia function. Supported by NS19620 (A.R.) and the Huntington's Disease Society of America (K.D.A.)

34.12

THE STRUCTURE OF NEURONS AND AFFERENT AXONS IN THE THALAMIC RETICULAR NUCLEUS: OBSERVATIONS WITH INTRACELLULAR LUCIFER YELLOW INJECTIONS AND PHA-L LABELING. by C. Asanuma. Laboratory of Neurophysiology, National Inst. of Mental Health, Poolesville, MD 20837.

The structure of neurons in the thalamic reticular nucleus (TRN) was investigated in lightly fixed slices of rat thalami by injecting the fluorescent dye, Lucifer Yellow (LY), under direct microscopic control, into individual TRN neurons. TRN neurons are large multipolar neurons, usually with elongated somata (range: 25-50 μm in longer diam.), whose dendritic arborizations are quite distinctive. The dendrites of each neuron radiate extensively in one or two tiers of restricted thickness (<10 μm) within the sheet-like plane of the TRN (approx. 200 μm in thickness). Each cell body has 3-5 primary dendrites, which rapidly give rise to 2-4 secondary dendrites. The primary dendrites are smooth, while secondary and tertiary dendrites are beaded; filamentous spine-like appendages are rarely seen. Many dendritic processes extend over 250 μm in length.

Retrograde tracer injections (FB,WGA-HRP,or FG) into the region of the TRN label neurons predominantly ipsilaterally in the parabrachial nucleus, the pedunculopontine nucleus, and in the magnocellular nucleus basalis, in addition to neurons in the locus coeruleus, raphé, dorsal thalamus, and cortex. However, anterograde tracer injections (WGA-HRP or PHA-L) into the parabrachial nucleus or the pedunculopontine nucleus label axonal arbors within the closely adjacent zona incerta, but not, to any significant extent, within the TRN. PHA-L injections into the nucleus basalis show widespread terminal arbors within the TRN; thin, varicose axons extend tortuously for considerable distances. The details of the relationship between these potentially cholinergic axons and the dendrites of TRN neurons remain to be investigated.

34.13

LAMINAR ORIGIN OF PATCH- AND MATRIX-DIRECTED CORTICOSTRIATAL PROJECTIONS IN THE RAT. C.R. Gerfen and A. Rutherford*. Lab of Cell Biology, National Institute of Mental Health, Bethesda, MD and MRC Brain Metabolism Unit, Edinburgh, Scotland.

Corticostriatal projections of medial frontal cortical areas in the rat are examined with the *Phaseolus vulgaris*-leucoagglutinin (PHA-L) anterograde axonal tracing method. Previous studies have suggested that corticostriatal inputs to the striatal patch and matrix compartments are determined by the cortical area of origin; i.e. the prelimbic cortex projects to the striatal patches whereas the anterior cingulate and medial agranular cortices project to the striatal matrix (Gerfen, 1984; Donoghue and Herkenham, 1986). The present more detailed analysis suggests a variation on this scheme. PHA-L labeled corticostriatal efferents of the infralimbic, prelimbic, anterior cingulate and medial agranular motor cortices each provide inputs to both the striatal patch and matrix compartments that are related to the laminar origin of the projection neurons. PHA-L injections into each of these cortical areas that label the efferents of cortical layer VI and deep layer V neurons, which provide a relatively restricted input to the deeper layers of the contralateral homotypic cortex, project relatively specifically to the striatal patch compartment. Conversely, injections that label efferents of more superficial cortical layers, which label a robust input to superficial layers of the contralateral homotypic cortex, label inputs to the striatal matrix. Also, qualitative observations suggest that inputs to the patch compartment are denser from the infralimbic and prelimbic cortices, whereas matrix directed projections are denser from anterior cingulate and medial agranular motor cortical areas. Thus the relative density of patch- and matrix-directed corticostriatal neurons may vary among cortical areas.

34.14

STRIATAL COMPARTMENTALIZATION IS PRECISELY PRESERVED ACROSS THE MAMMALIAN ORDER. **J. G. Johnston[1], C. R. Gerfen[2], K. Andruschak*[3], S. Haber[3] and D. van der Kooy[1], [1]Dept. of Anatomy, University of Toronto, Toronto, Canada, M5S 1A8, [2]Lab. Cell Biology, NIMH, Bethesda, Maryland, [3]Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, New York.**

The mammalian striatum is a compartmentalized structure that can be subdivided on the basis of different neurotransmitters, receptors and connections. Calcium-binding protein staining marks the matrix compartment, while enkephalin staining and opiate receptor binding mark the patch compartment, which is embedded in the matrix. Measurements of the patch compartment were made at rostral, mid and caudal striatal levels using opiate receptor binding in rat, enkephalin immunostaining in rhesus monkey and neurologically normal human and calcium-binding protein immunostaining in rhesus monkey sections. Although the human striatum is approximately 20 times larger than the rat striatum, the patch compartment still comprises 10-15% of the area of the striatum in the rat (10.6%), monkey (13.0%) and human (14.6%). The relative maintenance of the size of the patch compartment over a large increase in absolute striatal area could be achieved either by 1) increasing the number of patches, 2) increasing the area of individual patches, or 3) a combination of both strategies. We found that the area of the average patch enlarges along with increases in striatal area, while the average number of striatal patches does not vary in transverse sections of rat, monkey and human striatum. We suggest that lineage mechanisms (eg. equal increases in the number of patch stem cells and matrix stem cells) may underlie the maintenance of a specific ratio of total patch area to total matrix area across different mammalian species. However, epigenetic factors, such as adhesion and the establishment of afferent and efferent striatal connections, may play a role in the preservation of the absolute number of striatal patches across species.

34.15

PHARMACOLOGICAL AND MORPHOLOGICAL EVIDENCE FOR THE FORMATION OF CONNECTIONS BETWEEN TRANSPLANTED NEOSTRIATAL NEURONS AND SURROUNDING HOST NERVOUS TISSUE. N. Lee*, J.P. Walsh, C.D. Hull and M.S. Levine. MRRC, UCLA, Los Angeles, CA 90024.

Previous work from our laboratory demonstrated that transplanted striatal neurons (TSNs) possess normal, although immature, biophysical and morphological properties. TSNs also receive both excitatory and inhibitory synaptic inputs in response to stimulation of host striatal tissue. In the present study, neostriatal tissue from E14 fetuses was grafted into the intact host neostriatum. Recipient animals were sacrificed 4 to 10 weeks after transplantation and slices of their brains were studied in in vitro intracellular neurophysiological experiments. Excitatory postsynaptic potentials (EPSPs) elicited by stimulation of adjacent host tissue were blocked by the excitatory amino acid antagonist kynurenic acid. Since it is generally agreed that extrinsic neurons are the primary source of excitatory inputs to the neostriatum, these data indicate that the EPSPs resulted from an activation of synaptic contacts formed by the ingrowth into the transplant of extrinsic elements. Inhibitory postsynaptic potentials elicited by the same stimulus were blocked by the GABA antagonist biccuculine methiodide. Lucifer yellow injections into single TSNs demonstrated the outgrowth of processes from the transplant into the surrounding host tissue. Taken together these findings indicate that direct synaptic contacts may be formed between host and transplanted nervous tissue. Supported by USPHS Grant HD 05958.

34.16

COMPARATIVE FETAL DEVELOPMENT OF TYROSINE HYDROXYLASE AND SUBSTANCE P IN THE NEOSTRIATUM AND SUBSTANTIA NIGRA OF THE CAT. M.K. Boylan*, R. Gayek*, and R.S. Fisher. (SPON: D.L. Birt). MRRC and Dept. of Anatomy, UCLA, Los Angeles, CA 90024.

Dopamine is thought to play an organizational role in the fetal development of the neostriatum (NS), caudate (Cd) and putamen (Put). Tyrosine hydroxylase (TH), the rate-limiting enzyme of catecholamine synthesis and substance P (SP) develop early within the nigrostriatal and striatonigral pathways, respectively. In order to test the hypothesis that dopamine may influence the fetal developmental time course of SP in the NS, we compared the development of TH and SP immunohistochemistry in 16 fetal kittens from F30 through F60 in the NS and substantia nigra (SN) (gestation period in the cat is 65 days). The development of TH immunohistochemistry in the nigrostriatal pathway shortly preceeds the development of SP in the striatonigral pathway. At F45, TH undergoes its most dramatic increase in the number of cells and fibers in the SN and in TH innervation of the NS. At F50, SP undergoes its most noticeable developmental change in the NS; there is a large increase in the number of SP-positive cells, fibers and patches in the head of the Cd. At F55, SP fibers in the SN become significantly more dense. Also, the distribution of SP fibers growing into the SN closely matches the distribution of TH cells present in the SN. Thus, TH in the NS may influence the subsequent development of SP in NS cells, and may influence or guide the distribution of SP fibers growing into the SN. Supported by USPHS Grants NS 24596 and HD 05958.

34.17

NEUROPHYSIOLOGICAL DEVELOPMENT OF FELINE BASAL GANGLIA NEURONS IN VITRO: SUBSTANTIA NIGRA. M.S. Levine, J.P. Walsh, N.A. Buchwald, and C.D. Hull. MRRC, UCLA, Los Angeles, CA 90024.

Intracellular recordings were obtained from substantia nigra neurons in an in vitro slice preparation. To date, the ages of animals studied ranged from late fetal (E56) to postnatal day 39. Responses of individual neurons to intracellular injection of current and extracellular stimulation of local afferents were determined. Input resistance values obtained from current injection showed a gradual decline with age. In contrast to the large developmental changes in input resistance observed in other mammalian systems (i.e. caudate nucleus), values obtained from the youngest neurons examined were only slightly larger than those of the oldest neurons. The predominant form of synaptic input observed in the oldest neurons, an excitatory-inhibitory postsynaptic potential complex, was present in all neurons tested. Lucifer yellow (LY) was injected into neurons to study the development of single cell morphology. A variety of cell types were labelled by LY at each age. Dendrites and axons generally become more elongated and more highly branched with increasing age. The physiological and morphological information obtained indicates that substantia nigra neurons in the cat may mature earlier than neurons in the striatum. Supported by USPHS Grant HD 05958.

34.18

DYE-COUPLING BETWEEEN NEOSTRIATAL NEURONS: DEVELOPMENTAL REGULATION. J.P. Walsh, C. Cepeda*, R. Fisher, C.D. Hull and M.S. Levine. MRRC, UCLA, Los Angeles, CA 90024.

Intercellular communication via gap junctions has been shown to be developmentally regulated in the neocortex of rats and guinea pigs. The present study utilized Lucifer yellow (LY) injections and intracellular recording to determine if neuronal coupling among neostriatal neurons is under developmental control. Striatal slices were obtained by standard techniques from rats of 1 day postnatal age to adult (>35 days). Dye-coupling was found to be highest in the youngest neurons examined (1-5 day =70%). As age increased the incidence of dye-coupling declined. Six-ten day old neurons showed 60% coupling; 11-15 day old neurons showed 46% coupling; 15-20 day old neurons showed 36% coupling; 20-30 day old neurons showed 20% coupling and >30 day old neurons showed 2% coupling. In addition, the number of neurons labelled by each injection decreased with age. Single injections labelled large aggregates of neurons in slices obtained from 1-2 day old rat pups. After 5 days of age only 2-3 neurons were labelled per injection. Dye-coupled neurons also displayed fast-pre potentials or coupling potentials in response to orthodromic stimulation. These results indicate that striatal neurons in the immature rat are dye-coupled and can display electrotonic interactions. Coupling may function in the immature striatum to aid in the formation of neuronal connectivity by facilitating developmentally crucial electrical or chemical events. Supported by USPHS Grant HD 05958.

34.19

DYE-COUPLING BETWEEN NEOSTRIATAL NEURONS: MODULATION BY DOPAMINE. C. Cepeda*, J.P. Walsh, N.A. Buchwald, C.D. Hull and M.S. Levine (SPON: C.S. Bailey). MRRC, UCLA, Los Angeles, CA 90024.

Recent work in the retina suggests dopamine (DA) modulates the permeabilility of gap junctions. The present study determined if DA has a similar role in the mammalian neostriatum. Ten adult rats received unilateral electrolytic lesions in the substantia nigra (SN). After 3-5 weeks the animals were injected with ketamine (35 mg/kg i.p.). Six rats displayed turning to the side of the lesion. Neostriatal slices were prepared using standard techniques. Recording electrodes were filled with Lucifer yellow. Intracellular recordings were obtained from neurons in the striata ipsilateral and contralateral to the lesion in 23 slices. In the group with histologically verified SN lesions, 23 cells were filled in the ipsilateral neostriatum. Dye-coupling occurred in 43% of the injections. In the contralateral neostriata and in the striata ipsilateral to lesions that missed the SD, 31 cells were filled. Only 3% displayed coupling. Although no major electrophysiological differences were found between recorded cells on sides ipsilateral or contralateral to the lesions some cells in the ipsilateral striata displayed low-amplitude spontaneous depolarizing potentials. All animals that had SN lesions and a high incidence of dye-coupling displayed turning when injected with ketamine. This study suggests that DA may function in the neostriatum to uncouple gap junctions. Supported by USPHS Grants HD 05958 and AG 7462.

34.20

DEVELOPMENT OF CORTICOSTRIATAL CONNECTIONS IN THE CAT. R.S. Fisher and R. Gayek*. MRRC and Dept. of Anatomy, UCLA, Los Angeles, CA 90024.

The objective of these experiments was to determine the relationships between developing corticostriatal inputs and their target neurons. In fetal cats, retrograde and anterograde axonal markers (WGA-HRP) showed the initial ingrowth of corticostriatal axons at fetal age 55 (feline gestation = 65 days). As shown by electron microscopy and Golgi staining, this event coincided with the initiation of synaptogenesis in the neostriatum and began a period of dendritic outgrowth and elaboration. Immunochemistry also demonstrated that a massive increase in the expression of glutamatergic and GABAergic markers in the neocortex and neostriatum started at this time. Based on these data, it is reasonable to infer that the ingrowing corticostriatal axons exert an inductive effect on neostriatal target neurons. This influence may direct the morphogenesis of dendritic arbors in the neostriatum while coupling the expression of glutamatergic (neocortex) and GABAergic (neostriatum) fast amino acid neurotransmitters. Supported by USPHS Grants HD 05958 and NS 24596.

34.21

NEUROPHYSIOLOGICAL DEVELOPMENT OF FELINE BASAL GANGLIA NEURONS IN VITRO: CAUDATE NUCLEUS. *N.A. Buchwald, C. Cepeda*, J.P. Walsh, C.D. Hull and M.S. Levine. MRRC, UCLA, Los Angeles, CA 90024.*

Intracellular recordings were obtained from feline caudate nucleus neurons maintained in an in vitro slice preparation in 33 kittens ranging in age from fetal day 56 to postnatal day 64. Slices (400μm) from the caudate nucleus were obtained and incubated in Ringer's solution. Intracellular electrodes (50-120 mΩ) were filled with K-acetate. Some electrodes contained Lucifer yellow to identify the recorded cells. The passive and active membrane properties of 130 cells were studied. In very young neurons, spike amplitude was smaller than in older neurons. Caudate neurons recorded in slices from young animals showed high input resistances which progressively diminished with age. Excitatory postsynaptic potentials could be evoked in the youngest neurons tested (fetal day 56). Except for one large aspiny neuron all labelled cells were medium-sized spiny neurons. In cells obtained from prenatal slices and during the first postnatal week few dendritic spines occurred. A marked increase in spine density occurred during the second postnatal week. Caudate neurons showed dye-coupling more frequently in younger animals. These results in conjunction with our previously published in vivo studies demonstrate that both passive and active membrane properties of caudate neurons change during prenatal and postnatal periods. Supported by USPHS Grants HD 05958 and DA 3017.

SECOND MESSENGERS I

35.1

Non-coupling Between D_1 Dopamine Receptors and Adenylate Cyclase in the Amygdaloid Nuclear Complex. C. Kilts*, C. Anderson*, J. Lackowicz* and R. Mailman (SPON:R. Erickson). Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

Accumulated evidence indicates that amygdaloid dopamine (DA) receptors are not coupled to the stimulation of adenylate cyclase (AC) activity. Fenoldopam and DA produced a concentration-dependent (SCH23390-inhibited) stimulation of AC activity in homogenates of the micropunch dissected caudate nucleus, olfactory tubercle, nucleus accumbens and substantia nigra-pars reticulata. With the exception of the intercalated cell groups, neither DA nor fenoldopam significantly increased cyclic AMP synthesis in the component nuclei of the amygdaloid complex. AC activity in the central amygdaloid nucleus (CAN) was stimulated by GTP and Ca^{2+}/calmodulin in a concentration-dependent manner. The addition of Mg^{2+} and GTP to a partially purified membrane preparation of the microdissected caudate nucleus restored both basal and DA-stimulated AC activity. Similar attempts restored basal enzyme activity but failed to constitute a DA-stimulated system in the CAN. These findings indicate a mismatch between functional and radioligand binding site (Boyson et al., J. neurosci. 6:3177, 1986) markers of D_1 receptors in the amygdaloid complex. The results of on-going studies of the DA receptor regulation of G protein function and cyclic AMP efflux from slices of the amygdaloid complex will be presented. (NIMH MH-39967).

35.2

EFFECTS OF PERTUSSIS TOXIN ON DOPAMINERGIC MECHANISMS IN CAUDATE. P.C. Bickford-Wimer, C.L. Boyajian, M. Kim*, D. Cooper*, and R. Freedman. Depts. of Pharmacology and Psychiatry, UCHSC, and the VAMC, Denver, CO 80220

Differences in the transduction mechanisms for D1 and D2 receptors were exploited to study their electrophysiology in the caudate nucleus. Unilateral injections of pertussis toxin (PT) were made into the caudate nucleus of Sprague-Dawley rats. 12 to 24 hours later there was a significant attenuation of in vitro PT-induced incorporation of ^{32}P-NAD$^+$ into 39 and 41 k-Da substrates. Additionally, this treatment prevented the inhibition of adenylate cyclase activity by N-n-propyl-norapomorphine. Previous work has indicated that spontaneous discharge of caudate neurons is dependent on endogenous DA neurotransmission; therefore rats were prepared for electrophysiological recording under urethane anesthesia. Single barrel micropipettes filled with 3M saline were stereotaxically placed in caudate nucleus. Mean discharge rate increased from 4.1 ± 0.4 (SEM, 29 cells, 4 rats) to 8.7 ± 1.4 Hz (49 cells 6 rats). In addition, a higher percentage of interspike intervals less than 50 msec was observed in PT-treated rats. These electrophysiological changes were consistent with decreased dopaminergic neurotransmission. The data suggest that D2 mechanisms may underlie dopaminergic regulation of caudate neuron spontaneous rate. Work supported by USPHS grant NS-09199 and the VAMRS.

35.3

DOPAMINE-INDUCED INHIBITION OF SUBSTANTIA NIGRA NEURONS IS NOT DEPENDENT UPON ADENYLATE CYCLASE INHIBITION: AN IN VITRO ELECTROPHYSIOLOGICAL STUDY. A.L. Mueller and M.S. Brodie, Neurosci.Res.Div., Pharmaceutical Discovery, Depts. 47H & 47W, Abbott Labs., Abbott Park, IL 60064.

Dopamine (DA)-containing neurons within the substantia nigra zona compacta (SNC) are inhibited by DA via D-2 autoreceptors. D-2 receptors are known to be either negatively linked or unlinked to adenylate cyclase (AC). Previous reports have suggested the involvement of a pertussis toxin-sensitive G-protein in the mediation of DA-induced inhibition; however, the role of AC was not investigated. The aim of the present study was to determine the involvement of AC inhibition in DA-induced inhibition of DA neurons within the SNC. Submerged rat brain slices containing the SNC were superfused (2 ml/min), and presumed DA-containing neurons were recorded extracellularly. DA (1-30 μM) produced a dose-dependent reduction in spontaneous firing rate. Three treatments were used to increase intracellular levels of cyclic AMP (cAMP) in the slice: the AC activator, forskolin, and two membrane permeable analogs of cAMP, dibutyryl cAMP and 8(4-chlorophenylthio)-cAMP (cpt-cAMP). Pretreatment of slices with forskolin (1-10 μM), dibutyryl cAMP (100 μM), or cpt-cAMP (100 μM) typically resulted in an increase in the spontaneous firing rate (range 0-89%). This excitation was preceded occasionally by a transient reduction in firing. In all cases, DA-induced inhibition was not altered in the presence of these agents, i.e., elevation of intracellular cAMP levels did not prevent DA-induced reduction in firing. These results strongly suggest that AC inhibition with a resulting reduction in intracellular cAMP levels is not involved in DA-induced inhibition of neurons in the SNC.

35.4

8-BROMO-cAMP MIMICS THE EFFECTS OF NEUROTENSIN IN MIDBRAIN DOPAMINE NEURONS: A STUDY IN BRAIN SLICE. W.-X. Shi* and B.S. Bunney. Depts. Pharmacol. & Psychiat., Yale Univ. Sch. Med., New Haven, CT.

We have previously reported that neurotensin (NT) selectively attenuates the inhibition of midbrain dopamine (DA) neurons induced by microiontophoretically applied DA in vivo. In the present study, the effect of NT on DA neurons and the possible mechanism of its action are further studied using a brain slice preparation. The slices containing the ventral tegmental area and substantia nigra were submerged beneath a continuously superfusing medium. Extracellular signals were recorded from DA neurons based on established criteria. The activity of DA neurons was usually totally inhibited by 10 uM DA or less. Confirming our previous finding, NT (100 pM-5nM) significantly attenuated DA-induced inhibition of DA neurons with no or small increase in basal firing rate. Significant excitation was observed when higher concentrations of NT (>5 nM) were used. Application of the membrane permeable analogue of cAMP, 8-Br-cAMP (50 uM-1 mM) was found to mimic the effects of NT. Forskolin (0.1-1 uM), a direct activator of adenylate cyclase, produced the same effects as those of NT and 8-Br-cAMP. These results raise the possibility that the attenuation of DA-produced inhibition by NT may be mediated by intracellularly produced cyclic AMP. Preliminary results with IBMX, a phosphodiesterase inhibitor seem to support this hypothesis.

35.5

TUBULIN MODIFIES SYNAPTIC MEMBRANE ADENYLATE CYCLASE VIA NUCLEOTIDE EXCHANGE. Nan Wang* & Mark M. Rasenick
Dept. of Physiology & Biophysics & the Committee on Neuroscience, Univ. of Illinois College of Medicine Chicago, IL 60680 USA.

Tubulin, the primary constituent of microtubules, is a GTP-binding protein with structural similarities to other GTP-binding proteins. Whereas, microtubules have been implicated as modulators of the adenylate cyclase system, the mechanism of this regulation has been elusive. Tubulin, polymerized with the hydrolysis-resistant GTP analog, GppNHp, can promote inhibition of synaptic membrane adenylate cyclase which persists subsequent to washing. When tubulin is polymerized with the hydrolysis-resistant photoaffinity GTP analog, [^{32}P] AAGTP, and this protein is added to synaptic membranes, AAGTP is transferred from tubulin to the inhibitory GTP-binding protein, Gi. This transfer is blocked by prior incubation of the membranes with GppNHp or covalent binding of AAGTP to tubulin prior to exposure of that tubulin to membranes. Incubation of membranes with GppNHp subsequent to incubation with tubulin-AAGTP results in a decrease in AAGTP bound to Gi and a compensatory increase in AAGTP bound to the stimulatory GTP-binding protein, Gs. These data are consistent with synaptic membrane tubulin regulating neuronal adenylate cyclase by transferring GTP to Gi and, subsequently, to Gs. Further, domains on tubulin required for polymerization appear to be required for the transfer of nucleotide to Gi. This and other physical evidence suggest that tubulin forms complexes with G proteins and, in doing so, may alter neuronal adenylate cyclase.

35.6

MODIFICATION OF G_s-STIMULATED ADENYLATE CYCLASE IN BRAIN MEMBRANES BY LOW pH TREATMENT: CORRELATION WITH ALTERED GUANINE NUCLEOTIDE EXCHANGE. M.M. Rasenick and S.R. Childers (SPON.: H. Hamm). Dept. of Physiology, Univ. Illinois Coll. Med., Chicago, IL 60680 and Dept. of Pharmacology, Univ. Florida Coll. Med., Gainesville, FL 32610.

Pretreatment of rat brain membranes at pH 4.5 modifies function of G-proteins by eliminating G_s-stimulated adenylate cyclase (AC) while increasing opiate-inhibited AC (Childers & LaRiviere, J. Neurosci. 4, 2764 [1984]). To help characterize the molecular nature of the low pH effect, we labeled G_i and G_s alpha subunits in both control and low pH pretreated membranes with the GTP photoaffinity analog [32P]P3(4-azidoanilido)-P1-5'-GTP (AAGTP). Crude synaptosomal membranes were prepared from 21 day-old rats and preincubated at 0° for 10 min in either Tris buffer, pH 7.4 (control) or sodium acetate buffer, pH 4.5 (low pH pretreated). Membranes were first preincubated with unlabeled AAGTP after pH treatment and assayed for AC activity. Preincubation with unlabeled AAGTP produced a persistent inhibitory state of AC which was overcome in untreated membranes with high (>1 µM) concentrations of Gpp(NH)p. In low pH pretreated membranes, this inhibition could not be overcome, and stimulation by Gpp(NH)p was eliminated. Maximum inhibition of AC achieved by incubation with AAGTP was not altered by low pH pretreatment.

Stimulation of AC has been associated with a transfer of guanine nucleotides from $G_{i/o}$ to G_s alpha subunits (Hatta et al, PNAS 83, 265 [1986]). To determine the effect of low pH pretreatment on guanine nucleotide labeling and transfer, labeling with [32P]AAGTP was accomplished in control and low pH pretreated membranes. Incorporation of [32P]AAGTP into G_s (42 kDa) or $G_{i/o}$ (40 kDa) was unaffected by low pH pretreatment; however, transfer of [32P] from $G_{i/o}$ to G_s, which occured with low (10 nM) concentrations of Gpp(NH)p in untreated membranes, was severely retarded in low pH pretreated membranes. Both the potency and efficacy of Gpp(NH)p in producing exchange of [32P]AAGTP from $G_{i/o}$ to G_s was markedly reduced by low pH pretreatment. These results correlate the loss of G_s-stimulated AC with a loss of transfer of nucleotide from $G_{i/o}$ to G_s alpha subunits, and suggest that the nucleotide exchange participates in the modulation of neuronal AC.

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35.7

NOVEL MECHANISM OF REGULATION OF CYCLIC AMP ACCUMULATION IN A NEUROBLASTOMA CELL LINE. B. Baron and B.W. Siegel. Merrell Dow Research Institute, Cincinnati, OH 45215.

Muscarinic cholinergic (mAChR) and α_2-adrenergic receptors (α_2AR) share the ability to attenuate adenylate cyclase (AC) in diverse cell types, but are shown in the present study to exert opposite effects on AC in intact SK-N-SH (ATCC-HTB-11, passage 45-65) neuroblastoma cells.

The selective α_2 agonist, UK14304 (UK) reduced the level of forskolin (FORSK, 1 µM) stimulated cAMP formation by 36-56% (EC_{50} = 100 nM). The effect of 10 µM UK was antagonized by yohimbine, idazoxan, and phentolamine (IC_{50} = 0.25, 2.5, and 2 µM, respectively). In contrast, the α_1 adrenergic antagonist, prazosin (1 µM) was without effect. Carbamylcholine (CCh) increased both basal and FORSK-stimulated cAMP accumulation by 60% (EC_{50} = 10µM). The effect of CCh was sensitive to atropine and pirenzepine (IC_{50} = 0.03 and 20 µM, respectively).

Pretreatment of SK-N-SH cells with pertussis toxin (18h) led to a concentration-dependent loss of the ability of UK to attenuate cAMP accumulation (EC_{50} = 3ng/ml) while having no effect on the CCh facilitated response. Cyclic AMP formation elicited by the combination of CCh + FORSK was not reduced by 10 µM UK.

The presence of functional α_2ARs on SK-N-SH cells suggests that the biochemical requirements for receptor-mediated attenuation of AC activity are present. However, mAChRs in these cells do not utilize this signalling system. SK-N-SH cells may therefore provide a useful neuronal model system to study these receptor types.

35.8

CHOLINERGIC-LINKED, SECOND-MESSENGER INTERACTIONS BIMODALLY REGULATE ADRENAL OPIATE PEPTIDES. J.D. DeCristofaro and E.F. La Gamma. Dept. of Pediatrics and Neurobiology, SUNY @ Stony Brook, NY 11794-8111.

Physiologic stressors increase transsynaptic impulses to augment adrenal catecholamine release and synthesis. To determine the effects of stress on co-localized opiate peptides, we housed groups of 8 rats individually at 4°C. Cold stress (2 or 4d) slightly decreased enkephalin levels (ENK). More severe stress (wetting + cold) increased ENK levels by 95%; wetting alone (21°C) had no effect. To examine cholinergic mechanisms, rats were treated with nicotine (5 mg/kg sc BID or QID x 4d) or muscarinic agonist, carbechol (8.2 uM/kg sc BID or QID x 4d). ENK levels did not change. When given together ENK levels rose 3-fold. To examine cellular mechanisms, medullae were explanted in the presence of increased nicotinic or muscarinic receptor-linked, second-messengers, cAMP or cGMP, respectively. Increased cAMP prevented the rise in ENK levels relative to 4d control; db-cGMP (5 mM) had no effect. However, in the presence of both forskolin (10 uM) + db-cGMP (5 mM), ENK content rose 3-fold. Pertussis toxin blocked this increase. These data suggest that distinct from catecholamines, ENK pathways can be positively or negatively regulated by the severity of a stressful stimulus or by selective activation of cyclic nucleotide second-messenger pathways. Transmitter regulation may involve an interaction of multiple converging transsynaptic mechanisms. Sponsored by the Amer. Heart Assoc.

35.9

NEUROPEPTIDES INHIBIT cAMP PRODUCTION THROUGH A PERTUSSIS TOXIN-INSENSITIVE G PROTEIN IN THE NEUROBLASTOMA N1E115 CELL LINE. P. Kitabgi*, J.C. Bozou* and F. de Nadai* (SPON: A. Enjalbert). Centre de Biochimie du CNRS, Faculté des Sciences, 06034 Nice Cédex, France.

Neurotensin (NT), bradykinin (BK), somatostatin and Met-enkephalin all inhibited in a time- and concentration-dependent manner PGE_1- or forskolin-stimulated cAMP production in the neuroblastoma N1E115 cell line. Cell treatment with pertussis toxin (PT) for 6 hours partly reversed the inhibition elicited by peptides after short incubation periods (< 2 min) but, in contrast, had no effect after longer peptide incubations (> 3 min). Fluoroaluminate, a known activator of G proteins, also inhibited PGE_1-stimulated cAMP production in N1E115 cells, and this effect was not reversed by PT. The 6 hour treatment with PT was sufficient to ADP-ribosylate virtually all of the 41 kD substrate corresponding to the α-subunit of G_i. The peptides did not modify the rate of disappearance of cAMP in PGE_1-stimulated cells, indicating that the inhibition of cAMP production was not due to phosphodiesterase activation. Finally, protein kinase C which is activated by NT and BK in N1E115 cells did not interact with the cAMP-generating system, as shown by the lack of effect of phorbol ester on cAMP production. Altogether, these data point to the existence of a PT insensitive G protein that mediates neuropeptide-induced inhibition of adenylate cyclase in the neuronal N1E115 cell line.

35.10

ALUMINUM ALTERS CYCLIC AMP METABOLISM AND PROTEIN PHOSPHORYLATION IN RAT BRAIN. G.V.W. Johnson and R.S. Jope. Dept. of Pharmacology, Neurology, and Neuropsychiatry Res. Program, Univ. of Alabama, Birmingham, AL 35294.

The effects of aluminum (Al) on cyclic AMP metabolism and protein phosphorylation in rat brain were studied both in vivo and in vitro. Previously we demonstrated that chronic oral administration of Al to rats significantly elevates in vivo cyclic AMP levels in various brain regions. In our present studies we have demonstrated that the stimulation of cyclic AMP production in rat brain slices by 2-chloroadenosine was significantly potentiated when the slices were preincubated with 100µM $AlCl_3$. Al had no effect on basal or forskolin-stimulated cyclic AMP production.

The phosphorylation of certain proteins in rat brain is altered by Al. Previously, we demonstrated that orally administered Al significantly increased the in vivo phosphorylation of MAP2 and the 200-kD neurofilament protein. In our most recent study we measured protein phosphorylation in membrane and soluble fractions from the cortex and brainstem of control and Al-treated rats. In vitro endogenous protein phosphorylation was measured in the presence of cyclic AMP, Ca^{++}/calmodulin, Ca^{++}/phosphatidylserine or without additions. Al-induced alterations in phosphorylation were evident under several of these conditions. Supported by Fellowship #AG05382.

35.11

THE THALAMIC MIDLINE STRUCTURES PROJECTING TO THE AMYGDALA DISPLAY HIGH IMMUNOREACTIVITY FOR THE cAMP-RECEPTOR PROTEIN RII IN THE RAT

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The cAMP-receptor protein RII is the regulatory subunit of type II cAMP-dependent protein kinase. RII immunoreactivity is not homogeneous in the rat's CNS. In the thalamus, the positivity to anti-RII serum appeared concentrated in the midline. In particular, high RII immunoreactivity was evident in the neuropil of the paraventricular nucleus, and part of the rhomboid and reuniens nuclei. In order to further characterize this territory, a combined immunohistochemical and retrograde labeling procedure was here adopted. Fluorescent tracers were injected in two main targets of thalamic midline efferents, the amygdala and the hippocampus. It was thus evidenced that the distribution of cells retrogradely labeled from the amygdala largely overlapped the RII-immunoreactive territory. The latter appeared, instead, mainly segregated from the thalamic territory retrogradely labeled from the ventral hippocampus.

°°The authors are greatly indebted to Drs. S. Lohmann, U. Walter and P. De Camilli for the supply of anti-RII serum.

35.12

MODULATION OF THE SECRETORY RESPONSE IN CAROTID BODY CHEMORECEPTOR CELLS BY ADENYLATE CYCLASE ACTIVATING AGENTS.

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The carotid body (c.b.) chemoreceptors responds to pO_2 decreases. On hypoxic stimulation the chemoreceptor cells release dopamine (DA) in a Ca^{++}_O dependent fashion, being the release proportional to the O_2 decrease. It is known that B-agonists increase the electrical activity and cAMP levels in the organ. We explore the possibility that cAMP modulation of the transmitter release from type I cells is the nexus between both actions of the B-agonists.

Rabbit c.b. were loaded with ^{3}H-DA by incubation with ^{3}H-tyrosine and thereafter syperfused with salines equilibrated with either 100% or 5% O_2 in the presence or absence of forskolin, isobutyl-methyl-xantine (IBMX) and isoproterenol (Iso). We found: 1) Forskolin potenciates the hypoxic ^{3}H-DA release in a dose dependent maner (0.5 to 10 uM). 2) IBMX at 0.5 mM moderatly increases the hypoxic release. 3) Iso at 50 uM is equipotent to 5 uM forskolin. These findings support our working hypothesis.

Supported by DGICYT n°86/0325 and FISss 88/0944 grants.

35.13

ELECTROCONVULSIVE SHOCK INCREASES [^{3}H]-FORSKOLIN BINDING IN SUBSTANTIA NIGRA IN THE RAT.

L.J. Fochtmann, J.S. Gutkind, W.Z. Potter*. SCP,LCS,NIMH, Bethesda, MD 20892.

Electroconvulsive shock (ECS) affects a variety of neurotransmitter receptors, some of which are adenylate cyclase (AC) linked. In particular, cortical β receptors are decreased in number after chronic ECS with parallel decreases in forskolin and norepinephrine stimulated cAMP. Though brain homogenate studies have not revealed ECS-induced changes in dopamine receptors, we previously found increases in D1 receptor binding in the substantia nigra via quantitative autoradiography (QAR). We now report on QAR studies using [^{3}H]-forskolin to assess the catalytic subunit of AC after chronic ECS.

Chronic ECS consistently produced increases (30%-150%) in [^{3}H]-forskolin binding in the substantia nigra pars reticulata. In contrast, no consistent changes in the binding of [^{3}H]-forskolin were seen in other regions such as caudate and hippocampus. This parallels our finding of increased D1 receptor binding in the substantia nigra consistent with an upregulation of both the D1 receptor and the presumably D1-linked AC. Like the increase in D1 receptors, the increase in dopamine linked second messenger function may be relevant to the antiparkinsonian effects of clinically administered electroconvulsive therapy.

35.14

STIMULATION OF CATECHOLAMINE AND MET-ENKEPHALIN SECRETION FROM ADRENAL CHROMAFFIN CELLS BY ANGIOTENSIN: ROLE OF SECOND MESSENGERS.

A.M. Poisner*, H.K. Jiang*, M.K. Stachowiak, P. Hudson*, V. Owyang* and J.S. Hong (SPON: L. Lazarus). LMIN, NIEHS, Res. Triangle Pk., NC 27709.

To study long term regulation of the adrenal medulla, cultured chromaffin cells were exposed to Sar^1-angiotensin II (ANG) for 24 hrs. This caused a dose-dependent increase in the concentration of catecholamines (CAT) and met-enkephalin (MET) in the extracellular medium (ED_{50} 1 nM). Secretion was partially inhibited by nifedipine (calcium channel blocker), by TMB8 and dantrolene (inhibitors of calcium mobilization), by calmidazolium (calmodulin inhibitor) and by H7 (protein kinase C inhibitor). Phorbol ester (TPA) stimulated secretion. Forskolin (adenyl cyclase activator) potentiated the secretory effect of ANG. The results suggest that ANG effects are mediated by both extra- and intracellular calcium and may be modified by the level of cellular cAMP. Calcium may act in part by interacting with protein kinase C and calmodulin. These findings parallel our results on the effects of ANG on mRNAs for proenkephalin, tyrosine hydroxylase and PNMT (Stachowiak et al., 1988, this volume).

35.15

ROLE OF PHOSPHOINOSITIDE METABOLISM, CALCIUM AND ARACHIDONATE IN CYCLIC GMP SYNTHESIS IN N1E-115 CELLS.

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Addition of phospholipase C (PLC) from B. Cereus to neuroblastoma N1E-115 cells markedly stimulated polyphosphoinositide (PI) hydrolysis and also increased intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), cyclic GMP levels and arachidonate liberation in a time- and dose-dependent manner. The time profiles and dose-response relationships between the first three responses of PLC were well correlated, consistent with an inositol trisphosphate-mediated mobilization of intracellular Ca^{2+}. Ionomycin also stimulated intracellular cyclic GMP formation, in parallel to elevated $[Ca^{2+}]_i$, whereas it failed to induce arachidonate release. On the other hand, phospholipase A_2 markedly induced increased arachidonate formation in a time- and dose-dependent fashion (time-to-peak ~2-4 min) but only slightly elevated $[Ca^{2+}]_i$ and cyclic GMP levels. These results indicate that Ca^{2+} might play a more important role than arachidonate release in cyclic GMP synthesis in these cells. (Supported in part by NIH grants NS-24158, AG-07118 and AG-00344.)

35.16

MODULATION OF ATP RELEASE FROM ADRENAL CHROMAFFIN CELLS WITH CYCLIC GMP ANALOGS.

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Acetylcholine (ACh)-evoked catecholamine (CA) and ATP release from bovine adrenal chromaffin cells is initiated by the influx of Ca^{2+} into the cells through both voltage- and nicotinic receptor-gated Ca^{2+} channels. ACh acting on muscarinic receptors also stimulates the production of cGMP, but no clear function for cGMP has been described. We have found that 1 min preincubations with an analog of cGMP, 8-Br-cGMP (1 mM), approximately doubles the amount of ATP release stimulated by nicotine, ACh, elevated K^+, and Ba^{2+} while having no effect on basal release. ATP release was measured using an on-line luciferin/luciferase assay. In contrast, 1 mM 8-Br-cGMP produced no consistent enhancement of total CA release from chromaffin cells. Since total medullary CA reflects primarily epinephrine (E), a preferential enhancement of norepinephrine (NE) release by 8-Br-cGMP may explain this difference. Since NE secretory granules have been shown to have a two-fold higher ATP to CA ratio than epinephrine granules, an enhanced release of NE granules by 8-Br-cGMP would result in a greater relative increase in ATP release than total CA release.

35.17

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE 180 kD MEMBRANE GUANYLATE CYCLASE IN THE RAT BRAIN. A.A. Fedinec*, N.G.F. Cooper and R.K. [1]Sharma* (Spons: J.B. Schweitzer). Dept. of Anatomy and Neurobiology, [1]The University of Tennessee, Memphis, TN 38163, and [1]Section of Regulatory Biology Dept. of Brain and Vascular Research, Cleveland, OH.

A 180 kD protein which apparently contains atrial natriuretic factor (ANF) receptor coupled with membrane guanylate cyclase has been purified and biochemically characterized from rat adrenocortical carcinoma cells. Rabbit monospecific polyclonal antibodies to the 180 kD protein blocked up to 90% of the guanylate cyclase activity of the purified enzyme (Paul, A.K., Marala, R.B., Jaiswal, A.K., and Sharma, R.K. Science., 235:1224-1226, 1987). The distribution of the 180 kD guanylate cyclase was studied with a light microscope in vibratome sections of aldehyde fixed rat brains using a peroxidase immunocytochemical method. Controls for nonspecific binding were negative. Immunoreactivity of 180 kD guanylate cyclase in cerebellum was atributable to Purkinje cells. In these cells the dendrites, perykarya and axons demonstrated high concentration of immunoreactivity apparently associated with membranes. Deep cerebellar nuclei and cells in the brain stem, hippocampus, and cortex showed immunoreactivity. Supported by NIH:NEI EY02708 (NGFC) and NSF grant 8609867 and NIH grant NS23744 (RKS).

35.18

REGULATION OF CYCLIC GMP RESPONSE TO RAT ATRIAL NATRIURETIC FACTOR (99-126) DURING DEHYDRATION AND SALT-LOADING IN THE RAT SUBFORNICAL ORGAN. A. Israel, M.R. Garrido* and I. Becemberg*.(SPON: G. Viana) Universidad Central de Venezuela, Caracas-Venezuela.

Atrial natriuretic factor (ANF) is present in brain neurones. Binding sites for ANF are highly localized in the subfornical organ (SFO)(Brain Res. 408:31). ANF receptors are positively coupled to the guanylate cyclase system (GC) and they undergo regulatory changes with alterations in ANF plasma levels (PSEBM 182:559). We examined therefore the presence of functional- coupled to GC- brain binding sites for ANF by measuring the ANF-induced formation of cGMP in the rat SFO. In addition, the effect of water deprivation (WD)(reduced ANF plasma levels) and salt-loading (SL)(increased ANF plasma levels) on rANF-induced cGMP response was investigated. Male S-D rats (220-280g) were divided in the following groups: WS: water satiated, food and water provided; WD: water deprived for 4 days: SL: drinking water substituted for a 1% NaCl solution during one week. All animals were killed by decapitation and the brains were immediately removed. After removal, the SFO was homogenized and centrifuged. The supernatant assayed for GC activity. cGMP was determined with a radioimmuno-antibody procedure. ANF activated GC in crude homogenates of rat SFO (In pmol/5 min/mg protein: basal 97 $\pm$ 20 , ANF 100 nM: 166.3 $\pm$ 26). An elevated responsiveness to rANP-induced cGMP production was observed in crude homogenates of SFO after 4 days of water deprivation. WS: 83.4 $\pm$ 18 % and WD: 171.1 $\pm$ 43%. On the contrary, a reduced responsiveness to rANF-induced cGMP formation was observed in the SFO tissue from SL animals: 45.5 $\pm$ 17%. Our results suggest that cGMP mediates the central action of ANF and provide evidence suggesting that the receptors susceptible to the regulatory mechanism described in the SFO are the GC-coupled ANF binding sites. (Grants CDCH F.07.20.87).

SECOND MESSENGERS II

36.1

PHORBOL ESTER AND dbcAMP REDUCE TAURINE CONTENT AND EFFLUX IN PRIMARY ASTROCYTE CULTURES. R.A. PHILIBERT AND G.R. DUTTON, Dept. of Pharmacology, College of Medicine, University of Iowa, Iowa City, IA 52242.

We have studied the effects of chronic treatment of cerebellar astrocyte cultures with a variety of hormones and second messenger analogues on intracellular taurine content and K^+-stimulated taurine release. Both dbcAMP (1 mM), and to a lesser extent PDBU (1 µM), produced marked conversion of astrocyte morphology to stellate forms which were maximal at about 6 hrs and reversed partially (dbcAMP) and almost completely (PDBU) after 2 to 3 days. Two day exposure to PDBU (1 µM) or dbcAMP (1 mM) reduced basal taurine efflux by 47% and 49%, respectively, reduced 50 mM K^+-evoked release by 35% and 64%, respectively and also reduced the cellular taurine content by 24% and 42%, respectively. However, neither cellular glutamate or protein was affected. Time course studies showed that these decreases in taurine content were time-dependent with effects plateauing after 1 day of treatment. FGF, EGF and dbcGMP had no effect on morphology, cellular amino acid content or taurine release any time tested.

The reduction of taurine efflux levels and intracellular content by PDBU and dbcAMP, in the face of somewhat transient morphological changes, show that PKC and adenylate cyclase may potentially regulate these phenomena in astrocytes. Supported by NS 20632 and MH 15172.

36.2

MUSCARINIC RECEPTOR-MEDIATED INCREASE IN cAMP LEVELS: EVIDENCE FOR CROSS-TALK BETWEEN TWO SIGNAL TRANSDUCTION PATHWAYS. J. Baumgold. Lab. of Molecular & Cellular Neurobiology, NINCDS, Bethesda, MD 20892.

In contrast to the well described inhibition of adenylate cyclase resulting from muscarinic receptor stimulation, activation of muscarinic receptors in SK-N-SH human neuroblastoma cells resulted in a 2-4 fold increase in cAMP levels. This increase was observed regardless of whether basal, PGE1-stimulated, or forskolin-stimulated levels were measured. It was dose-dependent (EC50 of 6 µM) and atropine-sensitive (IC50 of 20 nM). The EC50 of this cabachol effect was similar to that for carbachol stimulation of phosphoinositide turnover in these cells, suggesting that the former may be mediated by the latter. However, exogenous activation of protein kinase C by phorbol ester treatment did not mimick the carbachol-induced increase in cAMP levels. Furthermore, protein kinase C down regulation did not interfere with this carbachol effect indicating that this effect is not mediated by diacylglycerol activation of protein kinase C. On the other hand, treatment of these cells with the calcium ionophore A23187 resulted in increased cAMP levels. In addition, the calmodulin antagonists TFP, R24571, and W7 all inhibited the carbachol-mediated increase in cAMP levels. These data indicate that the carbachol-mediated increase in cAMP levels is mediated by a calcium-calmodulin dependent process resulting from increased intracellular calcium levels in turn resulting from inositol trisphosphate release.

36.3

cAMP MODULATES INOSITOL POLYPHOSPHATE PRODUCTION AND CALCIUM MOBILIZATION IN NG108-15 CELLS. M.D. Campbell*, S. Subramanian*, M.I. Kotlikoff*, J.R. Williamson* and S.J. Fluharty*. Dept. of Animal Biology and Biochemistry/ Biophysics, Univ. of Penna., Phila., PA 19104 (SPON: R.O. Davies)

In the neuroblastoma x glioma (NG108-15) hybrid cell line bradykinin (BK) receptor stimulation induced a rapid accumulation of inositol polyphosphates (InsPs). $InsP_3$ production was maximal at 15 sec and continued agonist exposure resulted in diminished $InsP_3$ production. BK stimulation of $InsP_3$ was also dose-related with an ED_{50} of 9nM, and was maximal at 1µM. HPLC analysis revealed that $Ins(1,4,5)P_3$ levels increased markedly after receptor activation, as did $Ins(1,3,4,5)P_3$, $Ins(1,3,4)P_3$ and $Ins(1,4)P_2$. BK induced InsPs production was attenuated by pretreatment with forskolin or dibutyryl cAMP. Forskolin also reduced InsPs production elicited by $GTP\gamma S$ or sodium fluoride in membranes. Dose response characteristics for calcium mobilization, as measured with fura-2, were similar to that observed for BK induced $InsP_3$ production, as were inhibitory effects of forskolin. These results suggest that cAMP may inhibit receptor mediated and post receptor stimulation of phospholipase C, as well as the mobilization of intracellular calcium in neuron like cells. Supported by NS 23986, and HL 36150 and Univ. of Penna. Research Foundation.

36.4

THE CHANGES IN THE INTRACELLULAR CALCIUM AND ION CHANNEL ACTIVITY IN THE MUSCARINIC RESPONSE OF SYMPATHETIC NEURON. T. Iijima* and G. Matsumoto*(SPON:T. Shiida.) Electrotechnical Laboratory, Tsukuba, Ibaraki, 305 Japan.

The acetylcholine (ACh) response in an isolated superior cervical ganglion of rabbit largely depends on the applied ACh dose. The versatile signal transduction in this neuron is based on the variety of the ACh receptor (muscarinic) types and of the intracellular signal pathways. Intracellular calcium, which is known to regulate the ion channel activity directly or indirectly, is a key factor in the second messenger system. We investigated the relation between the calcium ion and the ion channel activity with a simultaneous recording of the intracellular calcium concentration ($[Ca^{2+}]_i$) change with a calcium sensitive dye and of the membrane current with whole cell clamping. The low dose of ACh (3 µM) caused 10 - 20 nM increase in $[Ca^{2+}]_i$ and an outward current in the membrane clamped at -60 mV. The high dose of ACh (30 -100 µM) caused a large inward current followed by an outward current and more than 50nM increase in $[Ca^{2+}]_i$. In the absense of Ca^{2+} in the external medium, the inward current and the calcium change were decreased, while the outward current remained. We may explain the results as follows: The low dose of ACh interacts with M1 type receptors and causes the Ca^{2+} release from the intracellular Ca^{2+} store, resulting in the activation of calcium dependent K^+ channel. The high dose of ACh further interacts with M2 type receptors and activates the cation selective channels.

36.5

CALCIUM LOCALIZATION ASSOCIATED WITH *c-fos* INDUCTION IN ACUTELY ISOLATED CA1 NEURONS. J.A. Connor, W.J. Wadman, and H.-Y. Tseng. AT&T Bell Labs., Murray Hill, N.J. 07974.

Acutely isolated CA1 neurons from hippocampus of mature guinea pigs, loaded with the indicator fura-2 in its membrane permeable form, showed a distribution of Ca^{2+} in which levels in the nuclear region were considerably higher than elsewhere in the cell following prolonged stimulation. This gradient developed after a prolonged period (5 to 10 min) in which dendritic Ca^{2+} levels were higher than those in the soma. Ca iontophore, ionomycin, in low Ca saline, abolished the nucleus-zone gradient and brought Ca^{2+} down to low uniform levels (<50 nM). Cells injected with ionic fura-2 did not show the nucleus-zone gradient after stimulation but did show the high dendrite, low soma gradient. Apparently the membrane permeable form of fura-2 is able to enter some cellular compartment near the nucleus and report Ca^{2+} changes there, but the ionic form does not. Loaded by either method, indicator entered and left the nucleus itself freely. Recent reports have demonstrated expression of the *c-fos* protooncogene following periods of intense electrical discharge in neurons of the mammalian CNS (c.f. Morgan, *et al.*, Science, 237:192, 1987). Reasoning that the *in vivo* electrical discharge might be producing similar Ca^{2+} loads to our *in vitro* experiments, and that the nucleus-zone Ca^{2+} elevation might be a signalling event, we examined, by immunostaining for *c-fos* product, individual CA1 neurons that showed the nucleus-zone gradient and compared them with neurons that had low uniform Ca^{2+} levels (<200 nM). Of the cells with high nucleus-zone Ca^{2+}, more that 90% showed nucleus specific staining for *c-fos* product. Low Ca^{2+} cells on the same coverslips remained unstained or else showed weak, diffuse staining. We thank T. Curran of the Roche Inst. for antibodies. Supported in part by F49620 from the AFOSR.

36.6

A CALCIUM-DEPENDENT, SHORT-LIVED, POST-RECEPTOR EVENT MEDIATES PROSTAGLANDIN E_2 (PGE) ACTION ON LHRH (LH- releasing hormone) NEURONS. K.R.Bhasker* and A.Barnea (SPON: R.M. Lebovitz). Depts. OB/GYN & Physiol., Univ. Tx. Southwestern Med. Ctr., Dallas Tx. 75235, U.S.A.

PGE is known to stimulate LHRH release from explants of the median eminence (ME) and copper (Cu) amplifies PGE action. To define the kinetics of Ca requirement for PGE action, ME of female rats were incubated with 150 μM Cu for 5 min in the absence of Ca and then with 10 μM PGE and 1.8 mM Ca for 10 min. LHRH release was 3.6±0.4 pg/5'/ME without PGE and 12±1.2 pg with PGE; this stimulated rate of release was attained within the 1st 5 min of PGE exposure and persisted for another 5 min. That PGE exposure for 2 min sufficed to induce this maximal release response indicates that desensitization to additional PGE action occurred by 2 min. Omission of Ca from the medium during the first 2 min of PGE-exposure did not alter PGE stimulation of LHRH release regardless of whether PGE-exposure lasted for 2 or 7 min. However, omission of Ca for the first 5 min of PGE exposure almost completely inhibited PGE action. Thus, PGE interaction with the LHRH neuron is rapidly (<2 min) manifested and it leads to desensitization. PGE binding to its receptor and the initial post-receptor events do not require extracellular Ca, whereas the subsequent event(s) require Ca and involve a rapidly turning over component.

36.7

ROLE OF INTRACELLULAR CALCIUM AND CELL VOLUME IN HETEROLOGOUS DENERVATION SUPERSENSITIVITY OF MUSCARINIC, α ADRENERGIC AND SUBSTANCE P RESPONSES IN RAT PAROTID ACINAR CELLS. M.K. McMillian and B.R. Talamo, Neuroscience Labs, Tufts Med. Sch., Boston, MA 02111

Parasympathetic post-ganglionic denervation of rat parotid acinar cells produces heterologous supersensitivity to muscarinic, α-adrenergic and substance P agonists, even though the corresponding neurotransmitter is not lost for all (NE is not reduced). Receptor stimulation leads to fluid and electrolyte secretion via activation of phospholipase C (PLC) and mobilization of Ca_i. After denervation, several responses to receptor activation were tested. The concentration-response curve for PLC activation and (^{3}H)inositol phosphate(s) (IP_n) formation was unchanged. However, other agonist-stimulated responses were sensitized. These included Ca_i elevation (quin2 and Fura2) and ^{22}Na uptake, as well as muscarinic stimulation of amylase release, which is mediated via diacylglycerol and C kinase. Denervation also decreased cellular volume and lowered resting Ca_i. These changes, coupled with previous findings that muscarinic receptor levels are not increased, suggest that sensitivity is regulated down-stream from the receptor and receptor-activated PLC. A decrease in surface area and volume under conditions where the number of receptors is unchanged and where each agonist concentration mobilizes the same amount of second messenger (diacylglycerol, IP_n, and hence Ca^{++}), would contribute effectively to shift the stimulation-response curve for elevating second messenger concentrations and therefore sensitize Ca-dependent ion transport and C-kinase stimulated exocytosis. Supported by NIH grant 1F32 DE05489 and NSF grant BNS-8710238

36.8

REGIONAL DISTRIBUTION OF A NON-MITOCHONDRIAL, ATP+Mg^{2+} DEPENDENT CALCIUM-UPTAKE POOL IN RAT BRAIN AND PERIPHERAL TISSUES. S. Supattapone, A. Verma*, C. Ross and S.H. Snyder. The Johns Hopkins University Sch. of Med., Dept. of Neuroscience, Baltimore, MD 21205.

Many neural functions are controlled by the level of free Ca^{++} within neurons. While much work has been done in defining the subcellular distribution and pharmacologic specificity of Ca^{++} sequestering pools, little is known of the histological distribution of these pools in the brain.

We have performed energy dependent uptake of $^{45}Ca^{++}$ by 10 µm rat brain sections and visualized its regional distribution using autoradiography. This $^{45}Ca^{++}$ pool appeared to be non-mitochondrial as it was inhibited by vanadate but not by ruthenium red, rotenone and oligomycin.

Of the organs examined only neuronal and smooth muscle tissues sequestered significant amounts of $^{45}Ca^{++}$. Relatively high levels were observed in the frontal cerebral cortex, striatum, thalamus, substantia nigra, subiculum, and molecular layer of the cerebellum. Low levels of accumulated radioactivity were seen in the hypothalamus and brain stem.

36.9

STUDIES ON NEUROTRANSMITTER INTERACTIONS ON CULTURED HIPPOCAMPAL NEURONAL CELLS — FROM VIEWPOINTS OF INTRACELLULAR CA ION. S. Kito, R. Miyoshi and M. Shimizu*. Third Dept. of Internal Med., Hiroshima Univ. School of Med., Hiroshima 734, Japan.

We have been studing on interaction between muscarinic acetylcholine receptors and somatostatin (SS) in the rat hippocampus through radioreceptor assay and measurement of phosphatidylinositol turnover. In the present study, cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) was examined by fura-2 fluorometry in cultured rat hippocampal neurons. Neurons were obtained from 18-day rat embryos and were cultured for 12 days. Changes of $[Ca^{2+}]i$ were monitored using a system in combination of fura-2, a fluorescence microscope, a video-camera and photometrical devices. Acetylcholine (ACh) of 10^{-5} M induced an increase of $[Ca^{2+}]i$, and heterogeneity in the responsiveness to ACh was observed in each cell. SS of 10^{-5} M also increased $[Ca^{2+}]i$. Addition of both ACh and SS induced an elevation more than the case of either ACh or SS alone. When 30 μM $LaCl_3$ was infused to block Ca^{2+} influx from extracellular fluids, the responses to ACh, SS and both were partially depressed with preservation of responses toward the same direction. Under this condition, an effect of KCl was abolished. These results suggest that the interaction between ACh and SS on $[Ca^{2+}]i$ occurs through both Ca^{2+} influx due to the opening of ion channels and mobilization of intracellularly stored Ca^{2+}.

36.10

PERTUSSIS TOXIN INDUCES ALTERATIONS IN SEPTO-HIPPOCAMPAL NEURONS PROPERTIES. P.Dutar, O.Rascol* and Y.Lamour. INSERM U.161, 2 rue d'Alésia, 75014, Paris, France.

The properties of septo-hippocampal neurons (SHNs) have been studied in vivo in rats pretreated with pertussis toxin (PTX)(n=12, urethane anesthesia). PTX (2µg) was injected bilaterally in cerebral ventricules 3 days prior to the experiment. Extracellular recordings coupled to iontophoresis were obtained from single neurons located in the medial septum, identified as projecting to the hippocampus by their antidromic response to the electrical stimulation of the fimbria-fornix. In PTX pretreated rats, the mean latency of the antidromic response was unchanged (1.4±1.3, n=121 vs 1.5±1.2ms, n=95). In contrast, the mean spontaneous activity was significantly higher (21.6±17.3, n=117 vs 13.2±11.6 spikes/sec., n=89) and the mean frequency of the rhythmic bursting activity was lower (2.7±0.4, n=42 vs 3.6±0.5Hz, n=40). The percentage of rhythmically bursting neurons was not different. Pharmacological properties of SHNs were differentially affected by PTX. Responses to acetylcholine (n=13), carbachol (n=51) and GABA (n=54) were unchanged. In contrast serotonin (n=28) and baclofen (n=83) -induced inhibitions were blocked.

In conclusion, these results provide evidence for a selective involvement of a PTX-sensitive G-protein in several physiological and pharmacological properties of septal neurons projecting to the hippocampus.

36.11

KAPPA OPIATE AGONISTS INHIBIT Ca^{2+} INFLUX AND INDUCE HETEROLOGOUS DESENSITIZATION IN RAT SPINAL CORD-DORSAL ROOT GANGLION COCULTURES: INVOLVEMENT OF G PROTEINS. Z. Vogel, D. Saya* and B. Attali*, Dept. of Neurobiology, Weizmann Institute of Science, Rehovot, Israel.

Basal $^{45}Ca^{2+}$ uptake in rat spinal cord-dorsal root ganglion (SC-DG) cocultures was potently stimulated (3.5-fold) by depolarization with 50 mM K^+ and the Ca^{2+} channel agonist Bay K8644. κ selective agonists such as U50488, tifluadom, dynorphin and MR2034 profoundly depressed the stimulated Ca^{2+} uptake, while μ (DAGO) and δ (DADL) agonists had no effect. κ agonist action was stereospecific and reversed by antagonists. Pertussis toxin pretreatment strongly affected the κ agonist inhibition of Ca^{2+} influx, suggesting that κ receptors are negatively coupled to voltage-dependent Ca^{2+} channels via a pertussis toxin-sensitive G protein. Chronic exposure of SC-DG to U50488 (10μM, 24h) desensitized the κ agonist inhibitory action. Moreover, this treatment desensitized the inhibition of Ca^{2+} influx observed with α2 adrenergic and muscarinic agonists (heterologous desensitization). The amount of a 41 kDa pertussis toxin substrate protein was decreased by 50 to 90% in U50488-treated cells, as compared to control. Using selective antibodies (kindly provided by Dr. A. Spiegel, NIH) we found a 30 to 80% down-regulation of the α subunit of Gi protein with no changes in Gαs, GαO and Gβ. Results suggest that the κ agonist-induced down-regulation of Gαi underlies the development of tolerance and cross-tolerance observed in spinal cord, following chronic exposure to opiates.

36.12

CALCIUM (Ca) AND GUANINE NUCLEOTIDE(G) REGULATION OF GABAb BINDING IN RAT CEREBRUM. R.H.Thalmann and M.I.Al-Dahan*
Baylor College of Medicine,Houston,TX77030

Using permeabilized(.05%Triton) thoroughly washed synaptic membranes, we confirmed that either Ca or G(GTPγs) controlled more than 90% of specific GABAb(^{3}H)--baclofen) binding. We then tested the hypothesis that the Ca effect might indeed depend upon a G protein. However: 1)Scatchard analysis indicated that Ca primarily affected Bmax while others had found that G primarily affected Kd; 2)Between postnatal day 1 and adulthood, the EC50 for Ca stimulation of binding remained constant at 1uM while that for the inhibition of binding by GTPγs declined from 1uM to 2.5nM; 3) Our permeabilized extensively washed membranes were probably unfavorable for phosphorylation by G protein dependent kinases, and 4) This Ca binding site does not so far behave as at least one Ca site that is known to be coupled to GABAb receptors via G proteins, namely the Ca channel.,e.g., 10uM nifedipine or 0.2uM w-conotoxin failed to antagonize the stimulation of GABAb binding by Ca. Although none of these experiments are themselves conclusive, none support the hypothesis. Thus Ca and G may regulate GABAb receptors by independent mechanisms and indeed we suggest that Ca may regulate the receptor directly, by a site on the receptor itself.
Supported by NIH Grant 21713.

36.13

INTERACTION OF PYRETHROID TOXINS WITH GTP-BINDING PROTEINS. **D.P. Rossignol*. (spon: J.C. Kauer). E.I. du Pont de Nemours & Co., Experimental Station, Wilmington, DE 19898**

Insecticidal pyrethroid neurotoxins have been shown to alter a variety of membrane receptors and enzymes including sodium channels, calcium channels, GABA-b (baclofen) receptors, and Na^+/K^+ and Ca^{++} Mg^{++}ATPases. In order to study the mechanism of pyrethroid activity, I have synthesized [3H]DeCAF (decyanoazidofenvalerate), a photoactivatable arylazide derivative of fenvalerate. SDS-PAGE analysis of rat brain membranes indicated that [3H]DeCAF covalently labeled a 36kDa membrane-bound protein. Labeling was sensitive to GTPγS and other G-protein modifiers. To determine if pyrethroids bind to GTP-binding proteins, DeCAF binding was studied in the bovine retinal transduction system. In crude retinal homogenates, a 36kDa protein was specifically labeled. This labeling was enriched five-fold in rod outer segments (ROS) and was nearly quantitatively solubilized (>95%) by treatment of ROS with 40uM GTP. Labeling was 1700-fold higher in the GTP-solubilized proteins than the pellet. It is likely that [3H]DeCAF covalently labels the β subunit of transducin because SDS-PAGE showed the [3H]-labeled protein to be 36kDa, distinct from [32P]-ADP-ribosylated α subunit and [3H]DeCAF-labeled protein was specifically immunoprecipitated by antibodies directed against either the β subunit of transducin or a synthetic peptide of β subunit (Mumby et al. PNAS 83,265-269). These experiments indicate that DeCAF binds to the β subunit of retinal GTP-binding proteins.

36.14

THE INTERACTION OF Gp WITH GUANINE NUCLEOTIDES. A. Tamir*, A. B. Fawzi* and J. K. Northup* (SPON: S. Amara). Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510

Gp is a major GTP-binding protein of human placental membranes purified by Evans et al., (1986) J. Biol. Chem. 261,7052-7059. This protein was also purified from human platelets and bovine brain. High affinity guanine nucleotide (GN) binding is associated with a protein that has identical migration on SDS-PAGE to H-*ras*. We characterized the interaction of Gp with guanine nucleotides by studing the thermodynamics as well as the kinetics of GN binding to Gp. Using [^{3}H] GppNHp we determined an apparent K_d of 0.2 μM. Apparent K_d values for other GNs were derived from EC_{50} for displacement of [^{3}H] GppNHp. These were 4.2 nM for GTPγS, 0.9 μM for GDPβS, 0.1 μM for GTP and 0.1 μM for GDP. [^{3}H] GppNHp, [^{3}H] GTP, and [^{35}S] GTPγS all identified the same site. The rate of GN binding to the placental Gp is much faster than that of the protein purified from brain. The former occurred on ice as well as at 30 and 37 ^{0}c, but did not occur in the absence of magnesium. Gp hydrolyzed [^{3}H] GTP with [^{3}H] GDP remaining tightly bound. Apparent hydrolytic rate of 0.03 $min^{-1}$was observed. The protein from the placenta and from the brain but not from the platelets copurifies with the βγ- subunits common to the identified G-proteins. Initial data suggest a functional interaction of the α subunit of placental Gp with the βγ complex that regulates GN affinity. These biochemical criteria support the inclusion of Gp in a family of signal transducing GTP-binding proteins.

36.15

MODULATION OF G-PROTEIN mRNA LEVELS IN NEUROBLASTOMA X GLIOMA HYBRID NG108-15 CELLS BY ADP-RIBOSYLATING TOXINS. E. A. Thiele*, S. H. Snyder, & K. M. Braas. (Sponsor: S. M. Sato) Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Guanine nucleotide regulatory proteins (G-proteins) are important in signal transduction mechanisms. G-proteins are heterotrimers consisting of α, β, and γ subunits. Membrane receptors act through G_s to stimulate adenylate cyclase activity, and G_i to inhibit adenylate cyclase activity. Cholera toxin and pertussis toxin catalyze the ADP-ribosylation of the α_s and α_i subunits, respectively, and both result in the activation of adenylate cyclase activity. Using cDNA probes to the α_s, α_{i2}, and β_1 subunits (Jones and Reed, J. Biol. Chem. 262:14241, 1987) we have examined by Northern analysis the regulation of G protein mRNA levels in NG108-15 cells after toxin treatment. In NG108-15 cells, we identified single mRNA spiecies for α_s with an apparent size of 1.8 kb, and α_{i2} of 2.2 kb. Two mRNA species were observed with the cDNA probe to the β_1 subunit, with apparent sizes of 3.0 and 1.6 kb. Treatment of NG108-15 cells with cholera toxin or pertussis toxin resulted in a decrease in G protein subunit mRNA levels. The relationship of intracellular cAMP levels and G-protein subunit mRNA levels following toxin treatment for different times is being investigated.

36.16

GTPase ACTIVITY IN NORMAL HUMAN MYELIN. *Chi Kin Chan*J. Ramwani, and*M.A. Moscarello (SPON: F. Coceani) Dept. of Biochemistry, Research Institute, The Hospital for Sick Children, Toronto, Ontario M5G 1X8

Myelin, a multilamellar membrane surrounding the axon of both CNS and PNS of vertebrates, is considered to function as an insulator. In the past decade, studies have shown that it is metabolically active and perhaps involved in cellular regulation by extracellular hormones and/or neurotransmitters. Myelin has been shown to consist of the following transmembrane signalling components: phosphophatidylinositol phosphates, phospholipase C, protein kinase C, adenylate cyclase, and muscarinic cholinergic receptors. Recently, we have demonstrated that myelin basic protein (MBP) specifically binds to GTP (Chan et al BBRC in press). It can be ADP-ribosylated by cholera toxin and also can be covalently labelled by azido-GTP at Gln residue of the N-terminus of MBP. In this communication, data are presented to show a GTPase activity in myelin. This enzyme activity is specific for GTP and has been shown to be inhibited by cholera toxin. Hydrolysis of GTP to GDP is time dependent with very little GMP formation. Immunoprecipitation of MBP from detergent extract of myelin by a polyclonal antibody caused a 50% reduction in myelin GTPase activity. Isolation of myelin GTPase is in progress to establish the relationship, if any, between MBP and GTPase activity in myelin.
In conclusion, although the enzyme has not been identified and isolated, MBP could be a factor that interacts with myelin G protein(s) to regulate the enzyme activity.

36.17

APPARENT LOSS OF Gi PROTEIN ACTIVITY IN DIABETIC RETINOPATHY
M. Hadjiconstantinou, Z.-X. Qu,* S.E. Moroi-Fetters,* G.-Y. Le,* and N.H. Neff. Depts. Pharmacology and Pathology, The Ohio State Univ. Col. Med., Columbus, OH 43210.

Recently it was reported that Gi protein is deficient in the liver of streptozotocin (STZ)-induced diabetic rats and that insulin treatment partially restores Gi protein activity (Gawler et al., Nature 327:229, 1987). We have evaluated whether the loss of Gi protein is a more generalized phenomenon involving receptor Gi protein-mediated signal transduction in neuronal tissue. Retinopathy is a common feature of diabetes, therefore we chose the retina for our studies. Stimulation of retinal muscarinic (M1) receptors results in inhibition of adenylate cyclase activity. Intraocular administration of pertussis toxin prevents this response suggesting that this is a Gi protein mediated activity. Rats were treated with STZ (85 mg/kg, i.p.) or saline and 3 weeks later acetylcholine (ACh)-induced inhibition of cAMP accumulation in rat retina measured. In control animals, ACh induced about a 30% decrease of cAMP accumulation. In diabetic animals, basal cAMP accumulation was elevated and ACh was unable to reduce it. In another experiment, insulin treatment, NPH (porcine) 2 U/100 g, was initiated 3 days after the induction of diabetes and continue for 14 days. Insulin treatment restored the ability of ACh to inhibit the accumulation of cAMP in diabetic retina. Our studies suggest that loss of Gi protein activity in diabetes may be a universal response which might be related to the extensive pathologies associated with the disease.

36.18

SECOND MESSENGER PATHWAYS INVOLVED IN THE REGULATION OF SUBSTANCE P IN RAT SYMPATHETIC NEURONS.
N.E. Kremer and J.A. Kessler. Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

The expression of the preprotachykinin (PPT) gene and of substance P (SP) in sympathetic neurons is influenced by the neuronal microenvironment. We have investigated the role of second messenger pathways in the regulation of SP expression in cultured sympathetic neurons of the neonatal rat superior cervical ganglion. Pure neuronal cultures contained no detectable PPT mRNA or SP peptide. Coculture of neurons with ganglion non-neuronal cells, however, induced expression of substantial levels of both the mRNA and the peptide. Potassium- or veratridine-induced depolarization reduced SP and PPT mRNA in cocultures to barely detectable levels. The role of calcium as a second messenger was examined by using the calmodulin inhibitor R24571. Treatment of cocultures with R24571 elevated levels of SP. Further, addition of R24571 reversed the effects of depolarization by KCl; R24571 at 0.3 μM partially restored SP levels, while R24571 at 1 μM raised SP to above control levels.

The role of protein kinase C was evaluated by examining effects of treatment with phorbol myristate acetate (PMA). PMA treatment did not induce expression of PPT mRNA or SP in pure neuronal cultures. However, treatment of cocultures with PMA resulted in a dose-dependent increase in peptide and PPT mRNA levels. Simultaneous treatment with PMA and KCl reduced SP to negligible levels. Thus PMA, unlike R24571, could not reverse the effects of potassium-induced depolarization. None of these or other manipulations of second messenger pathways induced expression of SP in pure neuronal cultures, raising questions about which second messenger mediated effects of nonneuronal cells. Once SP expression was induced by nonneuronal cells, there appeared to be a hierarchy of second messenger signals which modulated peptide levels.

SECOND MESSENGERS III

37.1

CALCIUM AND SODIUM DEPENDENCY OF PHOSPHOINOSITIDE HYDROLYSIS IN RAT CEREBRAL CORTICAL SYNAPTONEUROSOMES.
L. J. Chandler* and F.T. Crews. (SPON: W.R. Kem). Dept of Pharmacology, Univ. of Florida College of Medicine, Gainesville, FL 32610.

To more fully understand the role of calcium and sodium in phosphoinositide hydrolysis in brain, we examined potasium and norepinephrine-stimulated phosphoinositide hydrolysis in rat cerebral cortical synaptoneurosomes in the presence and absence of sodium and in the presence of various concentrations of calcium or EGTA. In medium containing 1 mM calcium and 96 mM sodium, the addition of 30 mM KCl caused a large increase in the accumulation of [^{3}H]-inositol phosphates. The fastest rate of accumulation occurred within the first 5 min of depolarization and reached a maximum after 40 min. Norepinephrine-stimulated phosophoinositide hydrolysis showed a similar time-course of accumulation of [^{3}H]-inositol phosphates. Basal, potasium- and norepinephrine-stimulated accumulation of [^{3}H]-inositol phosphates decreased upon a decrease in extracellular calcium. Both basal and stimulated accumulation was almost completely eliminated by the addition of EGTA in concentrations as low as 50 μM. Isosmotically replacing sodium with choline chloride caused a large increase in the basal accumulation of [^{3}H]-inositol phosphates that was almost twice that caused by either KCl or norepinephrine in the presence of sodium. The removal of sodium completely eliminated potasium stimulated but not norepinephrine stimulated accumulation of [^{3}H]-inositol phosphates. Basal accumulation in the absence of sodium decreased with decreasing amounts of added calcium and by the addition of EGTA (0.025 to 1 mM), but remained quite large even in the presence of 1 mM EGTA. This data will be discussed in relation to the effects of sodium and calcium removal upon the intracellular free calcium concentration measured with the calcium sensitive fluorophore fura-2. It is hypothesis that removal of sodium causes an increase in intracellular calcium in the absence of sodium-calcium exchange and this elivation of intracellular calcium stimulates phosphoinositide hydrolysis. (Supported by NIAAA Grant # AA06060.)

37.2

INOSITOL PHOSPHATES, GTP AND CAFFEINE RELEASE CALCIUM FROM BRAIN MICROSOMES.
L.C. Daniell and R.A. Harris. Dept. Pharmacology and VA Med. Ctr., Univ. Colorado Health Sciences Center, Denver, CO. 80262.

The effects of various calcium releasing agents on intracellular calcium stores were studied in mouse whole brain microsomes. Calcium release was monitored by determination of the extra-microsomal calcium concentration using Indo 1, a fluorescent calcium indicator. Inositol 1-phosphate (1-30 uM) and inositol 1,4-bisphosphate (3 uM) did not release calcium from microsomes. Inositol 1,4,5-trisphosphate (IP_3)-induced calcium release exhibited two components. One component was dependent on prior ATP-stimulated calcium loading and had an EC_{50} of about 100 nM. A second component of IP_3-induced calcium release was detected in the absence of ATP-dependent calcium loading (EC_{50} approximately 30 uM). GTP (1-10 uM) released a small amount of calcium and caffeine (1-3 mM) released a larger amount of calcium. Work is currently in progress to identify and characterize calcium stores of brain microsomes. Supported by the VA administration and grants GM11905, AA06399 and AA03527.

37.3

STIMULATION OF Ca^{2+} REUPTAKE AFTER INOSITOL 1,4,5-TRISPHOSPHATE INDUCED Ca^{2+} RELEASE FROM RAT CEREBELLAR MICROSOMES.
K.A. Stauderman. Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215.

The potential role of inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) to regulate intracellular Ca^{2+} fluxes in neuronal tissue was investigated with rat cerebellar microsomes as the model system. Using fura-2 fluorescence to monitor changes in extramicrosomal free Ca^{2+} concentration ($[Ca^{2+}]_e$), the microsomes accumulated Ca^{2+} in the presence of 500 µM ATP and an ATP-regenerating system. $Ins(1,4,5)P_3$ stimulated Ca^{2+} release in a concentration-dependent manner, producing half-maximal Ca^{2+} release at 60 nM after taking into account rapid metabolism of $Ins(1,4,5)P_3$. Following $Ins(1,4,5)P_3$-induced Ca^{2+} release there was a prompt reuptake of Ca^{2+} into the $Ins(1,4,5)P_3$-sensitive compartment, as judged by the lack of desensitization to subsequent additions of $Ins(1,4,5)P_3$. By measuring the rate of change of $[Ca^{2+}]_e$ as an index of Ca^{2+} uptake, the rate of Ca^{2+} reuptake after $Ins(1,4,5)P_3$-induced Ca^{2+} release was greater than after additions of Ca^{2+} standards, suggesting that Ca^{2+} reuptake was stimulated in the former case. The ability to detect this effect depends on whether $Ins(1,4,5)P_3$ can be metabolized faster than the rate of Ca^{2+} uptake into $Ins(1,4,5)P_3$-insensitive compartments. Ions other than Ca^{2+} may be involved in the process that stimulates Ca^{2+} reuptake.

37.4

PURIFICATION AND CHARACTERIZATION OF INOSITOL-1,4,5-TRIPHOSPHATE BINDING SITES IN RAT PERIPHERAL TISSUE.
R.J. Mourey*, S. Supattapone and S.H. Snyder. Dept. of Pharmacology and Molecular Sciences, Dept. of Neuroscience, Johns Hopkins University Sch. of Medicine, Baltimore, MD 21205.

An inositol-1,4,5-triphosphate (IP_3) receptor from rat cerebellum has recently been purified (Supattapone, S., et al., JBC, 263:1530, 1988). We were interested in identifying and characterizing IP_3 receptor from other rat tissues. In a screen of rat tissue, lymphoid organs and renal cortex provided the second most abundant source of [^{3}H]IP_3 binding.

We have purified the IP_3 binding site from rat spleen and thymus utilizing heparin-agarose and concanavalin A-sepharose affinity chromatography. The spleen and thymus IP_3 receptor are similar to the cerebellar IP_3 receptor, having high affinity for 1,4,5-IP_3 (K_d = 40 nM), and binding that can be inhibited by calcium, heparin and N-ethyl maleimide. However, the spleen and thymus IP_3 receptor differ from the cerebellar IP_3 receptor, both in molecular weight and cross-reactivity with anti-cerebellar IP_3 receptor antibodies. Interestingly, the kidney IP_3 receptor differs from the cerebellar receptor by having lower affinity for IP_3 (K_d = 400 nM), 1,3,4,5-IP_4 and IP_6 compete for IP_3 binding, it does not bind to concanavalin A-sepharose and it is resistant to N-ethyl maleimide.

37.5

STIMULATION OF DIACYLGLYCEROL FROM SOURCES OTHER THAN INOSITOL CONTAINING LIPIDS. M. S. Pessin* and D. M. Raben* (SPON: M. Steinmetz). Department of Physiology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

α–Thrombin stimulates an increase in the mass of cellular diacylglycerol (DAG) in quiescent IIC9 fibroblasts. This generation is biphasic when stimulated by a high concentration of α–thrombin (500 ng/ml), with an early phase peaking at 15 seconds and a late phase peaking at 5 minutes. In addition, the DAG levels are sustained for at least 1 hour. We have analyzed the molecular species of DAGs generated 15 seconds, 5 minutes, and 1 hour after stimulation with α–thrombin and compared them to those contained in control, quiescent cultures. The data demonstrate that while the 15 second peak is temporally related with the hydrolysis of inositol lipids, these lipids contribute a small fraction of the total DAGs generated at this time. At later times the DAGs appear to be derived almost entirely from non-inositol containing lipids. (Supported by BRSG from JHU and GM0730 from NIH.)

37.6

EFFECTS OF PHENYLETHYLAMINE & NORADRENALINE ON POLYPHOSPHOINOSITIDE TURNOVER IN RAT CEREBRAL CORTEX. A.A.Boulton and L.E.Dyck (SPON: J.R.Doucette). Neuropsychiatric Research Unit, Dept. of Psychiatry, University of Saskatchewan, Saskatoon, Sask. Canada S7N 0W0

The trace amine, phenylethylamine,can excite or inhibit rat cortical neurons indirectly. In contrast, phenylethylamine can enhance the effects of noradrenaline (NE) directly, when it is applied simultaneously with NE. NE can alter neuronal excitability by stimulation of the α_1-adrenergic receptor which activates phospholipase C increasing polyphosphoinositide turnover (PIT) to produce the second messengers, inositol triphosphate and diacylglycerol. The ability of NE and phenylethylamine to stimulate PIT in slices of rat cerebral cortex was examined by using a direct assay which involves labelling with ^{3}H-inositol and assaying ^{3}H-inositol phosphates in the presence of lithium. 2.6 to 10 mM Phenylethylamine significantly stimulated the formation of ^{3}H-inositol phosphates (to 120 - 215 % of control), while 26 - 2600 μM PE dose-dependently inhibited (0.1 mM) NE-stimulated PIT. I mM NE was not inhibited by phenylethylamine. The effects of phenylethylamine & NE were inhibited by prazosin. Thus, phenylethylamine appears to stimulate basal formation of ^{3}H-inositol phosphates and to inhibit NE-induced formation of ^{3}H-inositol phosphates by acting as a partial α_1-agonist. (Supported by the M.R.C.)

37.7

CHANGES IN PHOSPHATIDYLINOSITAL CONCENTRATION AND MOTOR ACTIVITY ASSOCIATED WITH DENERVATION SUPERSENSITIVITY. C.A. Harrington and P.B. Silverman. Abbott Laboratories, Abbott Park, IL 60064 and Univ. of TX Ment. Sci. Inst., Houston, TX 77030.

The biochemical changes associated with post synaptic dopaminergic supersensitivity are poorly understood. Although receptor proliferation can be quantified after loss of presynaptic input, the time course increased receptor number and the behavior associated with denervation are significantly different. We have hypothesized that the changes in behavior may more closely parallel changes in inositol lipid metabolism. In order to test this hypothesis we injected intraventricularly two groups of mice with 6-hydroxydopamine (6 HDA) or vehicle. Mice were anesthesized with 400mg/kg chloral hydrate. Four days and eight days post injection motor activity was measured in both groups after administration of 5mg/kg apomorphine. The morning of the 10th day post lesion all animals were sacrificed and striata dissected from each brain and frozen on dry ice. Each pair of striata were divided at random, for catecholamine and phosphatidylinositol analysis.

Motor activity was increased 4 fold and 2.0 fold above control at 4 and 8 days respectively post lesion. Dopamine concentration was decreased to 40% of control values in animals given I.C.V. 6 HDA. The mass of phosphatidylinositol was increased 15% ($p < .05$) in the animals treated with 6 HDA. Therefore the time course of increases in motor activity, depletion of dopamine, and increased in phosphatidylinositol concentration may be related.

37.8

PHOSPHATIDYLINOSITOL TURNOVER IS PROPORTIONAL TO THE LEVEL OF EXPRESSION OF THE RAT M1 MUSCARINIC RECEPTOR GENE. D.T. Dudley*, C.R. Clark, F.Z. Chung*, C.M. Fraser* and J.C. Venter*, Parke-Davis Pharmaceutical Research, Ann Arbor, Mi 48105 and Section of Receptor Biochemistry and Molecular Biology, NINCDS, NIH, Bethesda, Md 20892.

To examine the second messenger coupling of muscarinic receptor subtypes, we have examined murine B82 cells transfected with, and expressing rat M1 muscarinic receptors (clone M1C2) and compared them with the previously characterized clones cTB9 and cTB10 (J. Lai *et al.* Life Sci., in press). As determined by [^{3}H](-)QNB binding to membranes, cTB9 cells expressed 64 fmol/mg protein and cTB10 cells expressed 170 fmol/mg protein (J. Lai, *et al.*), while M1C2 cells expressed 800 fmol/mg protein. The parent B82 cell exhibited no [^{3}H](-)QNB binding. In M1C2 cells labeled to equilibrium with [^{3}H]inositol and stimulated with 1 mM carbachol (CCh), there was a rapid increase in inositol trisphosphate as well as the tetrakis-, bis- and monophosphates. No effect of stimulation was seen on inositol pentakis- or hexakisphosphates. For routine studies, total [^{3}H]inositol phosphate (IP) accumulation in the presence of 10 mM Li^+ was monitored. CCh stimulated accumulation of IP in all three cell lines, with EC_{50} values of 42.3, 39.2 and 41.3 μM for M1C2, cTB9 and cTB10 cells, respectively. However, substantially more IP accumulated in M1C2 cells (10.1 fold) as compared to cTB9 (2.0 fold) and cTB10 cells (1.9 fold). These data indicate that expression of an appropriate foreign receptor protein in these cells can lead to the complete appearance of the phosphatidylinositol signaling pathway, and additionally suggest that the magnitude of the signal produced is proportional to the level of receptor present.

37.9

CARBACHOL STIMULATES INOSITOL-PHOSPHOLIPID METABOLISM IN PC12 CELLS BY MULTIPLE MECHANISMS. J. Horwitz* (SPON: N. Smalheiser). Pediatrics and Kennedy Mental Retardation Res. Ctr., The University of Chicago, Chicago, IL 60637

Muscarinic agonists are known to increase the hydrolysis of inositol containing phospholipids in PC12 pheochromocytoma cells. Here I report that activation of nicotinic receptors also increases inositol-phospholipid metabolism in these cells. The specific nicotinic agonist dimethylphenylpiperazinium and the mixed agonist carbachol both increased [^{3}H]inositol monophosphate ([^{3}H]IP_1) and diacylglycerol (DG) formation. The effect of carbachol was partly blocked by the muscarinic receptor antagonist N-methylscopolamine and was also partly blocked by the nicotinic receptor antagonist, mecamylamine. Activation of muscarinic receptors caused a two-fold greater increase in [^{3}H]IP_1 than did activation of nicotinic receptors. In contrast, nicotinic and muscarinic receptor stimulation had comparable effects on diacylglycerol formation. To understand the relationship between [^{3}H]inositol phosphate and DG formation I studied the time course of the effect of carbachol. [^{3}H]I-1,4,5-P_3, as measured by HPLC, was elevated at 15 sec and then began to slowly fall so that by 5 min levels had dropped by 50%. DG levels, in contrast, did not change for the first two minutes and then peaked at 5 min. In summary, the effect of carbachol on phospholipid metabolism is mediated by both nicotinic and muscarinic receptors. Phosphatidyl-inositol-4,5-bisphosphate is not the major source for the DG formed during carbachol stimulation. (Supported by NS-22694 and HD-04583)

37.10

CHARACTERIZATION OF RECEPTOR-STIMULATED TURNOVER OF INOSITOLPHOSPHATES IN THE RAT HIPPOCAMPUS AFTER COLCHICINE TREATMENT. P. Tandon, G.J. Harry, J. Peterson*, and H.A. Tilson. LMIN, NIEHS, P.O. Box 12233, Res. Tri. Pk., NC.

To study the effect of a neurochemical lesion on the muscarinic cholinergic system (mAch), colchicine was stereotaxically injected into the dorsal and ventral hippocampus of rats at a dose of 2.5 ug/site. The animals were sacrificed 1, 3 or 12 weeks after injection. Brains were removed and the hippocampus dissected out. Phosphoinositol (PI) hydrolysis was studied in hippocampal slices after the stimulation of mAch by carbachol. Colchicine was found to increase the sensitivity of the mAch to different concentrations of carbachol. Colchicine also produced an increase in the turnover of inositolphosphates (IPS) in hippocampal slices resulting in a significant increase in IPS released after receptor stimulation, together with an increase in ambient levels of IPS in the hippocampal slices. This effect was found to be time-dependent. The hyperstimulation of the IP metabolism was receptor mediated as mAch antagonists blocked the effect. The increased release of IPS was specific to mAch stimulation, as acetylcholine produced the same effect, while serotonin did not. These results suggest that colchicine alters the signal transduction process of the mAch and that these alterations are linked to the compensatory changes occurring after degeneration of the CNS.

37.11

CLONING, EXPRESSION AND DELETION ANALYSIS OF THE MOUSE M1 mAChR in Y1 ADRENAL CARCINOMA CELLS. R. A. Shapiro*, N. M. Scherer* and N. M. Nathanson. Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195.

We have cloned the gene encoding the mouse M1 muscarinic acetylcholine receptor and inserted it in an expression vector under the control of the zinc-inducible metallothionein promotor. When transfected into the Y1 adrenocarcinoma cell line, functional M1 receptor is produced in response to added zinc. The receptor is able to stimulate large increases in total inositol phosphate (InsP) production in response to the agonist carbachol but does not inhibit adenylate cyclase activity.

Mutations have been constructed that delete various portions of the putative third cytoplasmic loop, and the effects of these mutations on InsP metabolism and down regulation are being examined. 30% of the amino terminal portion of this loop can be deleted with no effect on agonist binding and minimal effect on coupling to InsP metabolism. Deletion of 75% of the loop results in substantial reduction in the ability of the receptor to stimulate InsP turnover. The deletions do not affect the affinity of the mutant receptors for agonist.

37.12

CNS RECEPTOR-COUPLED PHOSPHOINOSITIDE (PPI) HYDROLYSIS A.S. Chiu*, P.P. Li and J.J. Warsh. Clarke Institute of Psychiatry, Toronto, Ontario, CANADA M5T 1R8.

Understanding the mechanisms involved in brain receptor-coupled PPI hydrolysis is incomplete. We report here membrane preparation and incubation conditions combined with HPLC assay of inositol polyphosphates which permit more detailed characterization of receptor-PPI coupling mechanisms. Brain membranes were prepared from homogenates of rat cerebral cortical slices prelabelled with [^{3}H]myo-inositol. Carbachol (100 μM) induced a small increment in inositol bisphosphate (IP_2) (142% of control) formation in brain membranes without affecting insoitol monophosphate (IP) or inositol trisphosphates (IP_3). IP_2 accumulation was increased by 100 μM of GTP, GTPγS and GppNHp (142,520, and 220%, respectively). Carbachol-induced IP_2 accumulation was markedly potentiated by GTPγS and GppNHp (820 and 300%, respectively). Atropine (10 μM) inhibited the GTPγS potentiation of carbachol on IP_2 accumulation. IP and IP_3 did not show significant changes compared to controls for any of the above treatments. Rapid hydrolysis of exogenous [^{3}H]IP_3 to [^{3}H]IP_2 by the membrane preparation suggests IP_2 likely derives from metabolism of IP_3 and reflects agonist-induced hydrolysis of phosphatidylinositol 4,5-bisphosphate.

These data indicate that muscarinic receptor coupling to PPI hydrolysis remains intact in this cell-free preparation and provide evidence of GTP-dependent modulation of receptor-coupled PPI hydrolysis in CNS.

37.13

SIGMA RECEPTORS NEGATIVELY MODULATE AGONIST-STIMULATED PHOSPHOINOSITIDE METABOLISM IN BRAIN SYNAPTONEUROSOMES. B.N. Kirschner*[1], S.B. Hellewell*[1], A.H. Newman*[2], K.C. Rice*[2], and W.D. Bowen[1]. [1]Div. of Biology and Medicine, Brown University, Providence, RI 02912 and [2]NIDDK, Bethesda, MD 20892.

Several transmitters have been shown to mediate their effects by stimulating phosphoinositide (PI) turnover. We investigated whether effects of sigma ligands could be mediated by this system. Rat brain synaptoneurosomes were prepared and assayed for inositol-1-phosphate (IP_1) formation by the method of Gusovsky and Daly (Neuropharmacol. 27:95, 1988). (+)-Pentazocine, haloperidol, and DTG had no effect on PI turnover in the concentration range of 1-300 μM. However, above 300 μM, these ligands produced a slight (approximately 20%) inhibition of basal activity. This observation led to an investigation of the ability of sigma ligands to modulate stimulation of PI turnover by receptor agonists known to be coupled to this system. At 100 μM, the muscarinic cholinergic agonist carbachol produced a 2-fold increase in IP_1 formation. This value was set to 100% and the ability of various concentrations of sigma ligands to modulate this effect was determined. Sigma ligands produced a concentration-dependent attenuation in the ability of carbachol to stimulate PI turnover. The ED_{50} values correlated with binding affinities at sigma receptors (as determined in guinea pig brain). ED_{50} values (μM) were: (+)-pentazocine, 8.3; DTG, 25; dextrallorphan, 27; haloperidol, 35.4; and levallorphan, 155. (+)-Opiates lacking affinity for sigma receptors had no effect. Similar results have been obtained with norepinephrine-stimulated PI turnover. These results suggest that sigma receptors are coupled to an intracellular mechanism which negatively regulates some component(s) of the PI signalling system. Therefore, agonists at sigma receptors may play a neuromodulatory role in vivo by altering the efficacy of PI agonists. Effects of sigma ligands on the efficacy of other PI agonists will be discussed. (Supported by NIDA Grant #DA03776 and the Dystonia Medical Research Foundation.)

37.14

GLUTAMATE INHIBITS NOREPINEPHRINE-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS. X. Li* and R.S. Jope, Dept. of Pharmacology, Univ. of Alabama, Birmingham, AL 35294

Inhibition by excitatory amino acid agonists of norepinephrine (NE)-stimulated phosphoinositide hydrolysis was studied in rat brain slices. The distribution of ^{3}H-inositol and the production of ^{3}H-inositol phosphates in cortical slices were measured after 5 to 60 min of incubation, with some slices being exposed to NE, glutamate, NMDA, quisqualate, or to NE in the presence of each of the excitatory amino acid agonists.

Glutamate did not effect the slice content of free ^{3}H-inositol, but abolished NE-induced production of ^{3}H-inositol monophosphate, ^{3}H-inositol bisphosphate and ^{3}H-inositol trisphosphate. Quisqualate mimicked the effects of glutamate, whereas NMDA and kainate did not inhibit the response to NE, suggesting that only quisqualate-selective receptors affected α_1-adrenergic receptor-mediated phosphoinositide hydrolysis. Glutamate produced similar inhibitory effects in slices from hippocampus and striatum.

To test if the inhibitory effect of glutamate was the result of irreversible cell damage, cortical slices were incubated with glutamate for 60 min prior to exposure to ^{3}H-inositol and NE. Preincubation with glutamate did not inhibit NE-stimulated ^{3}H-inositol monophosphate production.

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37.15

NMDA DOES NOT INHIBIT CARBACHOL-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS. G.C. Ormandy* and R.S. Jope. (SPON: L. Beani), Dept. of Pharmacology, Univ. of Alabama, Birmingham, AL 35294

The effect of NMDA on carbachol-stimulated phosphoinositide hydrolysis was studied in hippocampal and cerebral cortical slices of rat brain. NMDA was added either after 1 hour preincubation of slices with ^{3}H-inositol or simultaneously with ^{3}H-inositol. NMDA (50-1000 μM, 10 or 60 min) had no effect on the slice content of free ^{3}H-inositol or on carbachol-induced ^{3}H-inositol monophosphate production. Although NMDA produced a 15% inhibition of lipid labelling when added simultaneously with the ^{3}H-inositol it appears that these lipids are not in a pool associated with the cholinergic receptors. Even preincubation of slices with NMDA (60 min) prior to exposure to ^{3}H-inositol did not inhibit subsequent carbachol-stimulated ^{3}H-inositol monophosphate production. Thus, it is concluded that NMDA does not affect carbachol-stimulated phosphoinositide hydrolysis in either hippocampal or cerebral cortical slices of rat brain under these experimental conditions. Supported by NIH grant MH38752.

37.16

BIDIRECTIONAL REGULATION OF PHOSPHOINOSITIDE METABOLISM BY GLUTAMATE RECEPTORS: STIMULATION BY TWO CLASSES OF QA RECEPTORS AND INHIBITION BY NMDA RECEPTORS. Elizabeth Palmer*, Daniel T. Monaghan, Jennifer Kahle and Carl W. Cotman (SPON David R. Stevens) Dept. Psychobiology, Univ. California, Irvine, CA 92717.

Analysis of excitatory amino acid receptor regulation of phosphoinositide (PI) metabolism in rat neonatal hippocampal slices reveals two classes of quisqualic acid (QA) receptors. One class corresponds to the excitatory actions of the well described QA-agonist, AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid), which is selectively blocked by the recently available QA antagonist CNQX (6-cyano-2,3-dihydroxy-7-nitro-quinoxaline). AMPA maximally elicits a 2-3 fold stimulation of PI metabolism which is readily blocked by CNQX. The second class of QA receptor is activated by 10 μM QA, resulting in a 15 fold stimulation of PI metabolism. The QA-induced PI metabolism is not antagonized by CNQX.

QA-induced stimulation of PI metabolism is inhibited by NMDA receptor activation NMDA (100 μM) inhibits the QA-induced stimulation by 75% and the inhibition is dependent on extracellular calcium. As expected, glutamate appears to be activating both QA and NMDA receptors since NMDA antagonists CPP (3-((±)2-carboxypiperazin-4-yl) propyl-1-phosphonic acid), AP5 (D-2-amino-5-phosphopentoate), and MK-801 enhance glutamate stimulation of PI turnover.

In the neonate and adult hippocampal slice it is the AMPA-sensitive QA receptor which mediates most of the Schaffer collateral electrically-evoked synaptic response. Therefore, if the CNQX-insensitive QA receptors are activated during synaptic transmission, at low stimulation frequencies the transmitter glutamate would activate both ion channels and PI metabolism via the two QA receptors. At high stimulation frequencies, NMDA receptors would become active and shut down PI metabolism, while promoting calcium entry. Thus the stimulation paradigm could be controlling the nature of the second messengers generated. During development such a mechanism would allow for synaptic activity to control two seperate second messenger pathways which could be exerting opposite regulation of synaptic stabilization as has been theorized by Bear and colleagues (Science 237: 42-48). Supported by ARO DAAL 03-86-K-0067.

37.17

INHIBITION OF INOSITOL PHOSPHATE PRODUCTION BY ADENOSINE IN GH3 CELL MEMBRANES. T.M. DELAHUNTY and J. LINDEN* Dept. of Neuroscience and Physiology, Univ. of Virginia, Charlott -esville, VA 22908

We have previously reported that adenosine and its analogues inhibit phosphoinositide hydrolysis and inositol phosphate (IPx) production in GH3 cells via a pertussis sensitive mechanism (FASEB J., 2:5, 1988). We now extend those findings to a cell free system. Both basal and TRH (thyrotropin releasing hormone) stimulated inositol phosphate production were inhibited and the potency order of adenosine analogues was similar to that observed in whole cells: N6-cyclopentyladenosine, phenylisopropyladenosine (R-PIA), 5'-N-ethylcarboxamidoadenosine, indicative of a response mediated by A_1 receptors. The selective adenosine receptor antagonists BW-A1433U and 8-phenylsulfyltheophylline blocked the response. The stable GTP analogue, GTP-gamma-S, stimulated IPx production in GH3 cell membranes and this response was reduced by R-PIA. These data rule out indirect regulation of phosphoinositide metabolism by adenosine receptor-stimulated second messenger production or ion channel modulation and suggest a more direct effect.

37.18

CHARACTERISTICS OF 5HT-MEDIATED PHOSPHOINOSITIDE (PI) TURNOVER IN RAT HIPPOCAMPUS: EFFECTS OF HIGH POTASSIUM. A.M. Mellow, D.L. Murphy,* J.M. Cossery,* and D.M. Chuang. NIMH/LCS and LPP; Bethesda, MD 20892 and Washington, DC 20032.

5HT-mediated activation of phospholipase C and promotion of PI turnover is an important mechanism of biochemical signal transduction in the brain and has not been well characterized in the hippocampus, where its properties differ from those in the cerebral cortex.

PI turnover (the production of inositol monophosphate (IP_1) in the presence of LiCl) was measured in 350-μm rat hippocampal slices prelabelled with [^{3}H]-myo-inositol. 5HT produced a biphasic increase in PI turnover, with a maximum (40% over control) occurring at a concentration of about 50 μM. At higher 5HT concentrations, PI turnover fell to near control levels. This phenomenon was not seen in the cortex. Treatment of slices with high potassium (15-25 mM) both potentiated the action of 5HT and abolished the biphasic nature of the dose-response curve. The selective receptor agonists m-CPP ($5HT_{1B}$), DOI ($5HT_2$), and 2-Me-5HT ($5HT_3$) all had weak activity. The $5HT_2$ antagonist, ketanserin, blocked the response poorly, and the $5HT_3$ antagonist, ICS-205-930, was ineffective, all suggesting possible involvement of both $5HT_2$ and $5HT_1$ receptor subtypes. These results indicate that 5HT-mediated PI turnover in the hippocampus is under complex modulatory control, possibly via opposing actions of 5HT at different concentrations and at different receptor sites, and can be further modified either by a direct effect of potassium ion or via the depolarization-induced release of an unknown modulator.

37.19

EFFECTS OF INDOLEAMINES ON POLYPHOSPHOINOSITIDE HYDROLYSIS IN RAT CEREBRAL CORTEX. L.E. Dyck Neuropsychiatric Research Unit, Dept. of Psychiatry, University of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0

5-Hydroxytryptamine (5HT) increases polyphosphoinositide turnover (PIT) in rat cerebral cortex via stimulation of $5HT_2$ receptors. The effects of tryptamine (TRA) on PIT in rat cortex is less clear. Some groups report that it stimulates PIT in cortex, but others report that it is inactive. The ability of these compounds to stimulate PIT in slices of rat cerebral cortex was examined by using a direct assay which involves labelling with ^{3}H-inositol and assaying ^{3}H-inositol phosphates in the presence of lithium. 5HT increased the formation of ^{3}H-inositol phosphates by cortical slices significantly above control levels. Its effects were rather weak compared to noradrenaline (NE). 1 mM 5HT was inactive, and 10 mM increased PIT to 120±6 % of control activity. By comparison,the response to 1 μM NE was 162±17 % of control and 401±20 % to 1 mM NE. TRA (1-10 mM) stimulated the formation of ^{3}H-inositol phosphates(to 142- 234 % of control). In agreement with previous findings, ketanserin did not appear to block TRA. Interestingly, when TRA or 5HT (1mM) were incubated simultaneously with a submaximal amount of NE (0.1 mM), they inhibited NE-induced increases in PIT. TRA inhibited NE by 59%, while 5HT inhibited NE by 24%. The mechanism of this inhibition is under investigation.

NEUROETHOLOGY I

38.1

TEMPERATURE COUPLING IN CRICKET ACOUSTIC COMMUNICATION: LOCALIZATION OF TEMPERATURE EFFECTS ON THE FEMALE SONG-RECOGNITION NETWORK. A. Pires* and R.R. Hoy. (SPON: J.A. Doherty). Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Temporal parameters of the song of the North American field cricket, Gryllus firmus, are strongly affected by environmental temperature. Chirp rate and pulse rate within the chirp both increase as linear functions of temperature. Female phonotactic preference is affected by temperature in a parallel manner, such that warmer females prefer faster songs. Thus this communication system is said to be temperature coupled.

Since male song is produced by a thoracic central pattern generator (CPG) and head ganglia are necessary for female song recognition, anatomical localization of the temperature effect on song recognition is a test of the hypothesis that neural elements homologous to the male song CPG are present in the female and are used as a template for song recognition.

We developed a technique for selectively manipulating and measuring temperatures in different body compartments while the animal walks on a spherical treadmill which records direction and velocity of movements in response to synthetic song.

An initial result of this study is that female phonotactic preference can be altered by raising the temperature of the whole animal but not by heating the head capsule alone, consistent with the CPG-template hypothesis.

38.2

SEXUAL DIMORPHISM IN THE AUDITORY SYSTEM OF THE PRAYING MANTIS. D. D. Yager* and R.R. Hoy (SPON. M. C. Nelson). Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

We have found physiological and anatomical sexual dimorphism in the metathoracic auditory systems of two mantis species, Stagmomantis carolina and Ameles heldreichi. In the latter, extracellular recordings from the connectives of males show ultrasonic tuning similar to that previously reported for M. religiosa, while we detect no auditory activity at all in the females. Male S. carolina have a W-shaped tuning curve with sensitivity at 2-4 kHz and at 25-45 kHz. The low frequency hearing is mediated by mesothoracic rather than metathoracic structures. The females of this species have low frequency curves identical to the males', but no detectable response to ultrasound.

Differences in the external anatomy of the ear region (the metathoracic groove) parallel the physiological findings. The males of both species have grooves of an anatomical type physiologically correlated with ultrasonic hearing in 10 of 11 species tested. The female grooves differ markedly from the males'. These groove structures correlate physiologically with absence or strong reduction of ultrasonic response in 10 of 10 species tested. The internal ear anatomy of S. carolina males and females is similar with respect to the tympanal organ, tympanal nerve, and auditory neuropile.

In museum collections we have examined the metathoracic groove (ear) anatomy in both sexes of species in 177 genera representing all major taxonomic groups in the suborder Mantodea and find sexual dimorphism in 66 cases. Further, the occurrence of dimorphism in groove anatomy is strongly correlated with dimorphism in wing length. The dominant pattern is that seen in both species discussed above: fully winged males have the ultrasound-correlated groove type while the short winged females of the same species have aberrant groove structures.

(Supported by NINCDS Grant NS11630 to R.R. Hoy and by the Eppley Foundation for Research.)

38.3

POSSIBLE ACTIVE SPACE OPTIMIZATION BY THE MALE CRICKET FROG, ACRIS CREPITANS, THROUGH ADJUSTMENT OF NEIGHBOR SPACING. J. H. Fox. Psychology Dept., University of Texas, Austin, TX 78712.

Computer models of hypothetical anuran choruses (Fox, J.H. and Wilczynski, W., Soc. Neuroscience Abst. 12:84.10, 1986) demonstrate that a male may increase the active space (AS) of its mating call by joining a chorus and optimize AS by spacing itself at an appropriate distance from neighboring males. Optimal neighbor spacing depends on chorus geometry type, approximate chorus population, call duty cycle (DC), the randomization factor (RF) for neighbor spacing, and the threshold distance (TD), over which the solitary male's mating call attenuates to the female's multiunit midbrain auditory threshold.

Acris crepitans choruses in the Austin area are usually linear but often planar and typically have less than 50 members. DC = .029, RF = .63, and TD= 18.4 M. Conflicting spacing optima exist, based on the above values, for the two chorus types. AS is optimized when neighbor spacing is .79 M in linear choruses and 8.15 M in planar choruses. Actual neighbor spacing is 4.8 M and does not seem to differ between chorus types. Perhaps 4.8 M represents a compromise between the conflicting linear and planar optima.

(Supported by NSF grant BNS 8606289.)

38.4

BEHAVIORAL AND NEURAL THRESHOLDS TO ADVERTISEMENT CALLS IN A NEOTROPICAL FROG. W. Wilczynski. Dept. of Psychology, Univ. of Texas, Austin, TX 78712.

Male Hyla microcephala produce a short, high frequency, advertisement call with two spectral peaks: the dominant frequency at ca. 5.8 kHz and a lower amplitude peak at ca. 3 kHz. Different call amplitudes characterize different male behaviors. The mean (±SD) threshold for antiphonal calling is 62.2 ± 2.99 dB SPL (fast RMS). Males maintain an intermale distance marked by neighbor call amplitudes of 75 ± 5.95 dB. At ca. 81 dB males switch to an aggressive call (estimated from Wells & Schwartz, Copeia, 1985: 27-38). Midbrain multiunit recordings reveal one broad area of auditory sensitivity below 1.2 kHz and a second marked by a V-shaped frequency-threshold curve with a mean best frequency of 5.3 kHz. Both peaks in the call fall within this upper sensitivity band, which presumably represents basilar papilla (BP) activity. BP thresholds at best frequency average 62.7 ± 10.8 dB, which is not significantly different from the mean threshold for antiphonal calling (t=0.51, p>0.25), but is different from the mean neighbor amplitude (t=10.0, p<0.001). In collaboration with W. S. Geisler, a computer model of sensory filtering is being applied to these data to investigate the relationship between behaviorally relevant call amplitudes and the intensity-dependent activity of BP neurons.

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38.5

TESTOSTERONE-INDUCED CHANGES IN HORMONE ACCUMULATION IN THE SONG SYSTEM OF FEMALE CANARIES. E.A. Brenowitz and A.P. Arnold. Depts. of Psych & Zool, Univ. Washington, Seattle, WA 98195 and Dept. Psych & Brain Res. Inst., UCLA, LA, CA 90024.

Adult female canaries (Serinus canarius) show extensive plasticity in the neural sytem that regulates song production. Normal females do not sing regularly, have smaller song control regions (SCRs) in their brains than do males (Nottebohm and Arnold 1976), and have smaller dendritic trees in one SCR than do males (DeVoogd and Nottebohm 1981). Treatment of females with testosterone (T) induces song and increases SCR volumes (Nottebohm 1980), neuron number (Bottjer and Dignan, submitted), and dendritic tree size (DeVoogd and Nottebohm 1981). In this study we asked whether such T-induced neural plasticity is accompanied by changes in the accumulation of T or its metabolites by SCR neurons.

Female canaries were implanted with silastic capsules containing either T (n = 5) or nothing (n = 3). Vocal behavior was monitored. One month later the pellets were removed, each bird was gonadectomized and then injected with tritiated testosterone for steroid autoradiography. In the caudal nucleus of the ventral hyperstriatum (HVc), we measured the proportion of cells that were labeled and the extent above background that cells were labeled (density ratio). Gross volume of HVc was also determined.

Song occurred in all T-treated but no control females. HVc volume was 75% greater in T birds. The proportion of HVc cells labeled by T or its metabolites did not differ between T-treated (X ± SD = 50.2 ± 7.8%) and control (50.3 ± 0.3%) groups. Density ratios of HVc cells, however, were greater for T females (6.5 ± 1.6) than for controls (5.5 ± 1.9; P<.01, t-test). The T-induced masculinization of vocal behavior is thus accompanied by an increase in the strength with which T/metabolites is accumulated by target cells but not in the frequency of such cells. Also, T target and non-target cells must be recruited at equal frequencies into HVc following T treatment.

38.6

NEUROCHEMICAL STUDIES OF THE NUCLEUS INTERCOLLICULARIS IN THE JAPANESE QUAIL. M. Gahr*, G.F. Ball, M. Schumacher*, & J. Balthazart. (SPON: J. Cohen). Univ. Kaiserslautern, FRG; Rockefeller Univ., New York, NY 10021 ; Univ. Liège, Liège, Belgium.

In the Japanese quail crowing and copulatory behavior are both suppressed by castration, but different steroid treatments reinstate these behaviors. Little is known about the reason for this difference. N. intercollicularis (ICo) has been identified as an important area mediating vocal behavior in the quail. We have therefore investigated neurochemical factors influencing steroid effects in ICo. Approximately 50% of the cells in the medial portion of the nucleus contain estrogen receptors (determined immunohistochemically), however, levels of the steroid converting enzyme aromatase are undetectable. Levels of the 5α reductase enzyme are high in ICo but do not appear to be regulated by testosterone (T). Steroid effects on crowing are presumeably mediated by the modulation of neurochemical activity in ICo. We have therefore studied the localization and modulation by steroids of two neurotransmitter systems in ICo. Dense α-2 adrenergic receptor binding ([^{3}H] Para-aminoclonidine [PAC]) and muscarinic cholinergic binding ([^{3}H]N-methyl scopolamine [NMS]) as determined by autoradiography is present in the nucleus. However, there are differences between the two ligands. For example, PAC shows a distinct sub-region of binding in medial ICo which resembles the dorso-medial region of ICo described in songbirds. PAC binding is sexually dimorphic although the density of receptors in the male is not affected by T treatment. In contrast, NMS binding is not sexually dimorphic but castration of the male seems to decrease the density of binding, especially in rostral ICo. The actions of steroids in ICo are different in several ways from those described for copulatory behavior in the medial pre-optic nucleus.

38.7

AUTORADIOGRAPHIC LOCALIZATION OF BRAIN PROLACTIN RECEPTORS IN A PARENTAL AND NON-PARENTAL SONGBIRD SPECIES. G.F. Ball, A.M. Dufty*, A.R. Goldsmith*, and J.D. Buntin. Rockefeller University, New York, NY 10021; Univ. of Bristol, Bristol, U.K. and Univ. of Wisconsin, Milwaukee, WI 53211

In several species of birds, such as the European starling (*Sturnus vulgaris*) plasma levels of prolactin are positively correlated with the onset and maintenance of incubation behavior. Brood-parasites such as the brown-headed cowbird (*Molothrus ater*) lay their eggs in other bird's nests and do not engage in any parental care. However, seasonal changes in prolactin secretion in this species show a pattern similar to that of parental birds. It has been hypothesized that cowbirds have evolved a central insensitivity to the action of prolactin. In order to test this idea we compared prolactin binding sites in the brains of male and female starlings and cowbirds using *in vitro* quantitative autoradiography. Sites were labeled using [^{125}I] ovine prolactin (55pM [^{125}I] oPRL ± 85nM oPRL). Sections were exposed to X-ray film and the autoradiograms were analyzed with an image analysis sytem. Plasma levels of prolactin were measured in all subjects. Specific [^{125}I]oPRL binding sites were observed in both species throughout the pre-optic area (POA) and in hypothalamic regions including such nuclei as the paraventricular nucleus and the infundibular nucleus. Extra-hypothalamic binding sites in both species include the n. intercollicularis and the n. taeniae. A sex difference in receptor density was identified in both species in the POA. The density of binding was similar between the two species in all hypothalamic regions but in the POA levels were significantly higher in starlings. This difference may be related to inter-specific differences in parental behavior.

38.8

CONNECTIONS AND FUNCTION OF AN "AROUSAL"-AREA OF THE CAUDAL FOREBRAIN OF ZEBRA FINCHES. H.-J. Bischof*, K. Herrmann*, J. Engelage*, J. Niemann*, E. Church* (SPON: European Brain and Behavior Society). University of Bielefeld, Dept. Ethology, POB 8640, D 4800 Bielefeld 1, FRG.

In previous studies (Bischof, H.-J., Herrmann, K.; Behav. Brain Res 21: 215, 1986; Behav. Neural Biol: in press), we demonstrated that four areas of the forebrain of zebra finch males are activated in situations which arouse the animal, e.g. if it is exposed to a female. We investigated the connections and responses to sensory stimuli of one of these areas, the archi/neostriatum caudale (ANC). In addition, we examined the effects of ANC lesions on courtship behaviour. Besides other afferents ANC receives input from the visual area pretectalis, locus coeruleus, and the reticular formation. Only one projection to the optic tectum could clearly be demonstrated. Evoked potentials within ANC occurred with visual and, very rarely, acoustical stimulation. The best responses were obtained with combined stimuli. Bilateral lesions to ANC reduce male song activity during courtship, but not courtship behavior in general. We propose that ANC may be involved in the regulation of song output according to the behavioral state of the animal. Supported by the DFG.

38.9

SEXUAL DIFFERENCES IN CONTROL OF THE ZEBRA FINCH DISTANCE CALL. H. B. Simpson and D. S. Vicario. The Rockefeller University, New York NY 10021.

Song production in birds has been extensively studied as a model for brain correlates of behavior and learning. The song control pathway includes central nuclei HVc and RA and the tracheosyringeal nerve. In addition to song, songbirds produce a variety of calls in different social contexts. In zebra finches, the distance (or contact) call is sexually dimorphic and learned in males (Zann, '84, '85). We wanted to know whether this call depends on the same central and peripheral pathway as that described for song in canaries.

In male zebra finches, cutting the tracheosyringeal nerves has a dramatic effect both on the distance call and on the song. However, in females, the distance call remains virtually intact after nerve section, suggesting that her call does not require active control of syringeal muscles. Lesioning either HVc or RA in males affects both his song and distance call. In contrast, the female's distance call remains virtually intact after HVc lesions. These data are consistent with anatomical work demonstrating that the connection between HVc and RA is present in males, but sparse or absent in females (Konishi and Akutagawa, '85).

Our data show that the male's song and distance call depend critically upon HVc, RA and the tracheo-syringeal path, whereas females can call without a significant contribution from these structures. We propose that there exists another system for vocalization used by female zebra finches that may play a role in male vocalizations as well.

38.10

CONTROL OF SYRINGEAL MUSCLES IN NUCLEUS RA OF ZEBRA FINCHES. D. S. Vicario, and H. B. Simpson. The Rockefeller University, New York NY 10021.

Telencephalic nuclei HVc and RA are critical for avian song behavior. HVc projects to RA, and RA projects to part of n. XII (nXIIts) that controls the vocal organ, or syrinx. We have recently shown that nXIIts is organized in control zones for syringeal muscles.

The present study mapped outputs from RA by injecting small amounts of retrograde tracers into physiologically identified subregions of nXIIts. In nXIIts, the control zones for the dorsalis and ventralis muscles (the two largest syringeal muscles) are located caudally and rostrally respectively. In RA, cells projecting to caudal nXIIts were located ventrally, whereas the projection to rostral nXIIts originated in mid RA. Dorsal RA contained almost no cells projecting to nXIIts; instead, the cells there project to DM of ICo, the other major output of RA. Anterograde tracers were injected into subregions of RA to confirm these projections. Thus, the overall pattern in RA consists of approximately horizontal slabs of cells with different targets.

The same methods did not show topographic organization in the projection from HVc to RA, so we made small injections of the anterograde tracer PHA-L in HVc. HVc axons arriving in the dorsal RA region (projecting to DM) bifurcate richly. In contrast, most axon trunks that enter mid and ventral RA run rostrally in the horizontal plane for long distances, giving off short branches. We suggest that the terminal field of each HVc axon within the nXIIts-projecting region of RA could select cells involved in the control of particular syringeal muscles.

38.11

'TIMBRE' CONTROL AND DISCRIMINATION IN ZEBRA FINCH SONG. H. Williams, J. Cynx*, F. Nottebohm. Rockefeller University Field Research Center, Millbrook, NY 12545.

Zebra finch song syllables include harmonically related frequency components which may be differentially suppressed or emphasized. We call these patterns of harmonic suppression `timbre'. A syllable's timbre is conserved over a period of at least 9 months, and young males imitate the patterns of harmonic suppression in a song model's syllables. Syringeal denervation dramatically alters the patterns of harmonic suppression in a manner suggesting that the vocal organ controls the relative amplitudes of harmonics within a syllable.

Zebra finches can also discriminate between two syllables differing only in timbre. In a go - no go operant paradigm, male and female finches rapidly acquired a discrimination between stimuli varying only in the amplitude of the harmonics. Discrimination performance was not affected by lesioning HVc, a forebrain nucleus that is essential for song production and possibly important for song perception.

The selective suppression of some harmonics creates a great number of possible timbre variants, which add signal diversity to the limited set of fundamental frequencies and frequency modulation patterns used in zebra finch syllables. Patterns of harmonic suppression and emphasis are also important in speech, and may previously have been overlooked in bird song. The neural mechanisms mediating the motor control and perception of 'timbre' in zebra finches may be of interest to all students of vocal communication.

38.12

A COMPARISON BETWEEN THE EFFECTS OF SYRINGEAL DENERVATION AND AIRFLOW OBSTRUCTION ON SONGBIRD PHONATION. S. Nowicki* (SPON: P. Marler). Rockefeller Univ., New York, NY 10021

The two sides of the songbird syrinx are thought to operate independently during phonation, providing a simple interpretation of peripheral function with respect to cerebral lateralization of vocal control in birds. In order to reassess this hypothesis, male swamp sparrows (Melospiza georgiana) were subjected to two different procedures, both intended to disable the syrinx unilaterally. One group of birds had either the right or left hypoglossal nerve sectioned. A second group had airflow to one or the other syringeal half blocked by insertion of a plug in the bronchus. Some birds were subjected to both procedures in series, receiving a nerve section ipsi- or contra-lateral to the side that was previously plugged.

Occluding airflow to one side of the syrinx produces qualitatively different phonological effects than those observed when that same side is disabled by nerve section. In general, unilateral blockage of airflow has remarkably little effect on song. Birds given nerve sections ipsilateral to a side that was previously plugged show vocal deficits comparable to those observed in the case of unilateral nerve sections alone. These data are inconsistent with previous interpretations of syringeal function. They suggest that mapping of lateralized central control onto the periphery is more complex than previously supposed.

Supported by PHS grant NS24651 and The Hirschl Trust.

38.13

CHANGES IN NEURON NUMBER DURING SENSORY LEARNING IN SWAMP SPARROWS. K.W. Nordeen, P. Marler, and E.J. Nordeen. Psychology, U. Rochester, Rochester, NY, 14627, and Rockefeller U. Field Res Ctr, Millbrook, N.Y. 12545.

During vocal learning in songbirds, neurons are added to some song nuclei and lost from others. Previous work has not distinquished whether these neural changes correlate with memorizing a song model (sensory learning) or practicing song-like vocalizations (sensorimotor learning). In this study we measured changes in neuron number within swamp sparrow song nuclei during sensory learning, but before the onset of sensorimotor learning.

Male swamp sparrows were tutored with taped songs from 20 days of age until they were sacrificed at 20, 40, or 60 days after hatching. Sparrows memorize about 60% of their song material during this period, but do not rehearse learned material until 6-7 months later. Brains were embedded in paraffin and serial sections (10um) were stained with thionin. Neuron number was determined for hyperstriatum ventralis pars caudalis (HVc), magnocellular nucleus of the anterior neostriatum (MAN), robust nucleus of the archistriatum (RA) and Area X.

Neuron number changed substantially during sensory learning in all areas examined except RA. In both HVc and Area X neuron number doubled between 20 and 60 days of age. In contrast, MAN lost about 30% of its' neurons between 40 and 60 days of age. If these patterns of neuronal addition and loss encourage learning, their timing may restrict the period of sensory learning.

38.14

NON-CLASSICAL AUDITORY PROJECTIONS TO SONGBIRD FOREBRAIN. A.J.Doupe and M. Konishi. Div. of Biology 216-76, Calif. Institute of Technology, Pasadena, CA 91125

Birdsong is a complex learned behavior which requires auditory experience and feedback during its development. The classical thalamo-telencephalic auditory projection in songbirds is from nucleus ovoidalis to Field L. In addition, projections from Field L to the vicinity of hyperstriatum ventrale, pars caudale (HVc) and the robust nucleus of the archistriatum (RA) are known.

Using single and multi-unit electrophysiological recordings, we have found a wide distribution of auditory responses in other areas of the forebrain of adult male bengalese finches. These units were located in anterior portions of all layers of the hyperstriatum, as well as in anterior neostriatum and lobus parolfactorius (LPO). In LPO some of the recording sites were within the song nucleus X. The auditory units had response latencies of 20-30 msec, preferred complex stimuli such as white and narrow band noise, and were broadly tuned to frequency. Preliminary results of horseradish peroxidase (HRP) injections into these auditory areas showed retrograde label in the posterior dorsal thalamus.

These results demonstrate a large area of auditory responsiveness in the songbird anterior forebrain, and raise the possibility of a separate thalamo-telencephalic auditory pathway, parallel to the classical pathway through nucleus ovoidalis and Field L.

38.15

AUDITORY EXPERIENCE OF MATURING BARN OWLS. T.A. Haresign* and A. Moiseff. Dept. of Physiology and Neurobiology, Univ. of Conn., Storrs, CT 06268.

We recorded short latency auditory evoked potentials (EPs) from the Barn Owl (Tyto alba) to study the changes in hearing during maturation. This non-invasive approach allowed us to follow the development of individual owls beginning about 1 week after hatching. Three electrodes were arranged with one located at the vertex and one at each ear canal. Stimuli consisted of click or click pips (2kHz - 12kHz), and responses were averaged over 50 or more repetitions.

Short latency EP components (less than 5 msec) were detected in response to free field stimuli in owls of all ages. The threshold of these components decreased as individuals matured. The ability to detect specific frequencies was independent of age, however, during maturation, sensitivity to particular frequency bands varied.

Longer latency (8-20 msec) components characteristic of adult owls' EPs were not apparent in very young individuals. As the animals aged these components gradually appeared. When the owls were about 4 months of age the EPs took their adult form.

(Supported by NS21480 and an Alfred P. Sloan Fellowship to AM)

38.16

MATING CALL CHARACTERISTICS AND LARYNGEAL MORPHOLOGY IN TWO POPULATIONS OF *ACRIS CREPITANS*. B.E. McClelland, W. Wilczynski and M.J. Ryan. Depts. of Psychology, Zoology, and Institute for Neurological Sciences Research, University of Texas, Austin, TX 78712.

Allopatric populations of cricket frogs (*Acris crepitans*) differ in average size and mating call characteristics such as dominant frequency and temporal pulse patterns. This study compares the anatomical components of the larynges with mating call characteristics in individuals representing two populations from Sabine (SA) and Lake Balmorhea (BA), Texas.

The dominant frequencies are lower (BA=2.78; SA=4.03 kHz), but the head widths (BA=.91; SA=.79 cm), arytenoid cartilages (BA=.881; SA=.665), vocal cords (BA=.065; SA=.048), constrictor (BA=1.077; SA=.767) and dilator muscles (BA=.27; SA=.178) are all larger in the BA population (laryngeal measurements in mm^3; all $p<.01$, Student's t-test). These size differences may account for dominant frequency differences between the two populations. However, within each population the relationship between morphology and call characteristics is more complex. Stepwise regression analysis using standardized data shows that the dilator muscle volumes were significantly correlated with the duration of the mating calls (SA=61.5 ms) ($r=.87$; $p<.03$) in the SA population, but not in BA. The number of pulses per call (BA=4.72) was negatively correlated ($r=-.66$; $p<.05$) with the constrictor muscle volume in the BA population. The volume of BA 's vocal cords was also related to the number of pulses per call ($r=.61$; $p<.04$). The only correlation involving the dominant frequency was a negative relationship ($r=-.75$; $p<.04$) with the arytenoid cartilages in BA. (Research supported by NSF grant BNS 8606289)

38.17

AUDITORY NEURONS SELECTIVE FOR COMBINATIONS OF BEHAVIORALLY RELEVANT SOUND PATTERNS. B. DIEKAMP* and H. C. GERHARDT* (SPON: F. vom Saal). Division of Biological Sciences, University of Missouri, Columbia, MO 65211.

We examined the temporal selectivity of single neurons in the torus semicircularis of two species of the gray treefrog complex (Hyla versicolor and H. chrysoscelis) to acoustic signals whose significance in intraspecific call recognition has been established. Two fine-temporal call properties underlying species recognition were changed parametrically: pulse repetition rate (PR) was varied from 10 to 70 pulses/s; pulse shape (PS) was varied from 35 to 95% rise time. The spectral characteristics and other temporal properties of the stimuli were held constant at species-typical values. A substantial proportion of neurons responded selectively to specific PRs; others were selective to particular PSs. A few neurons were tuned to a unique combination of these two parameters. That is, most of these neurons responded maximally to the conspecific PR only if the pulses of the stimuli also had the conspecific PS; they responded poorly to stimuli with a PS typical of the other species over the entire range of stimulus PRs. These data are consistent with the observed behavioral preferences for synthetic calls with a conspecific PR and PS. We propose that PR-, PS-selective and combination-selective neurons play a crucial role in intraspecific mate choice. PR is highly temperature-dependent, but both behavioral and neurophysiological studies indicate that the nervous system of the female compensates for her own temperature in selecting calls with an appropriate PR. Since PS is a temperature-independent parameter of the male's call, it should be a particularly reliable redundant parameter for species recognition.

38.18

CORTICAL NEURONS SENSITIVE TO THE HARMONIC STRUCTURE OF ECHOLOCATION SIGNALS EMITTED BY AN FM BAT. S.L. Shannon* and D. Wong. Department of Anatomy, Indiana University School of Medicine, Indianapolis, Indiana, 46223.

Neurons that are delay-sensitive to pulse-echo pairs, consisting of a constant frequency and FM sweep (CF-FM), are present in the auditory cortex of the FM bat, Myotis lucifugus. These neurons are interspersed with other neurons exhibiting delay-sensitivity to FM sound pairs. The cortical zone containing both CF-FM and FM-FM neurons overlaps extensively with the tonotopic zone.

Facilitative responses are elicited by CF-FM stimuli in which the constant frequencies of the pulse are centered near frequencies of 42 and 84 kHz. Sonograms of Myotis during the search phase display FM signals that sweep downward approximately an octave in about 4 msec. The second harmonic of the FM sweep starts with an average frequency of 83.7 kHz, with a corresponding fundamental of 42.3 kHz.

These data suggest a sensitivity of CF-FM neurons to harmonically related frequency components of the emitted sounds and echoes. The possible role of these cortical neurons in coding for target characteristics will be discussed.

Supported in part by BRSF RR 5371 from Indiana University.

38.19

TARGET DETECTION AND ACOUSTIC IMAGING IN ECHOLOCATING BATS. C.F. Moss and J.A. Simmons. Section of Neurobiology and Department of Psychology. Brown University, Providence, Rhode Island 02912

Echolocating bats emit ultrasonic sounds and use echoes of these sounds to detect and track flying insect prey. This experiment examined the manner in which small step changes in target distance (jitter) may influence target detectability.

Big brown bats (Eptesicus fuscus) were trained to detect electronically delayed echolocation sounds in a two-choice psychophysical procedure. These sounds simulated echoes from a target located either to the left or to the right of the bat's observing platform, and the bat learned to approach the side on which the target was presented. We measured detection performance for stationary targets located 57 cm from the bat (simulated by echoes returning at a fixed delay of 3.3 ms) and for jittering targets located at the same mean distance but changing by 1-7 mm from one sample to the next (simulated by echoes that underwent step changes in delay from 5-40 μs about a mean of 3.3 ms). The results indicate that target jitter improves detectability, particularly for values between 2.6 and 4.3 mm (echo jitter of 15-25 μs).

In the CNS of bats, neurons responsive to pulse-echo pairs are probably involved in the coding of target distance. These neurons show delay-tuning and are selectively activated by targets at particular distances (Suga & O'Neill, Science, 206: 351-353). If excitation of these cells is involved in target detection, then echo jitter may enhance target detectability by discretely activating subpopulations of delay-tuned neurons.

39.1

A MODEL FOR NEUROGENESIS IN THE MAMMALIAN NERVOUS SYSTEM: GENERATION OF AN OLFACTORY NEURONAL CELL LINE. Brian L. Largent, Ronald G. Sosnowski*, and Randall R. Reed, Johns Hopkins Medical School, Baltimore, MD

Among central neuronal systems, the vertebrate olfactory system is unique in that it contains a population of mature neurons constantly undergoing, even into adulthood, steady state turnover. This steady state process is accomplished by stem cells which differentiate and mature into primary olfactory neurons replenishing degenerated sensory neurons. Our approach to generate clonal cell lines has been to immortalize primary olfactory neurons through the introduction of a recombinant oncogene into the germline of mice. The recombinant oncogene was constructed to provide cell specific expression of the oncogene product within primary olfactory neurons, thereby selectively affecting those cells. Specifically, the hybrid gene consists of the early region of simian virus 40 (SV40) - encoding the oncogene product, large T-antigen - flanked 5' by the cell-specific regulatory elements (enhancers and promoters) of the rat gene for olfactory marker protein (OMP), a protein whose abundant expression is limited to mature olfactory sensory neurons. Cell immortalization and/or transformation can be induced by expression of SV40 large T-antigen. The regulatory elements of the OMP gene should target expression of T-antigen to primary olfactory neurons, thereby affecting those cells in a selective fashion within the transgenic mouse. Olfactory tissue-specific expression of T-antigen message has been demonstrated for each of the mouse lines. To date, one homozygous mouse (for wild type T-antigen) has developed a tumor apparently derived from olfactory tissue. We are currently establishing cell lines from this tumor.

39.2

CULTURES OF OLFACTORY EPITHELIUM: NEURONAL PROGENITORS? S.K. Pixley. Dept. of Anatomy and Cell Biology, University of Cincinnati, College of Medicine, Cincinnati, OH, 45267.

Basal cells of the rat olfactory epithelium can completely regenerate a damaged epithelium by differentiating into the primary receptor neurons and also, supposedly (currently debated), the sustentacular (supporting) cells. In order to study this remarkable neuronal regeneration, a culture system of putative olfactory basal cells has been developed.

Adult Spraque-Dawley rat nasal tissues, from areas containing predominantly olfactory epithelium, were dissociated and maintained in monolayer cell culture. Epithelioid and spindle shaped cells were present. The epithelioid cells were uniquely positive when immunostained with anti-keratin antiserum. In olfactory epithelium tissue sections, keratin was found only in basal cells and cells lining Bowman's gland ducts. Further characterization of cultured cells showed that subsets of the keratin-positive cells were positive for the monoclonal antibodies, A2B5 (a neuronal and glial progenitor cell marker) and 1D9.B9 (a novel cell marker for a subset of sustentacular cells). A subset was fibronectin-positive. The keratin-positive cells were negative for antibody markers of differentiated glial cells and mature neurons. The presence of keratin and the apparent multipotentiality suggest that these cultured cells are olfactory basal cells.

Optimal culture conditions were determined for enrichment of the putative basal cells. Pure populations of these cells could be obtained because the epithelioid and non-epithelioid cells were differentially adhesive.

These cultures will be used to identify factors which stimulate differentiation of a mature neuronal phenotype.

39.3

A SUBSET OF CENTRAL AND PERIPHERAL NEURONAL PRECURSORS EXPRESS AN INSULIN GENE CONSTRUCT PRIOR TO COMMITMENT IN TRANSGENIC MOUSE EMBRYOS. *S. Alpert*, D. Hanahan*, M. Moustakos*#, and G. Teitelman#.* (SPON: M. Ehrlich) Cold Spring Harbor Lab., Cold Spring Harbor, NY 11724 and #Neurobiology, Cornell Univ. Med. Sch. NY, NY 10021.

Transgenic mice harboring hybrid genes utilizing the insulin gene regulatory region express the transgene in β cells of the pancreas and in cells of the embryonic nervous system. In this study we used immunohistochemical procedures to examine the timetable of expression and distribution of labelled cells in the nervous system of one of these lines (RIP1-Tag-#2) during development. In the forebrain, cells expressing the transgene are first seen in the ventral region of the diencephalon at day 9.5 of development (e9.5, 18-24 somites), and persist until e16, the last stage examined. In the mesencephalon, stained cells are seen in the basal plate only at e9.5, while in the rhombencephalon, stained cells are seen in the basal plate from e9.5 to e12, and disappear at later stages. In the spinal cord labelled cells are first seen in the ventral plate of the apical region at e9.5, and appear in the posterior region at e12. These immunoreactive cells disappear from the spinal cord by e16. Neural crest cells expressing the transgene are first seen at e9.5, when the immunostained cells are distributed alongside the neural tube. At e10-e11 labelled neural crest cells are located in a segmented pattern between somites and ectoderm, and are also seen in clusters dorsolateral to the aorta. By e12, the neural crest cells no longer express the transgene. We conclude (1) the timing of expression of the insulin transgene is characteristic of each particular region of the central and peripheral nervous system and (2) the location of the immunoreactive cells suggests that the transgene is expressed by catecholaminergic precursors. Thus, the appearance of the transgene could signal the time of commitment of the different neuronal precursors to the specific pathways of differentiation.

39.4

CELL SIZES AND BIRTHDATES OF NEURONS IN THE OPOSSUM RETINAL GANGLION CELL LAYER. S.Allodi*, J.N.Hokoç and L.A.Cavalcante*. ICB and IBCCF Universidade Federal do Rio de Janeiro, 21941, Brazil.

The opossum retinal ganglion cell layer (GCL) contains large, medium and small ganglion cells plus homogeneously distributed displaced amacrine cells. We report here complex relationships between cell sizes and epochs of neuron production for GCL. Pouch young opossums received pulses of 3H-thymidine at ages from postnatal days 1 to 21 (PND1 to PND21) and were killed as adults. Soma sizes of heavily labeled (HLN) and adjacent unlabeled neurons (AUN) were measured in stained radioautographs of horizontal sections of the eye and histograms were plotted. The range of soma sizes of HLN was narrower than that of AUN in PND5 and PND21 with biases toward large and small diameters, respectively. Ranges of soma sizes of HLN and AUN were nearly overlapping and comparisions of median values revealed that HLN=AUN in PND14 and that HLN>AUN in PND1, PND3 and PND10. The results suggest that: 1) The peak of production of the largest ganglion cells is slightly delayed with respect to the onset of GCL genesis, 2) The production of amacrine cells persists to and, in fact, predominates in late stages of GCL genesis.

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39.5

NEURAL DISRUPTED - A DROSOPHILA GENE REQUIRED FOR EMBRYONIC NEUROGENESIS. D.A. Wright, E.K. Neumann*, and A.P. Mahowald*. Genetics Dept., Sch. of Med., Case Western Reserve Univ., Cleveland, OH 44106

While some genes required for neural morphogenesis have been recognized on morphological grounds alone (e.g. neurogenic loci) other important genes may be overlooked in mutants screens. When abnormal neural development secondarily disrupts general morphogenesis, the primary defect is masked by a complex mutant phenotype. In Drosophila we can identify such loci directly from their stage- and tissue-specific gene expression. We report the characerization of l(2)nd by this approach.

The locus was detected by differential screening using cDNA probes from cultured embryonic nerve cells vs other tissues. Its chromosomal location (23A) is close to the decapentaplegic complex; W. Gelbart and colleagues provided a genomic walk and collection of lethals in the region. From these we have provisionally identified the l(2)nd complementation group. Normal expression of nd peaks in mid embryogenesis and again during pupation, with a separate maternal transcript in ovaries and young embryos. We are now using fusion proteins from a fly head cDNA expression library (P. Salvaterra) to generate antisera for mapping the tissues in which l(2)nd is normally expressed.

39.6

BIRTHDATE IS MORE IMPORTANT THAN TISSUE TYPE FOR THE *IN VITRO* REASSOCIATION OF EARLY POSTMITOTIC CORTICAL AND STRIATAL NEURONS. L.A. Krushel and D. van der Kooy, Department of Anatomy, University of Toronto, Toronto, Canada M5S 1A8.

Selective adhesion between early postmitotic neurons underlies pattern formation in the telencephalon. The earliest born cortical and striatal neurons form adhesive compartments *in vivo* in the face of the migration of later born (and not selectively adhesive) neurons. The earliest born cortical and striatal neurons (that form the deep cortical and striatal patch compartments, respectively) will selectively adhere together within their own tissue type, after being embryonically removed, dissociated, and reaggregated. Is this selective adhesion specific to neurons within their own tissue type or is a common adhesion molecule expressed on the surface of all early born neurons in the telencephalon? To examine this we cocultured dissociated embryonic cortex and striatum in a reaggregate suspension culture. Would early postmitotic cortical and striatal neurons reassociate according to tissue type or to birthdate? Timed pregnant Wistar rats were injected with 1 mCi ^{3}H-thymidine or 160mg/kg bromodeoxyuridine (BrdU) on embryonic day 13. These injectons label primarily the striatal patch and deep cortical layer cells. Two days later the striata and cortices were separately dissected and dissociated into single cells. Striata containing one label were cocultured with cortices containing the alternate label. After five days in a reaggregate suspension culture, the reaggregates which formed contained relatively small numbers of both cortical and striatal labeled cells. All the labeled cells appeared in clumps within the reaggregates. Within the labeled clumps, the BrdU and ^{3}H-thymidine labeled cells appeared randomly interdigitated. This suggests the existence of an adhesive factor common to early postmitotic telencephalic cells; a factor which may be more fundamental than tissue type preferences and instrumental in the compartmentalization of the forebrain.

39.7

MICROENVIRONMENTAL FACTORS IN NEURAL PATTERN FORMATION: ADDITION OF NEURONS TO DEVELOPING ENTERIC GANGLIA. H.M. Mackey and M.D. Gershon. Dept. of Anatomy & Cell Biology, Columbia Univ. P&S, New York, NY 10032

Neurons are added to the enteric nervous system (ENS), which grows as the bowel enlarges during postnatal life. The organization of the ENS is different from that of other regions of the PNS. The current experiments were undertaken in order to investigate the means by which new neurons are incorporated into enteric ganglia that have already begun to form, and also to test the hypothesis that the enteric microenvironment plays a critical role in determining the uniquely enteric pattern of neural organization. Neuraxial segments were dissected from the trunk region (which does not normally colonize the bowel) of quail embryos (16-20 somites) and co-cultured for 7-10 days with explants of chick gut that contained forming ganglia (ages 4-20 days) as chorio-allantoic membrane (CAM) grafts. Quail cells readily entered the developing ganglia of the gizzard and intestine until day E9. Quail cells also invaded the esophagus until day E12 but localized in the mesenchyme and muscle layers as well as in ganglia. Ultrastructural examination of chimeric ganglia revealed that the chimeric ganglia within the gut had adopted a typically enteric pattern of organization, while those that formed outside the bowel displayed a structure typical of extra-enteric peripheral nerve. Neurons and glia of quail origin were completely intermixed with chick cells in the chimeric ganglia. It is concluded that acquisition of the enteric pattern of organization is critically dependent on the enteric microenvironment, that new neural and glial cells can be added to enteric ganglia even after ganglion formation has begun, that preferential pathways exist within the gizzard and intestine that lead crest-derived cells to appropriate locations in the bowel where ganglia form, and that these pathways are less well developed within the esophagus than in the rest of the bowel. Supported by NIH grant NS 15547 and the Robert Wood Johnson Fdn.

39.8

EXTERNAL GERMINAL CELLS ALIGN ALONG SUBSTRATES IN DEVELOPING CEREBELLAR TRANSPLANTS. E.B. Ezerman. Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

During cerebellar (CB) development in the rat, external germinal (EGL) cells may migrate on axonal surfaces (Hynes et al., 1986) and/or be in contact with basal lamina (Hausmann & Sievers,1985). In intact and reaggregate CB transplants, EGL appears to align along penetrating blood vessels or pial membranes. We have investigated EGL orientation in CB primordia grown in culture prior to transplantation. If CB primordia are grown as explants in culture for 10-14 days prior to transplantation the EGL is less extensively aligned along penetrating blood vessels than in non-cultured transplants. This may result from absence of blood vessels in culture during the early migratory/proliferative period of EGL development. In reaggregated CB primordia grown on a collagen substrate in vitro for 10 days prior to transplantation neither ingrowing blood vessels nor extrinsic axonal surfaces are available as substrates for EGL migration or growth. When such explants + substrate are transplanted to cavities in rat CNS, EGL is observed primarily adjacent to the collagen. There are also laminin-stained glial end feet and processes along the collagen. Thus, EGL cells, initially interspersed throughout the reaggregate transplant, either proliferate along the surface/substrate, migrate to the surface/substrate, or both.

39.9

EVIDENCE OF A SIGNIFICANT ORGANIZING PATTERN FOR THE DEVELOPMENT OF LONGITUDINAL TRACTS IN THE NEURAL TUBE. M.-C. Bélanger*, L. Bertrand*, R. Marchand. Lab. Neurobiol., Hôp. Enfant-Jésus, 1401, 18e Rue, Québec G1J 1Z4 Canada.

In the past, several neuroanatomists perceived that the longitudinal arrangement of the nuclei of the cranial nerves represented a fundamental pattern of organization in the brain stem. Neuroembryologists have also described longitudinal columns in the neural tube. Since the advent of autoradiography, there has been very few studies of the rat brain stem histogenesis. The objectives of the present work are 1) to study the formation of such columns, 2) to identify by ^{3}H-thymidine autoradiography the earliest generated neurons that contribute to their formation, 3) to determine if brain stem nuclei other than the traditional somatic efferent or special visceral efferent nuclei also contribute to their formation, 4) to follow their histogenetic transformations through the different stages of development, 5) to evaluate the possibility that during the very early stages of development, these columns provide a substrate pathway (Katz) for the growth of axons and the setting of various longitudinal tracts.

Many of the earliest generated neurons ($E11_{15}$) are found in the different nuclei of the somatic and visceral efferent columns, and in the motor nucleus of the trigeminal nerve. Neurons of other nuclei like the interstitial (Cajal) and prepositus nuclei are generated simultaneously and are very closely related to the nearby somatic efferent column. During the early stages, all these neurons constitute a continuous column which is intimately related to the developing medial longitudinal fasciculus (mlf). We suggest that this primitive column of neurons might provide a substrate pathway for the setting of the mlf. The development of many other cell groups will also be presented and discussed. (In accordance with CCPA Guidelines). Supported by CRM and FRSQ.

39.10

PROCESS OUTGROWTH IN SITU IS NOT REQUIRED FOR THE ESTABLISHMENT OF POLARITY BY HIPPOCAMPAL NEURONS IN CULTURE. T.L. Fletcher and G.A. Banker. Department of Anatomy, Albany Medical College, Albany, NY 12208.

Hippocampal neurons were labeled at distinct stages of development by administering ^{3}H-thymidine to pregnant rats on either embryonic day 15 (E15) or E18.5. All of the fetuses were sacrificed on E19. Some of the fetal brains were sectioned and examined by autoradiography to determine the location of labeled cells in the hippocampus. The remaining brains were used to prepare hippocampal cell cultures.

Neurons labeled on E18.5 were still confined to the ventricular zone on E19; those labeled on E15 had completed their migration to the cortical plate. Other data suggest that the former cells had not begun process outgrowth, while the latter had begun to elaborate both axons and dendrites. The two populations of cells were equally able to establish axons and dendrites in culture, as judged by morphology and by immunostaining for MAP2, a dendritic marker. Cells that incorporated ^{3}H-thymidine in situ at E18.5 and underwent mitosis in culture also developed axons and dendrites appropriately. These results demonstrate that neurons can establish distinct axonal and dendritic domains in culture regardless of whether they have previously established processes in situ. Cells acquire this ability early in their development, perhaps shortly after their commitment to the neuronal lineage. (Supported by NIH grant NS17112.)

39.11

DEVELOPMENT AND CHARACTERIZATION OF HUMAN NEURONAL CELL CULTURE LINES. J. Nye(1)*, G. Ronnett(1,2), L. Hester(1) and S.H. Snyder(1). Depts. of Neuroscience(1) and Neurology(2), The Johns Hopkins University Sch. of Medicine, Baltimore, MD 21205.

Cell culture lines of human neurons have been established from tissue obtained at the time of hemispherectomies for intractable seizure disorders. Grey matter was dissected, dissociated and plated in wells containing minimal essential medium (MEM)-D-valine with 15% dialyzed fetal calf serum. Of 12 wells, 2 wells, EB1 and EB4, demonstrated spontaneous continued growth and have been passaged for over 6 months. Cells exhibit either bipolar or multipolar morphology; by immunocytochemistry, 100% of cells stain for neurofilament, neuron-specific enolase, tubulin, and are negative for keratin, vimentin, S-100, GFAP, and myelin basic protein. Treatment with several agents, including dibutyryl cAMP, isobutylmethylxanthine and dexamethasone causes cells to stop dividing and develop putative axons with varicosities and dendrites with spines. These lines should be useful to investigate neuronal differentiation.

39.12

DEVELOPMENT OF THE TURTLE CEREBRAL CORTEX IN VITRO. A.M.Wilson*, P.H.Desan and A.R.Kriegstein (SPON: S.Ryan). Neurology Dept., Stanford Med. Ctr., Stanford, CA 94305.

Dorsal telencephalic wall explants were dissected from embryos, anchored to the floor of a Primaria petri dish with a plasma-thrombin clot and maintained in 290 mOsm MEM with 10% horse serum for 0-20 days at 30°C. The explants survived and showed continued morphological and physiological development. Cultures taken at Yntema Stage 16, prior to the formation of the cortical plate (CP), showed abnormal morphological changes, such as continued proliferation of ventricular layer cells without formation of a CP, or formation of a convoluted, discontinuous CP. Cultures explanted at Stages 17 or 18/19, during formation of the CP, frequently showed continued maturation of the CP, although areas of deranged cytoarchitecture also occurred. Cultures from all three stages showed electrophysiological maturation, including the appearance of synchronized burst discharges following bicuculline application, which appears at Stage 21 in normal development. Similar electrophysiological development occurred even in cultures continuously exposed to bicuculline (10 μM) or APV (1 mM). Cultures from Stage 21 maintained normal anatomical and physiological organization for at least 20 days in culture. These observations suggest that both excitatory and inhibitory cortical circuits can develop and be maintained in vitro, and can form despite excitatory and inhibitory receptor blockade. Supported by NIH grants NS00887 and NS21223.

39.13

CEREBELLAR NEURONS GROW AND DIFFERENTIATE RAPIDLY AS INDEPENDENT CELLS IN THREE-DIMENSIONAL CULTURE. P.W. Coates & E.Dunn*. Cell Biol.& Anat.,TTUHSC Sch. Med.,Lubbock,TX 79430.

Monodispersed cerebellar cell suspensions from late gestation rat fetuses were plated at low density onto three-dimensional hydrated native collagen lattices and examined after each of 3 days in culture in order to determine whether cerebellar neurons could grow and differentiate as single neurons without direct cell-cell interactions similar to cerebral neurons (Coates, Dev.Br.Res.25:11,1986). Microscopy revealed cells that were widely dispersed and had distinct neuronal morphologies with long, thin thread-like processes identified as axons or dendrites which continued to lengthen and differentiate further over time. An image analysis system coupled to a phase contrast microscope and a microcomputer was used to measure indices of growth and differentiation. Only phase-bright, healthy neurons with at least one process more than 2 cell diameters long and not in contact with cells of any other kind were selected for measurment of axon and dendritic length alone and combined in μm per neuron, number of primary processes, branch points, segments and terminals per neuron. Data were analyzed by Duncan's analysis of variance. Mean axon length increased significantly on each day in culture. Mean length of the dendritic tree was significantly elevated by day 2. On average, single cerebellar neurons regenerated over 100% more new process growth within three days. Other indices of neuronal differentiation increased significantly on each day of the test period. Results support the hypothesis that in this culture system cerebellar neurons have an inherent capacity to grow quickly and express basic neuronal characteristics as single cells. The system should prove useful for examining intrinsic electrical (see Fermini et al., this volume) and other properties of cerebellar neurons that are not influenced by cell-cell communication. Support from NS20802.

39.14

EARLY MOLECULAR DIFFERENTIATION OF CELLS GIVING RISE TO PERIPHERAL AUDITORY AND OLFACTORY STRUCTURES. S.M. Prouty and P. Levitt. Dept. Anatomy, Med. Coll. Pa., Philadelphia, Pa. 19129.

The peripheral sensory structures of olfaction and audition are in part derived from surface ectodermal placodes. Through a series of morphogenetic and inductive events, specific segments of unspecialized surface ectoderm are committed to differentiate along various pathways. This committment is presumed to be determined by selective expression of gene products which affect cell-cell interaction. In the periphery, a monoclonal antibody generated against membranes from fetal brain cells cross-reacts specifically with these differentiated structures. Immunocytochemical analysis in rats from embryonic day (E) 9 to E18 determined the spatial organization of expression of the 3C2 antigen before and during the period of formation of the sensory organs. Immunoreactivity in all regions and at each age appears to be cell surface associated, with no apparent cytoplasmic staining. We found that the 3C2 antigen is expressed during neurulation in ectodermal regions that give rise to the auditory, olfactory, and hypophyseal placodes. Rathke's pouch and gut epithelium stain transiently during this early period of development. By E12, expression in the periphery is restricted to the otocyst and olfactory pits. Various parts of these epithelia and their derivatives are positive through E18. The lens placode is never immunoreactive. The data suggest that restricted regions of the ectodermal epithelium exhibit molecular specificity early in development, prior to the formation of the neural tube, which may relate to the ultimate fate of the cells in a specific segment. Supported by March of Dimes Research Grant 1-919.

39.15

RELATIONSHIP OF HYALURONIC ACID AND CHONDROITIN SULFATE PROTEOGLYCAN TO THE FAILURE OF NEURAL CREST-DERIVED CELLS TO COLONIZE THE TERMINAL COLON OF *ls/ls* MICE. V.M. Tennyson, R.F. Payette*, H.D. Pomeranz, T.P. Rothman, and M. D. Gershon. Depts. of Anat. & Cell Biol., and Pathol., Columbia Univ. P & S, N Y, N Y. 10032.

The enteric nervous system is derived from precursors that migrate to the gut from the neural crest. The precursors fail to colonize the terminal bowel of the *ls/ls* mouse. Laminin, collagen type IV, and glycosaminoglycans accumulate in the abnormal zone. The ultrastructural distribution of proteoglycans was studied with ruthenium red in the terminal colon of control and *ls/ls* fetuses. Extracellular matrix granules (g; 15-50 nm) and microfibrils (mf; 3-6 nm) were visualized. Digestion with testicular hyaluronidase removed g and mf, but chondroitinase ABC removed only g; therefore, g probably represent chondroitin sulfate (ChS-g), and mf hyaluronic acid (HA-mf). At day E11 ChS-g were juxtaposed to the surfaces of all cells and were also associated with HA-mf. The total number of ChS-g was the same in aganglionic and control gut; however, the ChS-g in apposition to cell membranes were increased 2.5-fold ($p< 0.001$), the packing density of HA-mf was higher, and the average length of HA-mf was 1.5 times as long in the *ls/ls* gut ($p< 0.001$). At day E13 the total number of ChS-g was similar in control and *ls/ls* mice; however, the pattern of distribution of ChS-g and HA-mf differed. In the control, the HA-mf decorated by ChS-g formed a regular intercellular array. In the *ls/ls* gut, HA-mf were much longer, and the microfibrils folded over and became redundant. The average number of ChS-g per μm length of HA microfibrils in the *ls/ls* mouse, moreover, was 3.5-fold greater than in control ($p<0.001$). At day E16 the innermost circular muscle cells were hypertrophic and the presumptive longitudinal muscle cells were separated by lakes containing abundant ChS-g and HA-mf in the aganglionic region. These observations indicate that HA-mf increase, and that the distribution, rather than the amount, of ChS is distorted in the path of migrating crest-derived cells in *ls/ls* mice. Supported by grants #HD 17736, NS 15547, HD 21032, the March of Dimes and the Parkinson's Disease Fdns.

39.16

IS THE PURKINJE CELL AN INTRINSIC SITE OF WEAVER GENE ACTION. R. Smeyne & D. Goldowitz. Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA. 19107.

Several cellular defects have been reported in the cerebellum of the homozygous weaver mutant mouse. These include a virtual absence of midline granule cells, abnormal Bergmann glia, and an approximately 50% decrease in the number of midline Purkinje cells (PCs). The loss of PCs has been reported as early as post-natal day 5. It is not known, however, if the PC number deficit is the result of a primary (i.e gene action intrinsic to the PC) or secondary (i.e. gene action extrinsic to the PC) site of mutant gene expression. This can be answered through the analysis of chimeric mice. In order to determine what is/are the primary site(s) of mutant gene action we constructed tetraparental chimeras from wv/wv and +/+ embryos. Cells from these two genotypes can be differentiated by the high (Gus^b, wv/wv) or low (Gus^a, +/+) expression of β-glucuronidase activity. Four chimeric animals, 2-4 months of age were perfused, the brains removed, embedded in wax, sectioned at 8 microns and stained for the presence of β-glucuronidase activity. Every 10th section was analyzed for number of Gus^b and Gus^a PCs. Controls were age matched +/+ and wv/wv mice. The phenotype of the each of the 4 chimeras analyzed suggested about a 90% contribution of wv/wv cells. Two formulas were used to assess the intrinsic (only wv/wv PC affected) or extrinsic (both wv/wv and +/+ cells equally affected) action of the weaver gene upon the PC. Newman-Keuls analysis of the 4 chimeric cerebella demonstrate no statistical differences from that predicted by either equation. The results, however, fit the extrinsic model better than the intrinsic model of weaver gene action. Further analysis of wv/wv<--->+/+ cerebella, containing a higher percentage of +/+ cells should better clarify whether the weaver gene intrinsically or extrinsically affects PCs to result in a reduced PC number.

39.17

FINE STRUCTURE OF SURVIVING +/+ CEREBELLAR PURKINJE CELLS IN LURCHER CHIMERIC MICE. K.W.T. Caddy and K. Herrup. University College London, London, England and E.K. Shriver Center, Waltham, MA.

Lurcher is an autosomal dominant mutation in the mouse that causes the cell autonomous degeneration of all +/*Lc* Purkinje cells (PCs). Lurcher ↔ wild type chimeras, therefore, contain only wild type PCs. Previous Golgi analysis of such chimeras revealed that these surviving +/+ PC have a grossly malformed dendritic tree (Caddy et al., *Neurosci Abst.* 12:1583). Electron microscopic examination has now been performed and shows both subtle differences in the organelles of the PC soma (compared to wild type mice or non-mutant chimeric mice) and also confirms our impressions from Golgi images that there is a deficit of PC dendrites in the upper part of the molecular layer. The organelle changes involve primarily the quantity of lysosomes and the organization of the rough endoplasmic reticulum. There are only a small number of lysosomal profiles in PCs from control animals (1 or 2 per section); in the lurcher chimeras, the number is usually greater than 10 per section. Further, rough ER, organized into Nissl bodies, is difficult to find in PCs from lurcher chimeric mice. The upper molecular layer contains a qualitatively normal density of parallel fiber profiles despite a clear reduction in the number of PC dendrites. Synaptic profiles are apparent, but preliminary analysis suggests that they are parallel fiber:stellate cell contacts. An ongoing analysis will quantitate the changes in the PC dendrites and further analyze the synaptic diversity in the cerebellar cortex of the lurcher chimera.

Supported by Action Research for the Crippled Child (KWT) and the NIH (NS-20591) and the March of Dimes Birth Defects Foundation (KH).

39.18

GLIAL ORGANIZATION IN THE DEVELOPING REELER NEOCORTEX. J.F. Gadisseux*, Ph. Evrard*, J.P. Misson*, V.S. Caviness,Jr. E.K.Shriver Center, Waltham, MA 02254.

Radial glial fibers(RGF) were identified in the reeler developing murine neocortex by immunostaining with RC2 antibody and, for EM study, preservation of glycogen content. Glial fiber organization within the cerebral wall at E14-15 is essentially identical in reeler and normal mice. The RGFs are mostly grouped in fascicles which ascend radially or slightly obliquely across the cerebral wall. The oblique transcortical axonal bundles and the malorientation of the cortical neurons are not associated with abnormalities of RGF orientation and grouping. Complete transformation of the glial fascicles to single fibers which occurs in the normal animal after E15, does not occur in reeler neocortex. As late as P0, many glial fibers remain grouped in fascicles across the reeler cortical plate(CP). This glial pattern in the CP of the reeler neocortex is similar to the pattern encountered in the subplate of the normal animal.Reduction in RGF density across the span from the ventricular to the pial surfaces occurs to a lesser degree and in a non-strata specific, more gradual way in the reeler mice. Thus many RGFs, like their counterparts in the normal animal, appear not to span the full width of the cerebral wall. Rather a substantial complement appear to extend no further than the upper zone of the CP. Selectively stained large growth cones at the tips of the transcortical glial fibers are abundant at this level of the mutant CP and superplate.

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39.19

ELECTRON MICROSCOPIC ANALYSIS OF ECTOPIC GRANULE CELLS IN THE DENTATE GYRUS OF NZB/BINJ MOUSE. P.J. Costello,* M. Sekiguchi* and R.S. Nowakowski (SPON: N.L. Hayes). Dept. of Anatomy, UMDNJ-Robert Wood Johnson Medical School, and the Cell & Developmental Biology Program, Rutgers University, Piscataway, NJ 08854, and Dept. of Anatomy, Tokai University School of Medicine, Kanagawa, JAPAN.

In the molecular layer of the dentate gyrus of the NZB/BINJ mouse there are ectopic granule cells which occur singly or in clusters of two or more. In previous light microscopic studies we have demonstrated that in the clusters of ectopic granule cells the granule cells appear to be in direct contact with one another, and that glial cells are frequently found closely apposed to the granule cells. For this study, we have used electron microscopy to analyze the ectopic clusters. Electron micrographs showed that the plasma membranes of two ectopic granule cells are in direct contact with one another. The extent of granule cell to granule cell contact, however, seemed to be less than that which is found between normally positioned granule cells, presumably because of differences in the number of neighbors each granule cell has. In some places astrocytes extend processes which wrap around the peripheral aspects of the cluster, seemingly encasing it. Finally, our ultrastructural analysis reveals that on the surface of ectopic granule cells there are axosomatic synapses. Thus, we conclude: 1) that although the contacts of the ectopic granule cells in the molecular layer with each other and with astrocytes may be qualitatively normal it may not be quantitatively identical to those of normally positioned granule cells and 2) that the ectopic granule cells are contacted by synaptic inputs from, as yet, unidentified sources.

(Supported by NIH Grant NS23647 and a grant from the UMDNJ Foundation.)

39.20

A QUANTITATIVE STUDY OF THE DISTRIBUTION OF ASTROCYTES IN THE MOLECULAR LAYER OF THE DENTATE GYRUS IN C57BL/6J AND NZB/BINJ MICE. P.R. Patrylo and R.S. Nowakowski, Department of Anatomy, UMDNJ-Robert Wood Johnson Medical School, and The Physiology/Neurobiology Program, Rutgers University, Piscataway, New Jersey 08854

In the molecular layer of the dentate gyrus of NZB/BINJ mice, ectopic granule cells occur either as single cells or as clusters. In contrast, ectopic granule cells occur rarely in the molecular layer of the dentate gyrus of C57BL/6J mice. Previously, we have shown that in the vicinity of ectopic granule cells in NZB/BINJ mice astrocytes display a non-stellate morphology and are seen to aggregate around the cluster. Here we show that the distribution of astrocytes in the molecular layer of NZB/BINJ and C57BL/6J mice is quantitatively different. For this study, the astrocytes were stained by the Cajal gold chloride-sublimate technique, and camera lucida drawings were made of the granule cell and molecular layers. The molecular layer was divided into thirds, and the astrocytes in each third were counted. In every C57BL/6J mouse studied the inner third of the molecular layer had fewer astrocytes than the mean of the outer and middle thirds, whereas in NZB/BINJ mice the inner third of the molecular layer, in general, had more astrocytes than the mean of the outer and middle thirds. A Mann-Whitney U test established that this difference in the distribution of astrocytes is significant (U=36, $p<0.003$). Experiments are currently being done to determine the developmental events which may lead to such a situation.

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EXCITATORY AMINO ACIDS I

40.1

KAINIC ACID INCREASES INTRACELLULAR CALCIUM IN MOUSE STRIATAL NEURONS BY TWO MECHANISMS . Shawn N. Murphy* and Richard J. Miller, (SPON: D. U'Prichard) Univ. of Chicago, Chicago, IL 60637

$[Ca^{2+}]_i$ in single mouse striatal neurons grown 14-28 days was measured using the fura-2 technique. Changes in $[Ca^{2+}]_i$ during application of kainic acid (KA) were measured in the presence of 1 μM TTX and 10 μM MK-801 while the contents of the bath were rapidly exchanged. KA caused a rapid increase in $[Ca^{2+}]_i$ to >1 μM which decayed over the next 5-10 minutes to a sustained level. Part of this increase was due to voltage sensitive calcium channels (VSCC's) and could be substantially reduced by predepolarization (70 mM K^+) in Ca^{2+} free media in the presence of 10 μM nitrendipine. Increases in $[Ca^{2+}]_i$ due to the opening of VSCC's could also be prevented by maintaining the cell in 70 mM K^+ and allowing $[Ca^{2+}]_i$ to come to a new equilibrium prior to the application of the agonist. When either of these two methods were used to block VSCC's, KA still caused increases in $[Ca^{2+}]_i$ in these neurons. The increases in $[Ca^{2+}]_i$ caused by KA under these conditions had a slow onset and reversal (30-60 secs). They were dose dependent with an IC_{50} of 201 μM and a maximal $\Delta[Ca^{2+}]_i$ of 360 nM. They were increased when all Na^+ was replaced with N-methyl-D-glucamine. Sustained increases in $[Ca^{2+}]_i$ due to activation of KA receptors were at least as large as increases in $[Ca^{2+}]_i$ seen upon activation of NMDA receptors and could be involved in KA induced neurotoxicity.

40.2

A UNIQUE GLUTAMATE RECEPTOR REGULATES INTRACELLULAR CALCIUM MOBILIZATION IN HIPPOCAMPAL NEURONS. Richard J. Miller and Shawn N. Murphy*, (SPON: P.Huttenlocher) Univ. of Chicago, Chicago, IL 60637.

$[Ca^{2+}]_i$ in single mouse hippocampal neurons cultured 3-14 days was measured using the fura-2 technique. Activation of glutamate receptors with kainate (KA), N-methyl-D-aspartate (NMDA), or a-Amino-3-hydroxy-5-methylisoxazole propionate (AMPA) increased $[Ca^{2+}]_i$ in Ca^{2+} containing medium. However, on removal of Ca^{2+} and addition of EGTA (20 μM) these glutamate agonists were no longer effective in increasing $[Ca^{2+}]_i$. In contrast, transient (15-30 sec) increases in $[Ca^{2+}]_i$ (300±100 nM) could be obtained with quisqualate (QUIS) (EC_{50}=100 nM), ibotenate (EC_{50}=100 μM), and glutamate (EC_{50}=30 μM). Similar transients were produced by the a_1 agonist phenylephrine (PhE) in Ca^{2+} free medium. Following the production of a single response, subsequent additions of QUIS were ineffective in producing responses unless intracellular stores were refilled by loading cells with Ca^{2+} following depolarization in Ca^{2+} containing medium. Responses to QUIS showed no tendency to desensitize while responses to PhE did. Responses induced by 1 μM QUIS could not be antagonized by 1 mM AP4, GDEE, or GAMS; nor could they be attenuated by co-application of QUIS with NMDA or KA. The responses were not decreased in Na^+ free medium or sensitive to TTX. The responses did decrease to ≤10% of their original value when bathed in Ca^{2+} free medium for >9 minutes possibly reflecting the depletion of Ca^{2+} stores. Thus hippocampal neurons appear to possess a unique glutamate receptor which is linked to Ca^{2+} mobilization rather than an ion channel. It is likely that this is a consequence of glutamate stimulated synthesis of IP_3 previously described.

40.3

INTRACELLULAR HIGH-ENERGY PHOSPHATES PREVENT WASH OUT OF NMDA CURRENTS IN CULTURED HIPPOCAMPAL NEURONS. J.F. MacDonald, M.W. Salter & I. Mody. Playfair Neurosci. Unit, The Toronto Hosp.& Dept. Physiol., Univ. of Toronto, Toronto, Ont., M5T 2S8

Whole-cell recordings from cultured mouse hippocampal neurons were used to study changes during intracellular dialysis of currents evoked by NMDA receptor activation. Recording electrodes (3-5 MΩ) contained (in mM): 120 CsCl, 35 CsOH, 10 HEPES, 11 EGTA, ± 1 mM $CaCl_2$. Pressure applications of L-aspartate (ASP; 100-250 μM) were used to elicit NMDA responses. A *support system* [2 TEA, 2 $MgCl_2$, 4 Tris-ATP, 20 Creatine Phosphate and 50 U/ml Creatine Phosphokinase], found to retard the gradual decline ("wash out") of voltage-dependent Ca^{2+}-currents, was added to the recording electrode in some cases.

When the *support system* was omitted, ASP-currents washed out by about 50% to a steady state value within 20 min from the start of recording. This wash out was prevented or reversed by inclusion of the *support system*. In all cases, ASP-currents could be blocked by NMDA antagonists or channel blockers. Currents evoked by kainate were stable in the presence or absence of the *support system*.

Thus, NMDA currents likely have two components: one which requires the presence of intracellular high-energy phosphates and another which does not.

Supported by the MRC of Canada. IM is an MRC Fellow; MWS is an Eastburn Fellow.

40.4

RELEASE OF INTRACELLULAR CALCIUM FOLLOWING ACTIVATION OF EXCITATORY AMINO ACID RECEPTORS IN CULTURED HIPPOCAMPAL NEURONS. I. Mody[1], J.F. MacDonald[1] & K.G. Baimbridge[2]. [1]Playfair Neurosci. Unit, The Toronto Hosp. & Dept. of Physiol., Univ. of Toronto, Toronto, Ont. and [2]Dept. of Physiol., Univ.of Brit. Columbia, Vancouver, B.C.

Intracellular calcium ($[Ca^{2+}]_i$) was measured using fura2 fluorescence in 7-12 day old cultured rat hippocampal neurons. After fura2-AM loading and a 30 min wash, neurons were placed in a small volume (<400 μl) rapid (2 ml/min) perfusion chamber at 22°C. The extracellular solution contained (in mM): Na-Isethionate or NaCl 144, KCl 5, D-Glucose 5.6, HEPES 10, $CaCl_2$ 1.8, TTX 0.5-1 μM and glycine 1 μM. Small volumes (25-50 μl) of excitatory amino acids (3-100 μM) dissolved in extracellular medium were rapidly injected into the chamber.

L-glutamic, L-aspartic, and N-methyl-D-aspartic acid caused rapid, reversible and concentration-dependent two- to ten-fold increases in $[Ca^{2+}]_i$. Perfusion of dantrolene-Na (15-20 μM) or 2,3-butane dione-monoxime (10-20 mM), drugs that antagonize release of Ca^{2+} from the sarcoplasmic reticulum, reversibly blocked the amino acid induced increases in $[Ca^{2+}]_i$. In voltage-clamp studies this effect could not be attributed to blockade of the receptor/channel. Thus, a significant portion of the rise in $[Ca^{2+}]_i$ produced by excitatory amino acids may be due to the release of Ca^{2+} from intracellular stores, possibly from the endoplasmic reticulum that has been known to function as a Ca^{2+} reservoir in neurons.

40.5

ACTIONS OF GLUTAMATE RECEPTOR AGONISTS ON INTRACELLULAR FREE CALCIUM $[Ca]_i$ IN CULTURED TURTLE CEREBELLAR NEURONS.

A.W. Krause, I.C. Haralabatos, and T.J. Sejnowski. Department of Biophysics, The Johns Hopkins University, Baltimore, MD 21218

Glutamate (GLU) receptor activation, particularly the N-methyl-D-aspartate (NMDA) subtype, is thought to affect long-term neuronal plasticity via modulation of ($[Ca]_i$). We have investigated this modulation using the $[Ca]_i$-sensitive fluorescent dye fura-2, in cerebellar cultures from the turtle.

Turtle cerebella were dissected from 2-6 week embryos, minced, then dissociated by trituration. Cells were incubated at 33° C in 3% CO_2 at pH 7.4-7.6 in a modified Basal Eagle's Medium supplemented with 10% fetal bovine serum on coverslips coated with polylysine and/or polyornithine. Cells at 2-25 days in culture were stained 1-2 hours with 5 μM fura-2AM, postincubated without dye at least 30 min and then observed on an inverted microscope at 22-24° C with continuous perfusion of turtle Ringer saturated with 95% O_2/5% CO_2. Koehler epifluorescence excitation (with 10 nm bandpasses) at 380 nm and either 345 or 350 nm was used with aperture-limited detection of fluorescence by a photon-counting PMT for maximum sensitivity.

Granule and Purkinje neurons and glia were studied as identified by morphology and parallel examination of cell-specific antibody staining. GLU, NMDA, kainate, and aspartate were applied from pressure micropipettes or by bath perfusion. In these neurons, GLU and NMDA frequently induced long-term $[Ca]_i$ elevation (>30 min) from resting levels (60-120 nM) to from 250 nM to >1 μM. Kainate and aspartate generally elicited fewer and more transient (10 sec - 5 min) $[Ca]_i$ increases. Surprisingly, 2-amino-5-phosphonovalerate (APV) also increased $[Ca]_i$, even in turtle Ringer without calcium (including 5 mM of the $[Ca]_i$ chelator EGTA), as did GLU and NMDA. Thus, these compounds can cause calcium release from intracellular stores. Glia rarely responded to these compounds and $[Ca]_i$ changes were usually slight (<50 nM). Oscillations in neuronal $[Ca]_i$ were sometimes observed with or without drug application. (Supported by NSF grant to TJS)

40.6

DEVELOPMENT OF EXCITATORY AMINO ACID AND SUBSTANCE P RECEPTORS IN RAT SPINAL CORD CELLS MONITORED BY CHANGES IN $[Ca^{2+}]_i$.

A.B. MacDermott, I. Schieren*, M. Womack*, and T.M. Jessell. Howard Hughes Medical Inst., Columbia U, CPS, New York, NY 10032.

Presumptive neurons in the rat spinal cord go through final cell division from embryonic day 10 (E10) to E15. As the neurons differentiate they begin to form synaptic connections with appropriate inputs and targets. We have studied the development of the response to excitatory amino acids (EAA) and substance P (SP) in populations of dissociated spinal cord cells from E13 to postnatal day 6 (P6) rats by following agonist-induced changes in $[Ca^{2+}]_i$. Voltage-gated calcium channels are an important route of calcium entry during agonist activation. The development of these channels was studied using high K^+ for activation. Measurements have been made by loading spinal cord cells, acutely dissociated by incubation in 0.06% trypsin, with the calcium indicator indo-1 and measuring responses in populations of cells using a flow cytometer.

At E13, there was no response to 72 mM KCl or to any agonist tested. By E15, >30% of the cells responded to K^+ while by E18, up to 65% responded. The development of the responses to kainate, quisqualate and glutamate shows a similar time course, although the increase in $[Ca^{2+}]_i$ elicited by kainate is much greater than that elicited by the other two agonists. 0.5 mM La^{3+} blocked the calcium response to K^+, kainate, quisqualate and l-glutamate. The response to NMDA/glycine was minimal at all ages suggesting that this class of receptors is trypsin sensitive, or selectively localized on the neurites or both. The number of cells responding to SP also increased from 0 to >35% of the population between E13 and birth. Thus, between E13 and P6, EAAs and SP caused elevation of $[Ca^{2+}]_i$ in an increasingly large subpopulation of spinal cord cells, with the response reaching a maximum near E18–E20.

40.7

DIFFERENTIAL DISTRIBUTION OF EXCITATORY AMINO ACID RECEPTORS ON EMBRYONIC RAT SPINAL CORD NEURONS IN CULTURE.

O. Arancio and A.B. MacDermott. Dept. of Physiology and Howard Hughes Med. Inst., Columbia U., CPS, New York, NY 10032.

The consequences of transmitter action depend not only on the properties of the receptor/channel, but also on the location of the receptor on the postsynaptic cell and its proximity to the presynaptic release site. To study the physiological localization of excitatory amino acid (EAA) receptors, we have measured the relative sensitivity to kainate, quisqualate and NMDA at the soma and along the neurites of rat spinal cord cells, plated at E14 and grown in culture for 8–18 days. Neurons were placed in a rapidly flowing bath (24°C) which kept the cells healthy for >8 hours. Recordings were made using whole cell patch recording under voltage clamp while drugs were applied through thin-tipped (<1μ id) puff pipettes. Two agonists were compared at each activation site, with each cell serving as its own control. Localization of drug application was confirmed with a fluorescent tracer dye.

All of the cells studied in culture responded to all three agonists when applied on the soma or on the neurites. As a rough indication of response decay over distance, we made local applications of 150 mM KCl along the neurite. The amplitude decreased >50% over 50 um from the soma. In contrast, responses to EAA agonists were often larger on the neurite than at the soma, even at 125 um distance. In 31% of the cells, the response to 1 mM NMDA was larger on the neurites than on the soma, and in 44% of the cells the response was larger at a more distant site on the neurite than closer to the soma. Similarly, with 100 uM kainate and 10 uM quisqualate, there were apparent hot spots on the neurites for each agonist. In addition, the variability of agonist sensitivity along the neurite was usually different for the two agonists tested on each neuron. These data support the notion that postsynaptic EAA receptors are differentially distributed on the surface of spinal cord neurons in culture.

40.8

CALCIUM CURRENT THROUGH CHANNELS GATED BY NMDA RECEPTORS.

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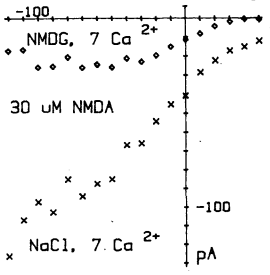
Whole-cell and single channel currents activated by NMDA were recorded from rat neocortical neurons maintained in cell culture. Whole-cell: NMDA and glycine were dissolved in either (mM) 160 NaCl + 0-20 $CaCl_2$ or 160 *N*-methyl-D-glucamine-Cl + 0-20 $CaCl_2$. In 160 NaCl, increasing $[Ca^{2+}]_o$ reduced I at negative potentials. In 160 NMDG-Cl, which did not support current by itself, inward current increased with $[Ca^{2+}]_o$. Inward current carried by Ca^{2+} was best observed with electrodes containing NMDG-methane sulfonate (Fig.). When electrodes contained Cs-methane sulfonate, outward current at positive potentials was much larger in NMDG-Cl + Ca^{2+} than in NaCl + Ca^{2+}. Outward current was larger still in Ca^{2+}-free NMDG-Cl, which suggests that both external Ca^{2+} and Na^+ impede outward flow of Cs^+. Single channel openings of 5-10 pS were observed at negative potentials in outside-out patches exposed to NMDA in 110 Ca^{2+}; channel activity could not be resolved in NMDG-Cl + 2-10 Ca^{2+}. These results suggest that Ca^{2+} carries a small fraction (<10%) of the current recorded in physiological solutions. Supported by Harvard University Society of Fellows.



40.9

MULTIPLE ACTIONS OF CNQX ON EXCITATORY AMINO ACID RESPONSES IN HORIZONTAL CELLS ISOLATED FROM CATFISH RETINA. T.J. O'Dell* and B.N. Christensen. (SPON: B. Haber) Dept. of Physiology and Biophysics, Univ. of Texas Medical Branch, Galveston, TX, 77550.

The effects of 6-cyano-2,3,-dihydroxy-7-nitro-quinoxaline (CNQX) on voltage-clamped horizontal cell responses to kainate (KA), quisqualate (QA), and n-methyl-d-aspartate (NMDA) were examined. CNQX blocked responses to EC50 concentrations of QA (2 μM), KA (75 μM), and NMDA (40 μM) with IC50's of 0.39, 1.29, and 12.1 μM respectively. At QA concentrations ≥ 50 μM, CNQX (≥ 50 nM) both potentiated the peak response and slowed desensitization. CNQX (0.2 μM) enhanced the peak response elicited by 100 μM QA by 31 ± 9% (mean ± S.D., n=7). The time course of desensitization of 100 μM QA responses could be described by a single time constant of 12.48 ± 5.3 msec. (mean ± S.D., n=15). In the presence of 0.2 μM CNQX the current decay was best described by the sum of two time constants with values of 12.54 ± 3.5 and 85.04 ± 16 msec. (mean ± S.D., n=6). A hypothesis accounting for these multiple effects of CNQX on QA-induced currents will be discussed. Supported by grant EY-01897 from Dept. of Health and Human Services to B.N.C. We are grateful to Dr. Tage Honore for a gift of CNQX.

40.10

MECAMYLAMINE ANTAGONIZES THE N-METHYL-D-ASPARTATE ACTIVATED CHANNEL IN ISOLATED HORIZONTAL CELLS FROM THE CATFISH RETINA. B.N.Christensen and T.J. O'Dell* Dept. of Physiology and Biophysics, Univ. of Texas Medical Branch, Galveston, TX. 77550.

Mecamylamine, an ACh channel blocker, was applied under concentration clamp conditions to voltage clamped isolated horizontal cells. This drug completely abolished the inward NMDA or aspartate (ASP) evoked currents at micromolar concentrations. The block was voltage dependent and relieved when the membrane was stepped to positive potentials. The block was dependent on the channel being open by the agonist. MECA did not block membrane currents during application of kainate or quisqualate supporting the notion that the channel activated by these agonists are different from the NMDA receptor/channel complex. Concentration-response curves measured for ASP before and after 50 or 100 μM MECA showed a simple depression in the response indicative of a non-competitive interaction. The K_i calculated from data obtained from a double reciprocal plot gave a value of 38.5 μM. Supported by grant EY-01897 from Dept. of Health and Human Services to B.N.C.

40.11

DIFFERENTIAL EFFECTS OF KA/QA, NMDA and APB RECEPTOR ANTAGONISTS ON OPTIC AND TORAL AFFERENT-STIMULATED ACTIVITY OF GOLDFISH TECTAL NEURONS IN VITRO. E. B. van Deusen and R. L. Meyer, Developmental Biology Center, University of California, Irvine, CA 92717.

We have isolated single goldfish tecta with intact optic and toral marginal fiber tracts in a perfusion chamber where the effectiveness of excitatory amino acid antagonists on tectal field potential responses to stimulation of each afferent system could be tested.

Kynurenate (KYN, 1.0 mM), a mixed antagonist of the kainate (KA) and quisqualate (QA) receptor subtypes, only moderately blocked retinotectal synaptic activity represented by the optic field potential (FPO). 2-amino-5-phosphonovaleric acid (APV, 1.0 mM) which selectively binds the NMDA receptor had minimal effects on either its amplitude or time course. In contrast, 2-amino-4-phosphonobutyric acid (APB, 1.0 mM), an antagonist of the Cl-dependent APB receptor was most effective in blocking 90% of the FPO amplitude. This greater sensitivity to APB than to KYN or APV indicates that dominant retinotectal excitatory transmission may not be mediated by the classical GLU receptor subtypes KA, QA or NMDA.

The marginal fiber field potential (FPM) was more complex, having four major synaptic components. KYN (1.0 mM) selectively blocked the first (4 ms) and third (20 ms) components of the FPM. APV (0.1 mM) simultaneously potentiated the third (20 ms) and blocked the slowest 30 ms component. APB (1.0 mM) was ineffective in blocking the FPM. These results demonstrate that the absence of APB effects on the FPM as well as the incomplete effects of KYN on the FPO were not due to poor drug penetration of the tectum, and support the conclusions that GLU is a transmitter of the marginal fibers and that NMDA may be used by intrinsic tectal circuitry. (Supported by NIH Grant EY06746 to R.L.M.)

40.12

N-METHYL-D-ASPARTATE RECEPTOR-MEDIATED COMPONENT OF FAST AND SLOW EXCITATORY SYNAPTIC TRANSMISSION IN THE RAT SPINAL DORSAL HORN. I. Kangrga, G. Gerber* and M. Randic, Dept. of Vet. Physiology and Pharmacology, Iowa State University, Ames, IA, 50011.

Synaptic transmitters released during activation of primary sensory neurons in the rat spinal dorsal horn may elicit both fast and slow excitatory responses in a single neuron (Urban and Randic, Brain Res., 290:336-341, 1984). Excitatory amino acids (EAA), are believed to be fast excitatory neurotransmitters released during low frequency stimulation of sensory afferents, whereas tachykinins were suggested to be slow excitatory transmitters released following the high frequency stimulation. However, the possible participation of EAA in the high-frequency evoked synaptic responses of individual dorsal horn neurons, that express receptors for EAA and peptides, has not been investigated.

Transverse spinal cord slices (300μm thick) with attached dorsal rootlets prepared from lower lumbar segments from 19day-old rats were used. Intracellular recordings were obtained from the dorsal horn cells using electrodes containing CH_3COOK (4M). The primary afferents were stimulated with single shocks at 1s intervals to elicit "low frequency" synaptic responses and for periods of 1s at 50ms intervals to evoke "high frequency" responses. L-glutamate and N-methyl-D-aspartate (NMDA) were locally ejected by positive pressures from micropipettes. Two antagonists of EAA, kynurenic acid and D-2-amino-5-phosphonovaleric acid (D,APV), were administered via the perfusion medium. In the presence of Mg^{2+} stimulation of the ipsilateral dorsal rootlet at low frequency (6-10V/0.04-0.2 ms) evoked a fast e.p.s.p. which was supressed by kynurenic acid ($2-5x10^{-4}$M) and APV ($0.5-1x10^{-4}$M). During high frequency stimulation the both EAA antagonists abolished the early component of the slow depolarizing potential. In the Mg^{2+}-free medium there was an increase in the peak amplitude, and especially the duration, of the low frequency-evoked e.p.s.p. and the early component of the high-frequency evoked depolarization.

These results indicate that the low-frequency excitatory synaptic transmission in the immature rat spinal dorsal horn is in part mediated by Mg^{2+}- and APV-sensitive NMDA receptors. In addition, tachykinin-mediated presynaptic release of EAA or postsynaptic activation of NMDA receptor system may participate in the high- frequency excitatory synaptic transmission. Supported by NSF and USDA.

40.13

PHORBOL ESTERS ENHANCE RESPONSIVENESS OF RAT DORSAL HORN NEURONS TO N-METHYL-D-ASPARTIC ACID. G. Gerber*, P. D. Ryu and M. Randic, Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames, IA 50011.

Excitatory amino acids (EAA), L-glutamate and L-aspartate, appear to be the major excitatory neurotransmitters in the mammalian spinal cord. Since molecular mechanisms underlying the EAA-induced depolarization of the rat dorsal horn neurons are not presently understood, we analyzed possible involvement of protein kinase C in regulation of signal transduction at various subclasses of EAA receptors by using phorbol esters.

Transverse spinal cord slices from 16-20-days-old rats were used. We have recorded intracellularly from spinal dorsal horn neurons while L-glutamate, N-methyl-D-aspartate(NMDA), kainate and quisqualate were ejected extracellularly by positive pressure from microelectrodes. Two exogenous activators of PKC, 4-β-phorbol-12,13-dibutyrate (PDBu) and 4-β-phorbol-12,13-diacetate (PDAc), and an inactive phorbol analogue, 4-α-phorbol-12,13-didecanoate, were applied by bath perfusion. The bathing fluid contained tetrodotoxin (TTX) to block indirect synaptic interactions.

Dorsal horn neurons were effectively depolarized by all EAA used.PDBu and PDAc ($5x10^{-8}$ to 10^{-6}M for 3 min) produced a small, but prolonged membrane depolarization occasionally preceded or followed by a hyperpolarization. In the presence of TTX, the phorbol esters induced a persistent enhancement of the depolarizing responses of dorsal horn neurons to NMDA(in about 60% of tested cells, n=18), and L-glutamate(in about 25% of cells, n=8), but not to kainate and quisqualate. The enhanced responsiveness to NMDA is potentiated in Mg^{2+}-free solution. 4-α-phorbol-12,13-didecanoate did not modify the responsiveness of dorsal horn cells to either NMDA or kainate or quisqualate.

Our results suggest that the spinal transduction at NMDA receptors might be regulated through the C-kinase system and that this modulation may have physiological relevance. The molecular mechanism whereby phorbol esters enhance signal transduction at NMDA receptors remains to be elucidated. The postsynaptic mechanism, by an increase in receptor binding affinity or in receptor density or an action on the cationic channel associated with NMDA-sensitive receptors, might be considered. Supported by NSF and USDA.

40.14

ELECTROPHYSIOLOGICAL INTERACTIONS OF NMDA AND PCP/SIGMA RECEPTOR AGONIST AND ANTAGONIST IN THE RAT CEREBELLAR PURKINJE NEURONS. Y. Wang and H. K. Lee*. Dept. of Pharmacology, National Defense Medical Center, Taipei, Taiwan (10700), Republic of China

The purpose of this experiment was to study the electrophysiological interactions of PCP and NMDA receptors. Drugs were directly applied to a cerebellar Purkinje neuron of urethane-anethetized rats through a multibarreled pipette. Neuronal activity was recorded extracellularly. We found that NMDA majorly produced excitation in the Purkinje neurons although sometimes it caused inhibition or excitation followed by depression. NMDA-induced responses were blocked by (+)PCMP or dexoxadrol. Interestingly, quisqualate-induced excitation was also antagonized by (+)PCMP although quisqualate had a lower sensitivity to (+)PCMP than did NMDA. We previously found that metaphit irreversibly acylated and blocked PCP-induced reactions, at least, in the cerebellum. After the application of metaphit, we found that metaphit did not antagonize NMDA's reactions whilst PCP-induced depression was blocked. In conclusion, our findings suggested that PCP/sigma agonists antagonized NMDA and quisqualate reactions whereas the presence of intact PCP/sigma receptor was not essential for the NMDA-mediated response in the cerebellum.

40.15

THE NOVEL NEUROTOXIN H_{12}-HISTRIONICOTOXIN (H_{12}HTX) BLOCKS THE N-METHYL-d-ASPARTATE (NMDA) RECEPTOR OF CULTURED HIPPOCAMPUS OF THE RAT. M.T. Lima-Landman* & E.X. Albuquerque. Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201.

H_{12}HTX was initially described as a neurotoxin which noncompetitively blocks the nicotinic receptor, increases the affinity of ACh for its binding sites and causes desensitization (see Ion Channels Vol. 1, Ed. T. Narahashi, Plenum Press, 1988, pp. 95-162). Because some homology has been suggested among different receptors, the effects of H_{12}HTX were tested on NMDA receptors of CNS neurons. Using patch-clamp technique in the out-side-out configuration, it was observed that the amplitude of NMDA-activated currents was voltage-dependent, i.e. amplitude increased with membrane hyperpolarization. In the presence of NMDA (5-20 μM) plus H_{12}HTX (1-100 μM), alteration in the kinetics of the receptor-ion channel coupled with a significant decrease in the frequency and a reduction of about 50% of the mean channel open time were observed. Since H_{12}HTX was dissolved in ethanol, and this agent has well known effects on lipid membrane, the vehicle was tested alone, and a shortening of channel open time was observed at concentrations above 0.5%. Therefore, the final concentration of ethanol was kept below 0.1%. The results of this study suggest some homology between different types of receptor-ion channel macromolecules. (Support: NIH Grant NS25296)

40.16

EXCITATORY TRANSMITTER ACTION IN OLFACTORY BULB CULTURES. Paul Q. Trombley*,† and Gary L. Westbrook. †Dept. Biology, Univ. of Oregon, Eugene & Vollum Institute, Oregon Health Sciences Univ., Portland, OR 97201.

The identity of the excitatory transmitter(s) and postsynaptic receptor/channels in the olfactory system are not well defined. Although L-glutamate is the likely transmitter at afferent and efferent pathways of the olfactory bulb, several small peptides including carnosine and N-acetylaspartylglutamate have been considered as possible transmitters. Likewise although behavioral plasticity in odor-stimulated responses is well documented, the synaptic site and mechanisms mediating this plasticity are also unclear; e.g. both norepinephrine and L-glutamate (via NMDA receptors) have been implicated in olfactory plasticity within the bulb.

Pharmacological studies using extracellular recording have suggested that kainate/quisqualate receptors mediate excitatory transmission in the bulb since kynurenic acid (KYN), but not 2-amino-5-phosphonovalerate (AP5) blocked evoked synaptic responses. However, since NMDA receptors play a neuromodulatory role in hippocampus, it is conceivable that similar mechanisms exist in the olfactory system. To begin to examine this question, we have used dissociated neurons from olfactory bulb of neonatal rats.

Neurons were plated on confluent feeder layers of astrocytes which had been previously prepared from olfactory bulb. Standard methods of whole-cell recording were used to record agonist- and synaptically evoked responses. Extracellular saline contained Mg 0 or 1 mM, and glycine (1 μM). Under current clamp, spontaneous EPSPs were observed which were markedly reduced by KYN or 6-cyano-2,3-dihydroxy -7-nitro-quinoxaline (CNQX) whereas AP5 often reduced, but did not eliminate synaptic activity. In Mg-containing saline, application of NMDA evoked an inward current with a region of negative slope under voltage clamp. Carnosine or homocarnosine(100 μM) did not evoke a response. Supported in part by the McKnight Fdn.

40.17

B-N-OXALYLAMINO-L-ALANINE (BOAA) AND QUISQUALATE ACTIVATE SIMILAR IONIC CURRENTS [1]C.N. Allen, [2]P.S. Spencer and [1]D.O. Carpenter. [1]Wads. Ctr. for Labs & Res., NYS DOH & Sch. of Pub. Hlth., Albany, NY 12201 & [2]Dept. Neurosci., Albert Einstein Col. Med., Bronx, NY 10461.

B-N-oxalylamino-L-alanine (BOAA) is a potent neuroexcitatory amino acid found in the seed of the chickling pea Lathyrus sativus. Use of the chickling pea as a staple food is the etiologic al agent of a form of spastic paraparesis known as lathyrism. Experimental evidence suggest that BOAA's neurotoxic action is mediated by activation of quisqualate or kainiate type excitatory amino acid receptors. Whole cell voltage clamp recordings were made from cultured hippocampal neurons. Quisqualate (50 um), BOAA (50 um) and kainiate (20 um) activated inward currents. Kainate currents activated more slowly and remained at the peak value for as long as the agonist was being applied. The quisqualate current was characterized by rapid activation, decay to a steady state level then further slow decay to baseline following removal of agonist. BOAA had a two phase current similar to that of quisqualate, however the current decay much faster following termination of agonist application. (Supported by NIH Grant NS23087 to D.O.C.)

40.18

NMDA-MEDIATED SYNAPTIC TRANSMISSION IN THE CA1 PYRAMIDAL CELLS OF THE GUINEA-PIG HIPPOCAMPAL SLICE. Y. Sahara, A. Miwa* and N. Kawai*. Dept. Neurobiol., Tokyo Metropol. Inst. Neurosci., Fuchu-city, Tokyo 183, Japan.

Synaptic transmission between Shaffer collateral-commissural pathways and CA1 hippocampal pyramidal cells is considered to be mediated by non-NMDA receptors. Recent studies, however, on cultured hippocampal cells (Forsythe, I.D. & Westbrook, G.L., J. Physiol., 396:515, 1988) suggested that NMDA receptors can be activated during synaptic transmission. In this study, we have characterized NMDA-activated synaptic responses in the guinea-pig hippocampal slice by use of a spider toxin (JSTX), which preferentially blocks quisqualate receptors (Abe, T. et al., J. Physiol., 339:243, 1983), combined with NMDA-antagonists, APV and zinc. Excitatory postsynaptic currents (EPSCs) were recorded from CA1 pyramidal cells by employing a single electrode voltage-clamp. In the vast majority of pyramidal cells, the peak amplitude of the EPSCs increased with membrane hyperpolarization, with a linear current-voltage relationship. JSTX reduced a considerable portion of the EPSCs. The remaining EPSCs showed a marked non-linearity, and were suppressed by APV or zinc. In a small number of pyramidal cells, however, the EPSCs had a non-linear current-voltage relationship. APV or zinc effectively blocked these EPSCs. Our results demonstrate that APV-sensitive NMDA receptors, as well as non-NMDA receptors, are involved in the synaptic transmission of the hippocampus.

40.19

EFFECT OF (-)PHYSOSTIGMINE (PHY) AND NARCOTIC ANTAGONIST (+)BENZYLCARBAMOYLESEROLINE (BCE) ON NMDA AND QUISQUALATE RECEPTORS IN MAMMALIAN BRAIN. S.S. Deshpande* & E.X. Albuquerque. (SPON: P. Sokolove) Dept. Pharmacol. & Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

The beneficial effect of PHY in reducing convulsions and lethality has been suggested to result from blockade of peripheral AChR (*Ann. N.Y. Acad. Sci.* **505**:226, 1987). OPs and carbamates indeed have shown blockade of ion channels associated with glutamate receptors of insect muscles (*J. Pharmacol. Exp. Ther.* **239**:279, 1986). Hippocampal and brain stem pyramidal neurons (E18) in tissue culture were used to study single channel currents (under outside-out condition) activated by NMDA (5-10 μM) and quisqualate (20 μM). Single channel conductance and mean open times of these currents in brain stem neurons were similar to those observed in hippocampus. As in the hippocampus, NMDA channels in brain stem neurons were sensitive to block by Mg^{++} (0.1 mM) and PCP (5 μM). PHY and BCE (40 μM) apparently did not affect channel conductance or open time for both agonists tested but caused a reduction in frequency of openings of about 30% for NMDA. The low efficacy of these drugs on excitatory synapses in mammalian CNS suggests that blockade of glutamate receptors is not a primary mechanism responsible for protection from OP toxicity. Initial experiments suggest that PHY was able to alter the kinetic properties of the AChR in the CNS. (Supported by U.S. Army Med. Res. & Dev. Comm. Contract DAMD17-84-C-4219)

40.20

DISTINCT ACTIONS OF PHENCYCLIDINE (PCP) AND SOME OF ITS ANALOGUES ON NMDA-ACTIVATED SINGLE CHANNEL CURRENTS OF RAT HIPPOCAMPAL NEURONS. E.X. Albuquerque and A.S. Ramoa (SPON: J.E. Warnick) Dept. Pharmacol. Exp. Ther, Univ. MD Sch. Med, Baltimore, MD 21201 USA, & Lab. Mol. Pharmacol. II, Inst. Biophys. "Carlos Chagas Filho", Fed. Univ. of Rio de Janeiro, Brazil.

PCP is known to block the neuronal excitatory effect of N-methyl-D-aspartate (NMDA). We have examined the action of PCP and some of its analogues on the response properties of single NMDA receptors. Application of NMDA (2-40 μM) to outside-out patches (n=43) of membrane excised from neonatal rat hippocampal neurons in culture elicited ion channel openings which were markedly reduced in frequency and duration in the presence of PCP (2-20 μM) and its behaviorally active analogue m-amino-PCP (5-10 μM). These effects were relieved at positive potentials and were prevented by the simultaneous application of $MgCl_2$ (4-8 mM). MK-801 (PNAS **85**:1307, 1988) and SKF-10,047, which apparently compete for the same binding site as PCP, also showed such effects. In contrast, the behaviorally inactive analogue m-nitro-PCP displayed two distinct effects on NMDA receptors. At low concentration, m-nitro-PCP (5-20 μM) unexpectedly increased the frequency of NMDA-induced openings but had no effect on channel lifetime. At a higher concentration (100-300 μM) m-nitro-PCP reduced channel lifetime and frequency. This blocking effect was relieved at positive potentials. In conclusion, the binding sites for PCP and its analogues have a complex role in the regulation of NMDA receptor function, and their activation may lead either to enhanced or decreased NMDA response. (Support: CNPq-Brazil, U.S. Army Med. Res. & Devel. Comm. Contract DAMD17-84-C-4219, & NIDA Grant DA02804)

HORMONAL CONTROL OF BEHAVIOR I

41.1

INDUCTION BY TESTOSTERONE OF THE AROMATASE IN THE SEXUALLY DIMORPHIC PREOPTIC MEDIAL NUCLEUS AND ACTIVATION OF COPULATORY BEHAVIOR IN QUAIL. J. Balthazart, C. Surlemont*, A. Foidart *and M. Schumacher*. (SPON: FNA), Lab. General and Comparative Biochemistry, Univ. of Liège Belgium.

In Japanese quail, the preoptic area (POA) contains a sexually dimorphic nucleus, the preoptic medial nucleus (POM; bigger in males than females) whose size, as observed in Nissl stain, is decreased by castration and increased by testosterone (T) treatments in adulthood. POM contains high levels of aromatase activity (AA). By the Palkovits punch technique combined with radioenzyme assays, we have shown that AA in the POM is controlled by T. It decreases after castration and is restored to intact levels by T treatments. The same effects are observed in other parts of POA but the magnitude of the changes and the absolute levels of enzyme activity are much smaller and do not always reach significance. The 5α- and 5β-reductases are not affected by these treatments. The AA induction by T in POA is dose and time dependent. Levels of AA seen in sexually mature males are restored in castrated birds by a treatment with 20 to 40 mm silastic T capsules which produce physiological levels of steroid in the plasma. Significant effects are observed within 8 hours of the implantation and the induction is maximal after 48 hours. Activation of copulatory behavior follows a similar time-course but occurs with a delay of 24-48 hours. If T-treated birds receive at the same time silastic implants filled with androstatrienedione, the activation of behavior and the increase of AA in POA are both suppressed. POM is probably implicated in the control of copulation because 27g stereotaxic implants of T in this nucleus but not elsewhere in the POA activate the behavior in castrated males. The increased Nissl staining (suggesting increased protein synthesis) and the induction of AA in POM following T treatment are probably critical steps mediating behavioral activation.

41.2

RELATION OF THE SEXUALLY DIMORPHIC DORSOMEDIAL NUCLEUS TO SEX BEHAVIOR IN MALE MICE. C.K. Wagner and L.G. Clemens. Neuroscience Program and Department of Zoology, Michigan State University, E. Lansing, MI 48824.

The sexually dimorphic dorsomedial nucleus (DM), located in L5-S1 of the mouse spinal cord, innervates the bulbocavernosus muscle (BC) of the perineal area. This muscle has been shown to be important in penile reflexes and successful reproduction in male rats. The present study examined the DM in male B6D2F1 mice, of which a subpopulation continue to exhibit the ejaculatory response (ER) long after castration, (continuers). The percent of males in each group exhibiting the ER 15 weeks after castration was: shams: 100% (n=4), continuers: 52% (n=4), non-continuers: 0% (n=4). Following the last test, lul of 1% CT-HRP was injected bilaterally into the BC. The total number of labeled cells did not differ significantly between groups: shams: 92.5 (8.34), continuers: 101.5 (19.6), non-continuers: 98.0 (20.0). In addition, no decrease in cell number was seen following long term castration, an outcome that differs from that obtained using thionin staining, (Wee & Clemens, Brain Res., 1987). It is suggested that the decrease in cell number seen with thionin stain may reflect a reduction in Nissl substance (i.e. cell activity), rather than degeneration of motoneurons. This study demonstrates that the number of cells in the region of the DM does not reflect the behavioral response to castration.

41.3

EFFECTS OF PREOPTIC AREA LESIONS ON THE SEXUAL BEHAVIOR OF MALE FERRETS. J.A. Cherry and M.J. Baum, Department of Biology, Boston University, Boston, MA 02215.

The effects of bilateral preoptic area (POA) lesions on masculine sexual behavior were examined in adult male ferrets. Males (N=40) were castrated, administered a high (5mg/kg) dose of testosterone propionate (TP) daily for 12 days, and pretested with a receptive female. Ten males then received large cathodal tungsten electrolytic lesions (LL, 2.5 mA for 1 minute) at the border of the POA and the anterior hypothalamus (AH), the site of a small sexually dimorphic nucleus (MN-POA/AH; Tobet *et al.*, Brain Res 364: 249, 1986). Three males were given sham lesions (SH), and 27 males received small lesions (SL, 1 mA for 15 seconds) aimed at the MN-POA/AH. For 9 LL males, the lesions destroyed most of the medial POA between the anterior commissure and optic chiasm including all of the MN-POA/AH, and portions of the AH and lateral POA. Sexual behavior was impaired in these males: mean durations of neck gripping, mounting, and thrusting were reduced significantly in post-lesion behavioral tests after 12 days of TP. These results confirm the effects of large POA lesions seen in many vertebrates. 12 SL males sustained some damage (9 bilateral, 3 unilateral) to the MN-POA/AH, while the rest of SL males had lesions scattered nearby. No lesions completely destroyed this nucleus bilaterally. No deficits in sexual behavior were observed in any SH and 24 SL males. However, in 3 males with bilateral MN-POA/AH damage, thrusting was substantially reduced despite high levels of mounting and neck gripping. Thus, large lesions including but not restricted to the MN-POA/AH impaired all aspects of mating; incomplete MN-POA/AH damage had limited effects.

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41.4

CRUISING, AN INDEX OF SEXUALLY MOTIVATED SEARCH BEHAVIOR IN MALE RATS: ACQUISITION, EXTINCTION, AND DISRUPTION BY NEUROLPETIC DRUGS. J.G. Pfaus, S.D. Mendelson, and A.G. Phillips. Dept. Psychology, Univ. British Columbia, Vancouver, B.C. V6T 1Y7 Canada.

Sexual motivation in male rats is commonly inferred from measures of sexual behavior that are either confounded by the receptivity of the female (e.g., intromission latencies) or that preclude the full expression of copulatory behavior (e.g., maintenance of mounting behavior following penile anesthesia). In the present experiments, we characterized a form of sexually motivated search behavior, or cruising, that male rats exhibit by themselves during a 5 min habituation period prior to the introduction of a female in the bilevel testing chambers developed by Mendelson and Gorzalka (Physiol. Behav., 39:67, 1987). Cruising behavior consisted of regular bouts of level changes that developed in both sexually experienced and naive male rats only if sexually receptive females were presented after the 5 min habituation period. Once established, cruising behavior could be partially extinguished if nonreceptive females or no females were presented after the habituation period. Finally, cruising behavior was significantly attenuated by a dose of haloperidol (.1 mg/kg) that had no effect on intromission latencies. We conclude that cruising behavior represents a sensitive and unambiguous form of sexual motivation in male rats that depends, in part, on the functional integrity of brain dopamine systems.

41.5

Electromyographic activity of rat perineal muscles during sexual behavior. G. M. Holmes, B. D. Sachs, W. D. Chapple[1], R. E. Leipheimer. Depts of Psychology and [1]Physiology and Neurobiology, Univ of Connecticut, Storrs, CT 06268.

The bulbocavernosus muscle (mBC) augments erections of the glans penis, whereas the ischiocavernosus muscle (mIC) augments erection and anteroflexion of the penile body, which allows for intromission. The EMG signal from these muscles reveals that in mounts with intromission, mIC activity precedes the onset of mBC activity. Mounts without intromission are characterized by either strong activity in the mIC with minimal mBC activity, or strong mBC activity which precedes the onset of mIC activity. Rectified integrated signals demonstrate highly consistent mBC activity across different intromissions, whereas activity during mounts is quite variable in both the duration and the energy of the burst. Ejaculations are accompanied by consistently stronger mBC activity than are other copulatory events, and are followed by a series of post-ejaculatory bursts lasting for 10-20 sec. Comparisons are made between EMG activity during penile actions within and outside of the context of copulation.

41.6

Bulbocavernosus muscle activity during sexual behavior in estrogen-maintained castrated rats. B. D. Sachs, G. M. Holmes. Department of Psychology, University of Connecticut, Storrs, CT 06268.

Previous research has demonstrated that castrated male rats exhibit declining levels of copulatory activity, and their penises and perineal muscles atrophy. Administration of estradiol (E2) maintains the motor patterns of copulation. However, E2 does not prevent atrophy of the penis or the penile muscles, nor does it maintain reflexive erections tested outside the context of copulation. We analyzed EMG activity recorded from the bulbocavernosus muscle (mBC) in E2-maintained males that had been castrated 4-8 weeks earlier. This muscle displayed EMG activity during copulation, and peak amplitude and burst duration did not differ from those of intact males. In addition, mBC activity recorded during genital grooming bouts was accompanied by visually confirmed erection of the glans penis. These results suggest that E2-maintained males are capable of penile erection during copulation for at least several weeks after castration.

41.7

FACTOR ANALYSIS OF COPULATORY BEHAVIORS IN HAMSTER AND RAT. M. Miernicki, J.B. Powers and J.T. Clark. Dept. of Psychology, Vanderbilt Univ. and Dept. of Physiology, Meharry Med. Coll., Nashville, TN 37240

Male Syrian hamsters require olfactory stimulation to mate. This may be derived from their anogenital investigation (AGI) of females. The latter behavior may be controlled quite separately from copulation because specific hypothalamic lesions can abolish copulation without affecting chemoinvestigation. To further assess the relations between these 2 forms of reproductive behavior, we performed factor analyses on hamster data and on rat data for comparison.

Copulatory behavior (CB) of 209 hamsters and 653 rats was analyzed using BMDP principal components factor analysis with varimax rotation. Data were obtained from sexually experienced animals prior to any experimental intervention. Measures for analysis included ML,IL,MF,IF,EL,PEI,III and hitrate (HR). For hamsters, 3 chemoinvestigatory measures were also included: AGI rate (secs/min) during CB tests (AGR), the ratio of AGR to total body investigation (AGT), and the time spent investigating female hamster vaginal secretions (FHVS) in a separate test with no female present. Five factors were extracted from the hamster analysis. For 3 of these, CB measures loaded similarly to previous analyses of male rodent sexual behavior. These factors were Copulatory Rate (MF,HR,III,EL,IF), Initiation (ML,IL) and Intromission Count (IF,EL,PEI). Chemoinvestigatory measures loaded onto 2 additional factors; [a] (AGT,AGR) and [b] (FHVS,PEI). The rat analyses generated similar results except that 4 factors were extracted: Copulatory Rate (EL,III,PEI), Hit Rate (HR,MF), Initiation (ML,IL) and Intromission Count (IF,III,MF). These findings suggest, in contrast to our previous assumptions, that hamster chemoinvestigation does not reflect arousal or initiation.

41.8

AGE-RELATED DEFICITS IN MALE SEXUAL BEHAVIOR ARE ACCOMPANIED BY CHANGES IN ANDROGEN METABOLISM AND BINDING IN BRAIN. K.C. Chambers, J.E. Thornton, and C.E. Roselli. Dept. of Psychology, USC, Los Angeles, CA 90089 and Dept. of Physiology, OHSU, Portland, OR 97201.

Decreases in sexual behavior in aging male rats do not appear to be due to decreases in testosterone (T) levels as exogenous T cannot restore the behavior. To determine if these behavioral deficits are due to changes in neuronal sensitivity to T, androgen metabolism and binding were measured in the brains of 14 old (25 months) and 14 young (3 months) male Fischer 344 rats. Both groups were given 7 weekly tests of sexual behavior. Blood was then collected and brains and pituitaries were frozen on dry ice for subsequent simultaneous measurement of aromatase activity (AA) and cytosolic (ARc) and nuclear (ARn) androgen receptors in the preoptic area (POA), basal hypothalamus (BH) and amygdala (AMY). None of the old males mounted, intromitted or ejaculated in any of the tests whereas all of the young males ejaculated in at least 2 tests. T levels were significantly lower in old males ($p<.05$). AA was lower in POA ($p<.05$) but not BH or AMY of old versus young males. No age differences were observed in ARc levels in these brain areas, however old males had significantly lower levels of ARn in POA and BH ($p<.05$) than young males. To what extent the behavioral deficits in aged males are related to these changes in brain metabolism and binding is yet to be determined. HD 20970 and 23293

41.9

NEUROENDOCRINE CORRELATES OF AGGRESSIVE BEHAVIOR IN MALE GOLDEN HAMSTERS. K.M. Levy, B.N. Bunnell, E.H. Mougey, and J.L. Meyerhoff. Dept. Psychology, U. GA, Athens, GA 30602 and Dept. Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

In order to explore the neuroendocrine concomitants of various agonistic behaviors, two experiments were carried out. In the first, male hamsters were implanted with indwelling jugular catheters and paired with a dominant, ovariectomized female. Defeated males exhibited elevated plasma levels of adrenocorticotropin, cortisol, corticosterone, and beta-endorphin.

Several reports have suggested that pituitary-adrenocortical and opiate responses to defeat are greater in mice that are submissive than in those that are dominant. In Experiment 2, catheterized, male hamsters were paired, and the hormonal responses of the dominant and submissive animals were examined. The results did not support the hypothesis that losing is more "stressful" than winning in terms of pituitary-adrenocortical activation (using cortisol as an index). Cortisol was equally elevated in both dominant and submissive hamsters. The responses of various peptides are being explored to assess the extent of the difference between hamsters and mice and to characterize the correlates of both acute and chronic agonistic interactions in golden hamsters.

41.10

RETENTION OF COPULATORY BEHAVIOR AFTER CASTRATION AFFECTS THE EXPRESSION OF AGGRESSIVE BEHAVIOR IN THE MALE HYBRID B6D2F1 HOUSE MOUSE (Mus musculus). K.L. Sinchak*, B.E.F. Wee and L.G. Clemens (SPON: G. Lew). Dept. Zool., Mich. St. Univ. E. Lansing, MI 48224.

Male mice of the B6D2F1 hybrid strain retained the ejaculatory reflex up to one year after castration (McGill and Manning, An. Beh. 24:507-518, 1976; Clemens, et al. Phys. Beh. 42:69-76, 1987). In contrast, aggressive behavior in the B6D2F1 was nearly abolished 14 weeks after castration (Sinchak, Wee and Clemens Con. Repro. Beh. Abst., 1988). In the present study we investigated the influence of copulatory experience on the expression of aggression in castrated B6D2F1 males. When tested in a neutral arena, castrated males exhibited the ejaculatory reflex on 38% of the tests; when tested in their home cage, they achieved an ejaculatory reflex on 65% of the tests. Castrated males that copulated in the neutral arena were aggressive in 50% of the tests 23-28 weeks after castration. Those males that copulated only in the home-cage were aggressive in 28% of the tests. Males that exhibited minimal copulatory behavior in both the arena and the home cage were aggressive in only 12.5% of the tests. While these data indicate that continued copulation facilitates aggression, it is not clear whether these animals are responding to low levels of endogenous androgens or whether these behaviors are independent of hormone action.

41.11

THYROID STATUS AND THE ACQUISITION AND RETENTION OF AN IMMOBILE FLOATING RESPONSE. D. Jefferys and J.W. Funder, Medical Research Centre, Prince Henry's Hospital, Melbourne 3004, Victoria, Australia.

We have previously shown that adrenalectomized (ADX), hypophysectomized (HPX), or ADX/HPX rats show indistinguishable levels of progressive immobility over a 15 minute swimming test. In a 5 min retest 24 hours later, HPX rats show levels of immobility indistinguishable from intact controls (~70%); in contrast, ADX or ADX/HPX rats remain immobile for only ~30% of the time (Jefferys et al. Eur. J. Pharmacol., 92:99, 1983), an effect reversed by glucocorticoids or kappa-specific opioids around the time of test (Jefferys, D. & Funder, J.W. Endocrinol., 121:1006, 1987). In the present studies, rats were given PTU for 1-21 days before test. After 10+ days of PTU, the acquisition of immobility was significantly reduced, as was retention on retest; PTU for 1-5 days was without effect, as was thyroxine (T_4) given to intact rats. The effect of 21 days PTU was totally reversed by concurrent T_4 administration (15 µg/day), by a single 15 µg dose of T_4 1 hr before test, or by 1 mg SKF 94901 immediately before test. We interpret these data as evidence that thyroid hormone is required for both the acquisition and the incorporation of the response; whether the very rapid effect of T_4/SKF 94901 in restoring the response in hypothyroid rats is a central or peripheral effect remains to be systematically explored.

41.12

EFFECTS OF ESTRADIOL AND PROGESTERONE ON BODY WEIGHT AND FOOD INTAKE IN OVARIECTOMIZED (OVX) SYRIAN HAMSTERS. Anita J. Bhatia* and George N. Wade (SPON: W.G. Richards). Neuroscience and Behavior Program and Department of Psychology, Univ. of Massachusetts, Amherst, MA 01003.

The effects of estradiol benzoate (EB) and progesterone on body weight and food intake were examined in OVX Syrian hamsters (Mesocricetus auratus). Nine weeks following OVX, hamsters received subcutaneous injections of EB alone (5 ug/day), progesterone alone (1 or 5 mg/day) or EB (5 ug/day) in combination with progesterone (1 or 5 mg/day) for 44 days. Treatment with EB alone decreased both body weight and food intake. Differing from its effects in OVX rats, progesterone was found to increase body weight and food intake in the absence of exogenous estradiol. Both groups receiving EB and progesterone concurrently exhibited a similar initial increase in body weight without increasing food intake. This increase was followed by body weight losses and reductions in food intake until both of these groups stabilized at levels similar to that of hamsters receiving EB alone.
(Supported by NS 10873, AM 32976 and MH 00321)

41.13

THYROID HORMONE REGULATION OF ALPHA-2 ADRENERGIC RECEPTOR SENSITIVITY AS MEASURED BEHAVIORALLY WITH THE ACOUSTIC STARTLE RESPONSE. D.A. Smith and J.V. Cassella. Neuroscience Program, Oberlin College, Oberlin, OH 44074

Stimulation of $alpha_2$-adrenergic receptors inhibits the acoustic startle response. Inhibition of adenylate cyclase (by the guanine nucleotide binding protein, G_i) may mediate this effect. Alterations in in vivo thyroid hormone levels produce changes in the sensitivity of cells to the action of other neuromodulators and neurotransmitters. It is possible that this effect of thyroid hormone is directly on the G_i protein. In this experiment, the effect of thyroid hormone on the $alpha_2$-mediated inhibition of startle was investigated.

The acoustic startle response was assessed in three groups of rats - controls, hypothyroid [treated with PTU for five weeks while fed an iodine-free diet], or hyperthyroid [thyroid hormone (T4) for three days].Animals were startled (30 white noise bursts at a 30 sec interval) before and after treatment with the $alpha_2$ agonist, clonidine (10ug/kg). PTU treatment slightly reduced baseline startle amplitude while thyroid hormone slightly increased startle but these changes were not significantly different from controls. The inhibitory effect of clonidine on startle was significantly enhanced in the PTU-treated animals compared to controls. The treatment with thyroid hormone did not significantly alter clonidine's effect.

41.14

DEFICITS IN MOTOR / REFLEX DEVELOPMENT AND SEXUAL BEHAVIOR IN THE MALE RAT FOLLOWING PRENATAL EXERCISE. S.K. Elliott* and J.L. Voogt. Dept. of Physiology , Univ. of Kansas Med. Center, Kansas City, KS 66103.

There is evidence that the developing brain is influenced morphologically (and functionally) by the gonadal hormone environment. Differentiation of sexual behaviors depends on the secretion of testosterone by the fetal testes, and prenatal stress alters the perinatal release of circulating testosterone with concomitant changes in adult male sexual behaviors. The goal of this research was to determine if strenuous maternal aerobic exercise induces the prenatal stress syndrome (PS-S) and its sequelae of impaired reproductive behaviors in male offspring. Changes in neurodevelopmental (motor/reflex) behaviors of offspring were evaluated, as alterations in these behaviors have been previously described in studies of CNS damage. Female rats were divided into 3 exercise groups with appropriate controls. Group I was pre-conditioned for 8 weeks prior to mating and continued to run during gestation at 75-80% of maximal aerobic capacity. Group II ran during pregnancy without pre-conditioning. Group III ran only during the last trimester, also without pre-conditioning. Acquisition delays were found in the following pre-weaning neurodevelopmental tests: surface righting, negative geotaxis, reflex suspension, and pole descent. Decreased exploratory behaviors, as evaluated with spatial maze and continuous corridor, persisted in adults. Copulatory behavior of adult male offspring was altered for mount, intromission, and ejaculation potentials, as well as for time to satiety. Severity of all behavior differences increased Group I II III. These results suggest that exercise during the prenatal period is sufficient to induce PS-S as well as cause other subtle neurodevelopmental impairments. Morphometric analysis of the SDN-POA is underway, as well as a search for other general cortical changes. (Supported by NIH Grant HD22340)

41.15

EFFECTS OF CYCLOHEXIMIDE ON MATERNAL EXPERIENCE EFFECTS IN POSTPARTUM FEMALE RATS. A.S. Fleming* and Una S. Cheung*. (SPON: G. Eskes). Dept. of Psychology, Erindale Campus, University of Toronto, Mississauga, Ontario, L5L 1C6.

Two hours of maternal experience with pups during the postpartum period in female rats results in heightened maternal behavior (reduced onset latencies to foster pups) 10 days later. This study investigated the effects of intraventricular cycloheximide, a protein synthesis inhibitor, on this maternal experience effect.

Eight groups of C-sectioned primiparous females were tested. Six groups of females were permitted to interact with neonatal foster pups for a two hour period thirty-six hours after C-section. Groups received intraventricular infusions of cycloheximide (100-400 ug) or saline immediately before, immediately after, and twenty-four hours after, the experience. Two groups of inexperienced animals had no post-C-section contact with pups and were injected with either cycloheximide or saline. All groups were tested for maternal onset latencies ten days after the C-section.

Experienced groups of females injected with cycloheximide immediately before and after the maternal experience, but not twenty-four hours after, had longer maternal onset latencies than experienced saline animals and similar to those of inexperienced groups. Cycloheximide did not, however, block the short-term retention of the behavior. These results suggest that maternal experiences are consolidated into long-term memory and that structural proteins are involved in this process.

41.16

EFFECTS OF ACUTE OR PROLONGED PROLACTIN ON LONG-TERM MATERNAL BEHAVIOR IN VIRGIN RATS. Gail Orpen[1], Jacquie Sarker*[2], and A.S. Fleming*[2]. St. Joseph's Hosp. Res. Inst., Hamilton, Ont.[1] and Dept. of Psychology, Univ. of Toronto, Mississauga, Ont.[2]

Female rats who give birth care for their pups immediately. If pups are removed by C-section, females continue to show a high level of responsiveness to foster pups for about a week. A rapid onset of maternal behavior can also be induced in virgin females with estradiol (E) and progesterone (P), but this responsiveness declines very rapidly. Attempts to prolong maternal responsiveness by brief exposure to prolactin (PRL) at the end of the E & P schedule were only partly successful (Orpen, et al. 1987). The first study investigated the possibility that prolonged PRL would be more effective. The second study investigated the long term effects on responsiveness of increasing levels of P or of E.

In the first study OVX female rats were implanted sc with a 1mm E capsule and 3 30mm P capsules in a 22 day schedule (Bridges, 1984) and received either SAL or PRL injections during the last 3 days (acute condition) or 12 days (prolonged condition) of the schedule. Groups of females were given maternal induction tests either 1, 4 or 7 days later. In the second study OVX females received either additional P (4 30mm capsules) or E (a 4mm capsule) in the same 22 day hormone schedule.

In study one neither acute nor prolonged exposure to PRL sustained maternal responsiveness for either 4 or 7 days. In study two, additional E sustained responsiveness for 4, but not for 7 days while additional P had no facilitatory effect.

41.17

ESTROGEN AND PROGESTERONE INFLUENCE ODOR STIMULATED HAMSTER FLANK MARKING. H.E. Albers and C.M. Rowland. Lab. of Neuroendocrinol. and Behav., Depts of Biol. and Psychol., Georgia State Univ., Atlanta, GA 30303.

Hamster flank marking is a form of communication stimulated by social encounters or the odors of other hamsters. Since the amount of flank marking in response to the odors of male hamsters varies over the 4 day ovulatory cycle, the present study examined how ovarian hormones influence odor stimulated flank marking. Ovariectomy (OVX), but not sham OVX, eliminated the 4 day cycle of flank marking and significantly reduced ($P<0.05$) the frequency of flank marking by over 50%. Implantation of Silastic capsules containing estradiol benzoate (E) restored preOVX flanking marking levels, whereas flank marking levels remained significantly reduced ($P<0.01$) in hamsters implanted with blank capsules (B). Injection of progesterone (P) into OVX+E treated hamsters 6 h before testing significantly reduced ($P<0.05$) flank marking when compared to OVX+E oil injected hamsters. The effects of P were only observed in E treated hamsters; no differences were observed in the amount of flank marking between OVX+B hamsters injected with oil or P 6 hrs prior to testing. These data demonstrate that estrogen and progesterone influence odor stimulated female flank marking and may underlie the variation in flank marking during the 4 day ovulatory cycle.

(Supported by NSF BNS-8711373)

41.18

ESTROGEN'S INFLUENCE ON WHEEL RUNNING PATTERN IN FEMALE RATS. H. Looy & R. Eikelboom. Department of Psychology, Queen's University, Kingston, Canada, K7L 3N6.

Wheel running behavior occurs at night in episodes separated by nonrunning periods. For female rats with ad lib access wheel running declines over the night and is highest on the night of estrus. Running patterns were examined both over the night and the four-day estrous cycle, to see how changing estrogen levels affect running initiation and termination. In 8 intact females, duration of running episodes decreased over the night. On the night of estrus, episode duration was longer in the first three hours, but for the remainder of the night was comparable to that of nonestrous nights. The duration of nonrunning periods increased over the night, and were shorter on the night of estrus. In 8 ovariectomized rats total nightly running was reduced as duration of running episodes decreased, while length of nonrunning periods increased. No four-day cyclic pattern was evident. Injection of estradiol (20 µg/kg sc) every fourth day into ovariectomized rats (n=8) restored running by increasing episode duration and decreasing nonrunning periods, such that their running pattern was similar to that of intact females on nonestrous nights. In conclusion estrogen appears to increase the salience of wheel running in female rats.

41.19

EFFECT OF FLUID DEPRIVATION ON TESTOSTERONE LEVELS AND THE RATE OF EXTINCTION OF A CONDITIONED TASTE AVERSION IN MALE RATS. E.A. Brownson, C.B. Sengstake*, and K.C. Chambers. Departments of Neurobiology and Psychology, USC, Los Angeles, CA 90089 and Department of Psychology, PSU, Portland, OR 97207.

The rate of extinction of a conditioned taste aversion (CTA) is dependent on testosterone (T) levels; high levels slow extinction. Fluid deprivation increases the rate in male rats and this effect can be counteracted with exogenous T. In the present study, 48 male Sprague-Dawley rats were either fluid deprived (D, 1 hr/day access to fluid) or nondeprived (ND, 24 hr access to fluid); extinction rates and T levels were measured. Following the first presentation of a 10% sucrose solution, a CTA was induced by injection of LiCl (0.30 M, 20 ml/kg). Daily extinction trials began 2 days later and continued until criterion for extinction (100% of first day consumption) was reached. Half of the D and ND males were bled before the first extinction trial and the other half of each of these groups were bled after. Deprivation increased extinction rates [$F(1,43)=8.65$, $p<0.01$] and lowered T levels [$F(1,43)=25.87$, $p<0.01$]. The time of bleeding had no effect on T levels or on extinction rates and there were no significant interactions. These findings are consistent with the hypothesis that the increased rate of extinction exhibited by fluid deprived males is T dependent.

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41.20

ROLE OF ANGIOTENSIN IN THE PRECIPITATION OF SEVERE SELF-INDUCED WATER INTOXICATION IN A PSYCHOTIC PATIENT. C.S. Sebastian and A.S. Bernardin*. Department of Psychiatry, LSU School of Medicine, Shreveport, La 71130.

Increased incidence of benign hyponatremia in psychiatric patients is well known. While moderate abnormalities in vasopressin secretion can explain mild basal hyponatremia in psychotic patients, they are inadequate to explain the more severe episodes of self-induced water intoxication (Goldman et al., N Engl J Med 1988; 318:397-403). The polydipsia is primary, rather than the consequence of a defect in renal concentration (Berl, N Engl Med 1988; 318:442).

Angiotensin II, a potent dipsogen that also increases sodium appetite may play a role in the development of water intoxication. It was hypothesized that central Angiotensin II is secreted in response to the mild hyponatremia in some psychotic patients. This results in increased water drinking and vasopressin secretion which aggravates the hyponatremia resulting in further release of Angiotensin II. An accelerating vicious circle is formed, until convulsions and following medical intervention with sodium supplementation interrupts it. A case of severe water intoxication was recently treated with Angiotensin Converting Enzyme (ACE) inhibitors on an "A-B-A" design. ACE-inhibitors have prophylactic value in the management of self-induced water intoxication.

42.1

A QUANTITATIVE STUDY OF INTRACRANIAL CALCIFICATION IN DEMENTIA OF THE ALZHEIMER TYPE. R.P.Friedland, J.Luxenberg*, E.Koss. Laboratory of Neurosciences, National Institute on Aging, N.I.H., Bethesda, Md. 20892.

Various lines of evidence from neurochemical, neuropathological and epidemiological investigations have suggested that calcium transport and binding are important processes in the normal cytoskeleton, and that these processes are disturbed in Alzheimer's disease (AD). In order to evaluate these hypotheses we analyzed the size of pineal and choroid plexus calcifications, using x-ray computed tomography, without contrast injection, in 23 subjects with probable AD (age 66.6±8.3 years) and 18 heathy aged controls (age 69.0±10.2 years). The area occupied by calcification was estimated directly from hard copies of the data by two independent observers who were blind to the diagnosis. There was good agreement between raters, with an ICC of 0.90 ($p < 0.0001$). Total calcifications occupied 130.8±89.8 mm2 and 170.9±79.6 mm2 in the AD and control groups, respectively. There were no differences in the area of calcification between the two groups. In the healthy aged group, but not in the AD group, there was a significant correlation ($r=0.69$, $p < 0.01$) between area of pineal calcification and lateral and third ventricular volume, as measured by volumetric x-ray CT. This relationship was also demonstrated using a partial correlation removing the effect of age ($r=0.75$, $p < 0.001$). These data suggest that AD is not accompanied by alterations in intracranial calcium deposition.

42.2

ANTIBODIES IN HUMAN SERUM AND CEREBROSPINAL FLUID RECOGNIZE NERVE GROWTH FACTOR. M.A. Downen, K. Wieties*, E.J. Moticka* and E. Giacobini, Dept. Pharmacol., Southern IL Univer. School of Medicine, Springfield, IL 62794-9230

Neurodegenerative disorders may involve a disruption of the trophic regulatory mechanisms in the nervous system. The presence of antibodies in human serum and CSF that recognize 7S nerve growth factor (NGF) from mouse submaxillary gland (Sigma) was determined. This was measured using an ELISA. 96 well plates (Dynatech) were coated with 1.25 µg NGF diluted in a carb-bicarb buffer, pH 9.6. Serum or CSF was applied to the wells. Goat anti-human IgG and anti-human IgA-alkaline phosphatase (Sigma) diluted 1/500 in PBS was applied and incubated overnight at 4°C. Immune complexes were visualized following incubation with p-nitrophenylphosphate. Absorbance was measured at 405 nm using the Titertek microplate reader (Flow Labs). The presence of antibodies was determined in serum samples diluted 1:30 from 32 Alzheimer's disease (AD) patients and 21 normal aged controls. No difference was seen between normals and AD patients when the mean absorbence was compared. Anti-NGF activity tended to increase with age in AD ($\bar{x}$=72.6) but not in normal controls ($\bar{x}$=71.1). An increase in antiNGF activity with age in CSF from 12 AD patients was seen. When serum anti-NGF activity as plotted versus CSF antiNGF activity, AD patients with higher CSF antibody activity also showed higher serum antibody activity. We have demonstrated the presence of antibodies in human serum and CSF which recognize 7S NGF.

42.3

A POSSIBLE NEUROPHYSIOLOGICAL MARKER FOR ALZHEIMER'S TYPE DEMENTIA AMONG SUBJECTS WITH A POSITIVE FAMILY HISTORY. J.A. Coffman, M. Torello, E. Burns, K. Travis,* N. Andreano,* H.A. Nasrallah. Department of Psychiatry, Ohio State University, 473 West 12th Avenue, Columbus, OH 43210

Early identification of individuals at risk for dementia of the Alzheimer type (DAT) is important for the establishment of early intervention strategies. There have been a number of reports documenting changes in long-latency evoked potentials in Alzheimer patients compared to controls (Pfefferbaum et al, EEG & Clin Neurophys 59:104, 1984). However, no data exists which show yearly evoked potential changes in an at-risk population. In this study, we have tested a group of individuals who have at least one parent or sibling with DAT and are therefore at-risk for the development of this disease (St. Clair DM et al, Br J Psychiat, 147:702, 1985).

Ten medication-free subjects with a family history of DAT and ten age, sex, and handedness matched controls were studied using an auditory "odd-ball" evoked potential task. Each subject was required to count the number of infrequently occurring (15%) high-pitched (1500 Hz) tones presented in a series of high and low-pitched (500 Hz) (85%) tones. The evoked potential to the high frequency tone was averaged from 40 artifact-free trials. The results showed a striking amplitude difference between groups ($p < .01$) during the post-stimulus interval of 100-150 ms specifically increased amplitude of the N100 waveform in the at-risk group (mean = 12.1 µV versus -6.8 µV) over the right centro-parietal area. Moreover, during the interval of 300-400 ms specifically decreased amplitude over the right fronto-temporal area there was a between-group difference ($p < .01$) in amplitude over the right fronto-temporal area. There were no differences in counting performance between groups.

These preliminary results represent to our knowledge a new finding and suggest the existence of an early neurophysiological marker of DAT in persons who may be at-risk for later developing Alzheimer.

42.4

GENETIC LINKAGE STUDIES IN AUTOSOMAL DOMINANT FAMILIAL ALZHEIMER'S DISEASE: AN ANALYSIS OF 30 FAD PEDIGREES. Peter St. George-Hyslop*, James F. Gusella*, Jonathan Haines*, Massachusetts General Hospital, Boston, Massachusetts 02114; John Hardy*, St. Mary's Hospital Medical School, London UK W21PG (Spon: S. Fink).

We previously reported that 2 anonymous DNA markers (D21S1/D21S11 and D21S16) on chromosome 21 showed evidence of genetic linkage to the Familial Alzheimer's Disease (FAD) gene. 29 additional small pedigrees with autosomal dominant FAD have now been examined. We confirm the prior report of linkage to chromosome 21 ($\hat{Z}$=3.30; $\hat{\theta}$ = 0.17 at D21S1/D21S11). Multipoint linkage analysis suggests that the FAD gene is located closer to the centromere, proximal to both D21S1/D21S11 and D21S16 ($\hat{Z}$=4.89). The current data do not permit the discovery of genetic heterogeneity ($P > 0.10$). The issue of genetic heterogeneity in FAD will be better resolved by the examination of highly informative markers closer to, or at the site of the FAD gene.

42.5

NEUROPSYCHOLOGICAL DIFFERENTIATION OF NEUROPATHOLOGICALLY DISTINCT DEMENTIAS WITH A BRIEF MENTAL STATUS EXAMINATION. D. Salmon, P. Kwo-On-Yuen, W. Heindel, N. Butters and L. Thal. San Diego VAMC and Depts. of Neurosciences and Psychiatry, UCSD, La Jolla, CA 92093.

Patients with Alzheimer's disease (AD) and Huntington's disease (HD) were administered the Dementia Rating Scale (DRS), a standardized mental status examination which provides a global dementia score and subscores for attention, initiation, construction, memory and conceptualization capacities. DRS scores were highly correlated with scores on two other widely used mental status examinations the Mini-Mental State (AD:r=.78; HD:r=.72) and the Blessed Dementia Scale (AD:r=-.79; HDr=-.80).

An analysis of DRS subscore patterns was conducted. Although the total DRS scores of 23 AD and 23 HD patients were precisely matched indicating equivalent levels of dementia, the patterns of subscores differed markedly. The AD patients' impaired memory subscore was significantly poorer than that of the HD patients. In contrast, the initiation and attention subscores were significantly lower for HD than for AD patients. These results are indicative of qualitative differences in the cognitive impairment associated with neuropathologically distinct dementias and demonstrate that such differences can be elucidated with brief mental status examinations.

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42.6

CONTRIBUTION MADE BY INDIVIDUAL ITEMS OF THE MINI-MENTAL STATE EXAMINATION TO THE DIAGNOSIS OF ALZHEIMER'S DISEASE. [1,2]D. Galasko, [2]B.Lasker, [2]M.M.Pay, [2]D. Bauer, [1,2]L.J. Thal, (SPON: D. Jeste) [1]Dept. Neurol, VAMC, SD 92161, [2]Dept. Neurosci, UCSD, La Jolla, CA 92093

12 components of the Mini-Mental Status (MMS) examination were analysed for their contribution to the diagnosis of dementia in 97 patients with Alzheimer's disease (AD) and 85 elderly controls. A subgroup of the AD patients with mild dementia (MMS 24/30) was studied separately. AD was diagnosed clinically according to NINCDS-ADRDA criteria. The AD and control groups did not differ significantly with respect to age, sex or education. Mean MMS scores were 17.3 for AD, 25.2 for the mild AD subgroup, and 29.0 for controls. Total MMS score correlated well with the diagnosis of AD (r=.78). In the AD and mild AD groups respectively, delayed recall (r=.84 and .57), orientation for time (r=.79 and .58) and place (r=.75 and .64), construction (r=.59 and .35), and attention r=.53 and .40) correlated most strongly with the diagnosis of dementia. Stepwise logistic regression analysis produced an optimal model predicting the diagnosis of AD, using orientation for time and place, recall and construction.

42.7

SLEEP IS IMPAIRED IN EARLY ALZHEIMER'S DISEASE (AD)

DE Williams*, MV Vitiello*, PN Prinz & RK Ries* (Sponsor: C. Arnett). Dept. Psychiatry, U. Washington and American Lake VAMC, Seattle, WA 98195.

Sleep fragmentation is a common problem among AD patients. This change has been documented in polygraphic studies of mild to severe AD. The present study further documents this change in early stage AD. 46 healthy, early stage AD patients (possible or probable by NINCDS criteria; Mini Mental Status scores ≥21) were compared with 54 controls (matched on health and demographics). Polygraphic sleep/wake measures were averaged across the 2nd and 3rd consecutive nights of a 72 hour study during which napping was absent. Results: increased nighttime wakefulness (# awakenings; time awake) and decreased deep sleep (stages 3 + 4, when arousal thresholds are highest) was observed in early AD vs. controls.

	CONTROL	ALZHEIMER	
NUMBER OF WAKES	9.3±4.2	13.1±8.6#	
WAKE AFTER SLEEP ONSET (MIN)	50.5±39.3	78.9±47.5#	
% WAKE (TIB)	14.2±8.7	19.0±10.0#	
% 3 & 4 (TIB)	9.4±4.6	7.5±4.0#	
% REM (TIB)	19.2±5.2	17.7±6.0	#p<.05

These results suggest that neural pathways controlling sleep maintenance (thought to be predominately subcortical[1]) degenerate in early AD in parallel with the more commonly studied cortical and hippocampal areas.

1.Prinz, et al., Neurobiol. Aging, 3:361-370, 1982.

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42.8

COMPUTER AUTOMATED SLEEP EEG BIOMARKERS FOR EARLY ALZHEIMER'S DEMENTIA (AD)

PN Prinz, LH Larsen*, ML Akins* & MV Vitiello*. American Lake VAMC & Dept. Psychiatry, U. Washington, Seattle, WA 98195.

A growing literature suggests the clinical waking EEG is altered in AD such that lower (<8 hz) frequency activities increase at the expense of higher (>12 hz) frequencies(*). We report here the results of a computer automated procedure that examines this phenomenon in 3 states: REM sleep, slow wave sleep (SWS), & quiet waking (QW).

35 healthy aged control subjects, 35 early stage AD patients, and 23 major depression patients matched on health, age, education and other factors were studied over a 72 hour period. The all night EEG was digitized at 128 hz and analyzed using autoregressive and fourier transform techniques. ≥5 min of idealized, artifact-free QW, REM & SWS were automatically selected from 9+ hours of continuous EEG (C4 vs. A1).

Results: Beta activity (12-25 hz) was significantly decreased in the AD vs. non-AD groups regardless of state (QW, REM SWS). Delta and theta energy increased during REM and QW, and decreased during SWS in AD patients relative to the other two groups (all p<001, ANOVA). When the best of these variables (R12-16, R16-20, S12-16, W12-16, S6-8, ALPHA/ THETA) were employed in discriminant analyses they yielded correct overall classification rates of 90-92%. These results indicate that quantitative sleep EEG biomarkers may prove to be useful in early detection of AD. A prospective study to determine their usefulness is currently underway.

*Coben, et al., EEG Clin. Neurophysiol, 55:372, 1983.
Duffy, et al., Ann. Neurol., 16:439, 1984.
Pentilla, et al., EEG Clin. Neurophysiol., 60:1, 1985.

Supported by the VA and MH 33688 and RR-37.

42.9

USE OF A SEMANTIC P300 TASK IN EARLY ALZHEIMER'S DISEASE DETECTION.

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Event-related potential (ERP) studies have demonstrated prolonged P300 latencies in a majority of subjects with dementia. However, most studies have not controlled for dementia etiology, nor confounding effects of nonspecific attentional or perceptual deficits. The classic auditory tone (be-boop) paradigm was employed, and failed to detect altered P300 in mild cases of Dementia of the Alzheimer Type (DAT). In young intact subjects, tasks that require semantic processing of words enhance later memory for the word as well as its associated P300 amplitude. This technique was adapted for 6 patients with mild DAT (meeting NINCDS-ADRDA task force criteria for Alzheimer's Disease, probable), with mean age of 71.7, and mean Mini-mental State Exam (MMSE) score of 22. Subjects sorted names by gender, and were asked to attend to randomly inserted target names occurring 20% of the time. Memory for the targets was tested on a probe recognition task after a 1-min. distraction. Compared to 6 control subjects (mean age 69.7, mean MMSE score 29.5), mild DAT subjects showed a significant reduction of N2-P3 amplitude difference (p<.005), prolongation of P300 latency (p<.05) to target words, and increase in probe errors (p<.005). This paradigm may be useful in the clinical evaluation of dementia to enhance detection of early DAT (supported by AG 05354).

42.10

THE P300 EVENT RELATED POTENTIAL (ERP) AND REGINAL CEREBRAL BLOOD FLOW (RCBF) IN THE ASSESSMENT OF PATIENTS WITH DISORDERS OF SHORT TERM MEMORY.

E. Gordon*, C. Kraiuhin*, Y. Zurynski*, A. Howson*, R. Meares*. (Spon: W. McEntee) Psychiatry Dept, Westmead Hosp. Sydney, AUSTRALIA

Studies in our laboratory suggest that an abnormal delay (2SD) in latency of the P300 ERP is found in 80% of patients in the moderate to severe stages of a dementing process. However, P300 latency does not appear to discriminate among patients with mild to moderate Alzheimer's (n=30), the elderly depressed (n=10) and healthy, normal subjects (n=100).

We propose that the P300 component elicited via the "oddball" paradigm does not reflect substantially those cognitive prccesses which are impaired in mild Alzheimer's. Our current research involves eliciting P300 during a specific test of verbal short-term memory. We hypothesize that the latency and amplitutde of the P300 component thus elicited will prove a means of distinguishing among the three groups noted above. To our knoweldge, we are the first group to record the P300 component from cognitively impaired patients performing a specific test of memory.

We have also examined Xe-133 RCBF in mild to moderately impaired Alzheimer patients (n=15), elderly depressed subjects (n=10) and normal controls (n=100). Seventy percent of the Alzheimer patients had abnormally low global cerebral flows. Moreover, global blood flow was abnormally low in some of the Alzheimer patients who had normal P300 latencies.

42.11

A STRESS TEST FOR MEMORY: DIFFERENTIAL EFFECTS OF MEMORY LOAD ON THE AMPLITUDE OF EVENT-RELATED POTENTIALS IN AGING AND ALZHEIMER'S DISEASE.

L. deToledo-Morrell, S.Evers*, T.J.Hoeppner, F.Morrell and D.C.Garron*. Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, IL 60612.

The amplitude and latency of event-related potentials (P300) were assessed during a modified Sternberg memory scanning task in patients with early Alzheimer's disease (AD, N = 5), age matched controls (N = 7) and young adults (N = 8). This report is based on P300 potentials recorded from a midline parietal electrode (P_Z) referred to linked ears. On a TV monitor, subjects were first shown a set of letters to be remembered, memory set size varying from 1 to 5 letters. After each study period for a given set size, single letters were presented one at a time and subjects were asked to press one key if the letter had been seen before, and a second key if it was any other letter of the alphabet. Previously memorized letters (target) were presented with a probability of 24%.

Although AD patients did not differ from age matched controls in P300 amplitude when a memory load of 1 letter was used, their P300 amplitude was dramatically and significantly reduced with increasing memory load. Aged controls did not differ from young adults on this measure. AD patients and aged controls did not differ from each other in P300 latency, but both showed delayed potentials compared to young adults. We conclude that electrophysiological abnormalities in early AD are best detected when functional demand is imposed on the memory system. In addition, amplitude measures seem to be more sensitive than latency measures in assessing memory dysfunction.

42.12

DIFFERENTIAL VULNERABILITY OF THE TWO HEMISPHERES DURING THE EARLY STAGES OF ALZHEIMER'S DISEASE: ELECTROPHYSIOLOGICAL EVIDENCE.

T.J. Hoeppner, L. deToledo-Morrell, F. Morrell and S. Evers.* Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, Il 60612.

Event related potentials (P300) were obtained from scalp electrodes (including P3-A1, P4-A2) in patients with early Alzheimers disease (AD, N=5) and in age matched controls (N=7) during two oddball procedures involving spatial and letter discriminations. The spatial task required Ss to press one response key when the upper of two box outlines was illuminated on a TV monitor and another key when the lower box was lit. The second task was a modified Sternberg procedure. On a TV monitor Ss were first shown a target letter to remember and then asked to press one key when the target was presented and a second key when any other letter was seen.

In the spatial task, AD patients exhibited a significant hemispheric asymmetry with P300 amplitude being markedly reduced at the P4 electrode compared to that at P3. Age-matched controls revealed no such asymmetry. In the letter discrimination task, the interhemispheric difference in AD patients was not as pronounced, although they still showed significantly lower amplitudes at P4 compared to age-matched controls. These results suggest that AD, unlike normal aging, may be associated with an early and selective impairment of right hemisphere function.

42.13

EFFECTS OF PHYSOSTIGMINE ON IMMEDIATE VERSUS DELAYED RECALL IN FOUR ALZHEIMER'S PATIENTS.
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Four inpatients diagnosed as Alzheimer's dementia of mild to moderate severity were administered physostigmine (1.0 mg i.m.) accompanied by methscopolamine bromide (5 mg p.o.), and an intra-muscular placebo in a randomized double blind fashion, with forty-eight hours between drug and placebo treatments. Assessment began at 15 minutes after drug or placebo injection using digit span tasks, a semantic fluency task (recalling examples from a given category), and a Bushke list-learning task. Three of the four patients scored better on the digit span tasks following physostigmine as compared to the placebo. Three of the four patients also produced more correct responses and fewer incorrect responses on the Bushke task following physostigmine. However, far more reminders were necessary on the Bushke task after physostigmine so that the overall recall score (Correct responses - (incorrect responses + reminders)) was worse following physostigmine. Further, three out of the four patients did worse on the semantic retrieval task after physostigmine. These results suggest that while phyostigmine may improve memory on tests of immediate recall, the drug may actually impair performance on tests of delayed recall. Further, the similar results on the Bushke and semantic fluency tests suggest that retrieval rather than learning might be the focus of the drug-induced impairment.

42.14

EXTRAPYRAMIDAL SIGNS AND DEMENTIA PROGRESS INDEPENDENTLY IN ALZHEIMER'S DISEASE.
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Dementia is the hallmark of Alzheimer's disease (AD), and extrapyramidal signs (EPS) are the hallmark of Parkinson's disease (PD). Many AD and PD patients display clinical and pathologic characteristics of both diseases, raising the question of whether mixed presentations reflect separate, co-occurring disease processes or the multifaceted expression of a single process. The former hypothesis predicts that EPS and dementia develop independently: the latter hypothesis predicts a correlated development. We investigated the independence of EPS and dementia in patients with probable AD: EPS scores equaled the number of signs observed (maximum = 12), and dementia scores were taken from the Memory-Information-Concentration section of the Blessed Dementia Scale. Longitudinal data ($N = 64$) showed that EPS and dementia significantly increased with time ($p < .01$). Cross-sectional data ($N = 137$) showed that the presence of EPS was not associated with the severity of dementia ($p > .5$): further, among patients with EPS, EPS scores were uncorrelated with dementia. The statistical independence of the progression of EPS and dementia supports the view that joint AD and PD symptomatology reflects the effects of multiple disease processes.

42.15

CHOLINERGIC MANIPULATION: EFFECTS ON REGIONAL CEREBRAL BLOOD FLOW AND COGNITIVE FUNCTION
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The effects of scopolamine were examined to elucidate interrelations between cholinergic manipulation, cerebral perfusion and neuropsychological function and to evaluate these findings relative to Alzheimer's disease (AD). Previous studies from this laboratory, conducted in young normal subjects, demonstrated that scopolamine produced dose and time dependent impairments in mnestic function and a global decline in regional cerebral blood flow (rCBF), maximal in frontal cortex (Honer et.al., 1988). However in AD, characterized by presynaptic cholinergic deficit, a global rCBF deficit is observed, with a temporo-parietal locus. The global rCBF deficit may reflect inhibition of diffusely distributed cortical cholinergic projections. The frontal lobe deficit may be attributable to an antagonism of dopaminergic projections, which have a greater distribution in frontal cortex. Interactions between acetylcholine and dopamine have been demonstrated *in vitro* and are mediated by high affinity muscarinic receptors, which are not impaired in AD (de Belleroche et.al., 1985).

Physostigmine (PHY, 0.02mg/kg,IV) and neostigmine (NEO, 0.01mg/kg or 0.007 mg/kg, IV) were administered to reverse the effects of scopolamine (0.007mg/kg,IV), to coincide with its maximal effects at 25 minutes after infusion. rCBF was measured by the 133-Xe inhalation technique, with 16 detectors over each cerebral hemisphere. The memory deficit (as measured by the Buschke Selective Reminding Test), produced by scopolamine was reversed by PHY, not NEO. Consistent word retrieval across trials was most impaired.This may be analogous to "representational memory", which is impaired by lesions ofthe prefrontal dopaminergic projection (Goldman-Rakic, 1987). PHY reversed the frontal rCBF deficit but NEO did not. These findings represent a divergence between the sequelae of muscarinic cholinergic blockade and AD. The rCBF deficits differ in the two conditions and, whereas physostigmine reversed scopolamine induced deficits, it is not consistently efficacious in the treatment of AD.

Supported by NIH grant AG 05433

42.16

LANGUAGE PERFORMANCE AND RATE OF COGNITIVE DECLINE IN ALZHEIMER'S DISEASE (AD).
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Numerous reports have investigated the relationship between language function and the course of Alzheimer's disease. Some have found language disturbance to be an early prognostic sign of rate of cognitive decline. One recent study (Faber-Langendoen et al, Ann Neurol 23:365, 1988) found that early language dysfunction in AD subjects identifies a group with more rapid progression of dementia. In a recent study we conducted, however, we found that lexical-semantic impairment was unrelated to onset or progression of symptoms in 86 patients with probable AD (Becker et al, Arch Neurol 45:263, 1988).

In the present study, we further investigated this issue in 50 subjects with mild to moderate AD who where evaluated as part of a larger longitudinal study. We used more sensitive measures of language impairment, including a detailed analysis of a sample of spontaneous language. The language scores did not differ between those identified as "fast decliners" and "slow decliners" according to their change scores on the MMSE (Folstein et al, J Psychiatr Res 12:189, 1975). These findings suggest that the degree of initial language impairment in AD patients does not predict the rate of cognitive decline.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS I

43.1

INSULIN RECEPTORS AND NEUROPEPTIDES IN LIMBIC-HYPOTHALAMIC AREAS OF THE RAT BRAIN.
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Although insulin receptors have been demonstrated in brain, little is known of their exact anatomical location and further characteristics of cells that express the receptors. Insulin receptor-like immunoreactivity (IR) was demonstrated by using a specific antibody against a unique peptide within the carboxy terminus of the receptor beta-subunit. IR was found in a widespread distribution on neuronal perikarya and processes throughout the rat forebrain. Among limbic areas, the highest density of IR was found in the CA1 and CA2 pyramidal cell layer of the hippocampus, followed by CA3, CA4, dentate gyrus and amygdaloid complex. In the hypothalamus, densest IR was present in the arcuate, periventricular, dorsomedial, suprachiasmatic and supraoptic (SON) nuclei; moderate dense IR was found in the ventromedial nucleus and posterior hypothalamic area. The median eminence showed a topographical distribution of IR with the highest density in the lateral part of the external zone. Double-labeling with antisera against somatostatin (SOM) and vasopressin (AVP) revealed a subpopulation of SOM neurons in the periventricular nucleus and a group of AVP neurons in the ventromedial SON that were colocalized with IR. Our findings suggest that insulin and its receptor may be involved in the regulation of neuronal activity including the release of neurotransmitters and peptides in the central nervous system. (Supported by PHS grants DK32884, AG05445, AG00300 and DFG, Un 59/1-2).

43.2

AUTORADIOGRAPHIC DISTRIBUTION OF 2-[^{125}I]-IODOHISTIDYL^{1}-NEUROKININ A BINDING SITES IN RAT BRAIN.
T.V. Dam, E. Escher* and R. Quirion. Douglas Hospital Res. Ctr. McGill Univ. Montréal, Québec and Dépt. de Pharmacol., Fac. de Méd. Univ. de Sherbrooke. Sherbrooke. Québec. CANADA.

Recent studies have suggested the existence of three neurokinin (NK) receptors in brain namely NK-1, NK-2 and NK-3. However, using [^{125}I]-Bolton-Hunter conjugates of neurokinin A ([^{125}I]-BH-NKA) and eledoisin ([^{125}I]-BH-ED), it was found that the distribution of [^{125}I]-BH-NKA and [^{125}I]-BH-ED binding sites were very similar. This casts some doubt over the existence of specific brain NK-2 sites. In this study, we use 2-[^{125}I]-iodohistidyl^{1}-NKA ([^{125}I]-NKA) as an alternate probe to characterize the autoradiographic distribution of putative NK-2 binding sites in rat brain. Rat brain sections were prepared and incubated with 50 pM [^{125}I]-NKA as described before (Quirion, R. and Dam, T.V., J. Neurosci., 6: 2187-2199, 1986). The results show that the distribution of [^{125}I]-NKA sites is different from those observed with other NK ligands including [^{125}I]-BH-ED. High densities of [^{125}I]-NKA binding sites are found in the external plexiform layer of the olfactory bulb, hippocampus, lateral septum, certain amygdaloid nuclei and locus coeruleus. The most striking difference between the localization of [^{125}I]-BH-ED and [^{125}I]-NKA binding sites is seen in the cortex. There is practically no specific [^{125}I]-NKA labelling in this region while very high densities of [^{125}I]-BH-ED binding sites are found in cortical laminae IV and V. These data suggest the presence of a distinct population of NK-2 binding sites in rat CNS. (Supported by Scottish Rite Foundation for Schizophrenia (U.S.A.).

43.3

DISTRIBUTION OF BINDING SITES FOR BASIC FIBROBLAST GROWTH FACTOR IN RAT BRAIN. W.F. Herblin, R.G. Krause* and J.S. Schwaber, Medical Products Department, E.I. duPont Nemours & Company, Wilmington, Delaware 19898

Basic fibroblast growth factor (FGF) has been shown to support proliferation of cell lines and survival of neurones in culture. Its local concentration has been reported to increase after injury in the brain. While these results imply the likelihood of FGF receptor mediation in brain, such receptors have not been demonstrated. In this study, we examined adult rat brain to determine whether binding sites for FGF were present.

Brains from male CD1 rats were cryostat sectioned at 20 microns and the sections melted onto gelatin coated slides. Sections were taken in the saggital and coronal planes at 90 and 150 micron intervals, respectively. The slides were dried overnight and covered with Dulbecco's modified Eagle medium with 0.15% gelatin and 20 mM HEPES (pH 7.5). Recombinant FGF supplied by Synergen, Inc. and iodinated by the lactoperoxidase method was added at 50 pM. Non-specific binding was determined with 5 μM unlabeled FGF. After two hours at 4° C., the slides were washed in PBS, 2M NaCl and PBS, followed by X-ray film and emulsion autoradiography.

Film autoradiography indicated an increased density of specific grains over the cerebellum, hippocampus, parietal cortex and the dorsal and pontine medullary regions. Emulsion autoradiography demonstrated that the grains in the more densely labeled regions were not associated with a particular cell type but were diffusely distributed throughout the tissues. When 2M NaCl was used to remove low affinity binding, much diffuse labeling was removed, but the hippocampus, cortex and the molecular layer of the cerebellum were still relatively more heavily labeled. This technique can be used to study the distribution of FGF receptors in other tissues as well as the response of receptor expression to injury.

43.4

EXPRESSION OF YES PROTO-ONCOGENE IN CEREBELLAR PURKINJE CELLS. M. Sudol*, and A. Alvarez-Buylla*. (SPON: C. Ouimet). The Rockefeller University, New York, NY 10021.

We have investigated the expression of the cellular-yes oncogene, the normal homolog of the Yamaguchi 73 virus-transforming gene, viral-yes. Using affinity-purified antibodies raised against the amino-terminal portion of the viral-yes protein, we have identified cellular-yes protein, pp62$^{c\text{-}yes}$, and analyzed its expression in various chicken tissues. Adult brain, retina, liver, and kidney express relatively high levels of pp62$^{c\text{-}yes}$ whereas muscle, spleen, and bone marrow contain low levels of the protein. Using an immune complex kinase assay and immune blot analysis we have shown that the adult cerebellum contains the highest level of pp62$^{c\text{-}yes}$ among all chicken tissues tested (Sudol et al., Oncogene Res., 1988, in press). Immuno-histochemical staining with anti-yes IgG localized pp62$^{c\text{-}yes}$ in denritic trees and cell bodies of Purkinje cells. Diffuse and less intense staining was also observed in the granular layer. It is possible that pp62$^{c\text{-}yes}$ is associated with a signal-transducing complex, expression of which is elevated in Purkinje cells.

43.5

LOCALIZATION OF CANNABINOID RECEPTORS IN BRAIN M. Herkenham, M. D. Little*, M. R. Johnson*, L. S. Melvin*, A. C. Howlett, R. B. Rothman, B. deCosta*, and K. C. Rice*. Unit on Functional Neuroanatomy, NIMH, Bethesda, MD 20892; Clinical Neurosci. Branch, NIMH; Lab Chemistry, NIDDK; St. Louis University Medical School, St. Louis, MO; and Pfizer Central Research, Groton, CT

A potent cannabinoid analog, CP-55,940, synthesized at Pfizer, shows high enantioselectivity and is pharmacologically similar to Δ^9-THC but with 5 to 80 times the potency in animal tests of adenylate cyclase inhibition *in vitro* and analgesia, catalepsy and spontaneous activity *in vivo*. Related compounds (levonantradol, nabilone) administered to humans produce psychoactive effects traditionally associated with the "marijuana high." CP-55,940 was tritiated (79 Ci/mmole) and used in section binding assays with 50 mM Tris-HCl buffer, pH 7.4 and BSA to minimize nonspecific binding. Optimized binding was 70% specific. Unfixed slide-mounted sections of rat and marmoset brains were incubated, washed, dried and exposed to LKB Ultrofilm. Resulting autoradiography showed great heterogeneity of binding in a unique and conserved pattern. Areas in both species showing strikingly high levels of binding sites were the globus pallidus, substantia nigra pars reticulata, and cerebellar molecular layer. Elevated and heterogeneous levels were also found in the olfactory bulb, cerebral cortex, hippocampus, and striatum. Low levels characterized most of the brainstem and spinal cord. These distributions suggest involvement of cannabinoids in motor and cognitive functions.

43.6

PHENCYCLIDINE AND σ RECEPTORS IN RAT SPINAL CORD: BINDING CHARACTERIZATION AND QUANTITATIVE AUTORADIOGRAPHY L. M. Aanonsen and V. S. Seybold Dept. of Cell Biol. and Neuroanatomy and Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

The biochemical characterization of PCP and σ receptor binding sites has been completed in slide-mounted tissue sections from rat brain and spinal cord. ^{3}H-N-(1-[2-thienyl]cyclohexyl)piperidine (^{3}H-TCP) and ^{3}H-(+)-3- (3-hydroxyphenyl)-N-(1-propyl)piperidine (^{3}H-3-PPP) were used as selective ligands for the PCP and σ binding sites, respectively. The dissociation constant (K_D) for ^{3}H-TCP was found to be 20.15 ± 4.56nM with a B_{max} of 296 ± 22 fmol/mg protein in rat brain tissue. The K_D for ^{3}H-3-PPP was 15.05 ± 3.31nM with a B_{max} of 29.94 ± 6.08 fmol/mg protein. K_D's for these ligands were not significantly different in spinal cord tissue. The order of potency of drugs inhibiting the binding of ^{3}H-TCP was MK-801>PCP>>haloperidol>3-PPP>> N-methyl-D-aspartate (NMDA) while the order of potency for ^{3}H-3-PPP was (±)pentazocine=haloperidol>>PCP>TCP>>NMDA.

Autoradiographic studies were performed on adjacent, identified spinal cord sections in order to compare the localization of PCP and σ receptors. ^{3}H-TCP receptors were found to be localized primarily in the superficial dorsal horn and were significantly decreased in caudal compared to rostral segments. ^{3}H-3-PPP binding sites were found to have the highest density over large dorsal root ganglion cells and over ventral motor neurons. The density of ^{3}H-3-PPP binding sites was also high in lamina X and in the intermediolateral area in thoracic segments. The differential distribution of PCP and σ binding sites in the rat spinal cord suggest that two binding sites exist for PCP and σ agonists.

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43.7

REGIONAL DIFFERENCES IN PCP AND σ BINDING IN THE BRAINS OF SCHIZOPHRENICS. A.D. Weissman, M.F. Casanova*, J.B. Kleinman, E.D. London and E.B. De Souza. NIDA Addiction Research Center, Baltimore, MD 21224 and Clin. Brain Disorder Br., NIMH, Washington, DC 20032.

The psychotomimetic effects of certain cycloalkyls and benzomorphans that interact with PCP and/or σ receptors has led to the hypothesis that these sites may be important in the etiology of schizophrenia. To investigate this hypothesis, PCP and σ receptors were measured in homogenates of seven regions of postmortem brains of patients diagnosed as belonging to several subclasses of schizophrenia and age-matched controls. PCP receptors were measured with [^{3}H]TCP (5 nM), and specific binding was defined in the presence of 10 μM PCP. σ receptors were measured with [^{3}H]haloperidol (1 nM) in the presence of 50 nM spiperone to block D2 and S2 sites, and specific binding was defined by displacement with 1 mM d-NANM. Occipital and parietal cortices from schizophrenics had significantly lower (25 and 26%, respectively) specific PCP binding than controls. Specific σ binding also was significantly decreased compared to control in the parietal cortex (38%), temporal cortex (47%), and dentate nucleus (25%). In contrast, neither PCP nor σ receptors were altered in the cingulate and frontal cortices and putamen in schizophrenics. The data demonstrate selective and differential regional decreases in PCP and σ receptors in schizophrenia and suggest an involvement of both the PCP and σ systems in this disorder.

43.8

RECEPTOR PLASTICITY IN THE RAT VISUAL SYSTEM. D.T. Chalmers* & J. McCulloch, Wellcome Surgical Institute University of Glasgow, Glasgow G61 1QH.

The rat visual system provides an appropriate model in which to study neurotransmitter receptor plasticity. Using a fully quantitative autoradiographic technique, we have examined the responses of a number of receptors, implicated in visual neurotransmission, to reduced and prolonged functional deficit.

Black-hooded PVG rats (n=20) were unilaterally enucleated under 2% halothane anaesthesia. At 1,5,10 and 20 days post-enucleation, cerebral glucose use was measured using the (^{14}C)-2-deoxyglucose technique. (^{14}C)-2-deoxyglucose-6-phosphate was eluted from those sections required for receptor autoradiography and the binding of [^{3}H]-5HT (2nM), [^{3}H]-Ketanserin (2nM), [^{3}H]-QNB (2nM), [^{3}H]-Muscimol (10nM) and [^{3}H]-DHA (2nM) was measured.

Significant reductions in glucose use were evident in both primary and secondary visual structures in the visually deprived hemisphere at 24 hours. The responses in ligand binding levels were heterogeneous: [^{3}H]-QNB and [^{3}H]-Ketanserin unaltered throughout the time course; [^{3}H]-5HT and [^{3}H]-DHA reduced in primary visual areas at 5, 10 and 20 days and [^{3}H]-Muscimol levels reduced in primary and secondary structures only at 20 days.

The variation in receptor response may indicate the importance of the site in visual processing and/or susceptibility to altered neuronal activity.

43.9

EVIDENCE FOR A POSTSYNAPTIC COLOCALIZATION OF NMDA, GLYCINE AND PCP BINDING SITES IN THE CA1 REGION OF HIPPOCAMPUS. V.Crepel*, A.Represa*, M.Beaudoin-Kais * and Y. Ben-Ari, (spon:D.McKay) INSERM U29, 123 BD.Port Royal, 75014 Paris,France.

Recent studies suggest that NMDA receptors, a subtype of glutamate receptors, are subject to regulation by glycine and with a PCP site form a receptor channel complex. NMDA probably plays a critical role in epilepsy and ischaemic damage to the hippocampus. NMDA binding sites are widely distributed in the hippocampus, particularly in the CA1 region on the terminal field of CA3 pyramidal cell axons (the Schaffer collaterals). This zone is particularly vulnerable to ischaemic injury. We report here the long lasting changes that transient bilateral hemispheric ischaemia or kainic acid induced epilepsy produce in the distribution of these binding sites. The severity and the extent of the lesions were studied by Gallyas, Fink-Heimer and cresyl violet staining methods. The distribution of NMDA, glycine and PCP binding sites was studied by quantitative autoradiography.

After kainate treatement there was a selective destruction of CA3 pyramidal neurons and dentate polymorph cells. NMDA, glycine and PCP binding decreased in the lesioned zone but not in the terminal field of their axons (CA1). The ischaemically damaged hippocampus shows a clear destruction of CA1 pyramidal and dentate polymorph cells. The density of binding sites for the differents receptor markers was severly reduced in CA1(>70%) 10 days after transient ischaemia.

To conclude: 1/ our results strongly support the anatomical colocalization of NMDA, glycine and PCP binding sites on the pyramidal cells of CA1: 2/ there was no sign of presynaptic localization of NMDA receptors on Schaffer collaterals; 3/ we proposed that in CA1, 30% of NMDA binding sites must be located on interneurons.

43.10

GTP MODULATES MELATONIN BINDING AND ITS EFFECTS ON ADENYLATE CYLCASE IN HAMSTER BRAIN. L.P. Niles, F.S. Hashemi* and B.J. Mason*, Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

We have reported that the pineal hormone, melatonin, causes a GTP-dependent inhibition of adenylate cyclase activity in the rat hypothalamus (1). We now report a similar GTP-dependent effect in the hamster hypothalamus where melatonin, in pico-nanomolar concentrations, inhibits forskolin-stimulated adenylate cyclase activity.

In view of GTP's importance in eliciting melatonin's effect on enzyme activity, we have examined the effects of this nucleotide on the binding of 2-[^{125}I]iodomelatonin ([^{125}I]MEL) (2) in the hamster brain. Brain homogenates were incubated in the absence or presence of GTP which caused a dose-dependent inhibition of [^{125}I]MEL binding with a maximal effect of 40-50% occurring at a nucleotide concentration of 10^{-3} M.

These findings suggest that hypothalamic [^{125}I]MEL binding sites are functionally relevant receptor sites coupled to adenylate cyclase via a GTP regulatory protein. (Supported by the Ontario Mental Health Foundation and MRC, Canada)

1. Adv. Biosci. 53, 283 (1984).
2. BBRC 147 (3), 949 (1987).

43.11

THE PHARMACOLOGY OF 2-[^{125}I]IODOMELATONIN BINDING SITES IN HAMSTER HYPOTHALAMUS. D.S. Pickering and L.P. Niles, Dept. of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

Recently the ligand 2-[^{125}I]iodomelatonin ([^{125}I]MEL) has been used to label putative melatonin receptor sites. Its high affinity and specific activity make it far superior to the previously used ligand, [^{3}H]melatonin. We report here on the site specificity of [^{125}I]MEL.

The pharmacological profile of the site labelled by [^{125}I]MEL was examined in detail in hamster hypothalamus. A high affinity site (K_d= 0.18 nM) was found, similar to that in cerebral cortex and hippocampus. The profiles in all three regions were qualitatively similar. Cold IMEL was the most potent inhibitor of [^{125}I]MEL binding (IC_{50}≈ 0.5 nM) while only other closely related indoles exhibited significant potency: 6-chloromelatonin > N-acetyl-serotonin (NAS) > melatonin > 6-hydroxymelatonin > 5-methoxytryptophol >> 5-methoxytryptamine (5-MT) >> serotonin (5-HT). Prazosin was found to be a potent inhibitor in all brain regions studied (IC_{50}≈ 10 nM); however, other α_1-adrenergic agents (e.g. WB-4101), serotonergic and dopaminergic compounds were poor inhibitors. The indole-like ring structure of prazosin and its 6,7-dimethoxy groups likely are responsible for its affinity at these sites. It is interesting that while 5-MT and 5-HT are poor inhibitors, NAS is a good inhibitor. This suggests that removal of the positive charge of the amino group (by acetylation) is important for directing the specificity of indoles towards the putative melatonin receptor and away from serotonin receptors.

In summary, a unique, non-serotonergic, non-dopaminergic and non-adrenergic binding site is labelled by [^{125}I]MEL in hamster brain and proposed to be a melatonin receptor site.

(Supported by the Ontario Mental Health Foundation and Medical Research Council of Canada)

43.12

PROPERTIES OF ^{3}H-CARFENTANIL BINDING TO HUMAN AND RAT BRAIN OPIATE RECEPTORS IN VITRO. R.A. LYON, M. TITELER, L.T. RYDELEK*, A.E. BULLOCK*, J.J. FROST[1], R.F. DANNALS[1]*, and M.J. KUHAR[2]. Dept. Pharmacol. Toxicol., Albany Medical College, Albany, NY 12208. [1] Div. Nuclear Med., Johns Hopkins Med. Institutions, Baltimore, MD 21205, [2] NIDA Addiction Res. Ctr., Baltimore, MD 21224.

^{11}C-Carfentanil has been successfully used as a Positron Emission Tomography ligand in humans. Preliminary studies have shown that ^{11}C carfentanil interacts primarily with mu opiate receptors. Because the half-life of ^{11}C renders it difficult to use in in vitro binding studies, ^{3}H-carfentanil was synthesized (NEN) and its binding properties examined in rat and human brain membranes.

Preliminary saturation analyses indicate that ^{3}H carfentanil specific binding is saturable in rat diencephalon (105+/-5 fmol/mg protein) and whole cortex (82+/-4) and human thalamus (92+/-9) and pre-frontal cortex (43+/-8). Specific ^{3}H-carfentanil binding (determined in the presence of 1 uM naloxone) displays a K_D of 0.09 nM and is regulated by guanine nucleotides. Preliminary competition experiments indicate that the majority of specific binding is to mu opiate receptors. Data from completed saturation, kinetic and displacement studies in rat and human tissues will be presented. (Supported by USPHS NS150 80).

43.13

VALIDATION OF A POSITRON EMISSION PROBE FOR NEURO-RECEPTOR STUDIES IN HUMAN BRAIN K.J. Jeffries*, C.A. Tamminga, D.F. Wong*, H.H. Holcomb, K.H. Douglass*, J.M. Links*, R.F. Dannals*, H.N. Wagner* (SPON S. Presty). Maryland Psychiatric Research Center and Johns Hopkins Hospital, Balto., MD 21228

A dual detector positron emission probe developed by Bice et al. enables quantification without localization of positron emitter labeled tracers within an adjustable brain volume. This device consists of two sodium iodide scintillators which detect positron annihilation events by coincident detection of 511 keV gamma-ray pairs. The scintillators, placed on opposite sides of the head, provide a measure of activity in the delimited volume of tissue. Phantom studies demonstrate a linear response to increasing activity levels in the field-of-view. The radiation dose needed for a probe data acquisition is approximately 1/40 of that required for a PET scan, allowing for repeated studies to be performed over a short period of time. We have now developed a simple model based on geometry and attenuation in the field-of-view for interpretation of the probe signal to be used to calculate activity in a region-of-interest from probe raw data. This technique's most obvious application is in the study of CNS drug kinetics. A preliminary PET/Probe correlation study demonstrated a 46% displacement of C^{11}-NMSP by haloperidol in caudate/cerebellum as measured from PET scan data versus a 45% displacement measured from probe data. Multiple probe studies over 48 hrs demonstrated 25% and 21% displacement from baseline at 2 hrs and 48 hrs respectively after haloperidol. C^{11}-NMSP displacement by haloperidol at different dose levels and its correlation with plasma drug levels will be reported.

43.14

[3H]Ro5-4864 AND [3H]PK11195 LABEL FUNCTIONAL RECEPTORS IN MITOCHONDRIA. J.D. Hirsch, C.F. Beyer*, L. Malkowitz*, C. Loullis, B. Beer, and A.J. Blume. Molecular Neurobiology Group, CNS Res., Med. Res. Div., American Cyanamid Company, Pearl River, NY 10965.

By studying the distribution of enzyme markers, specific [3H]Ro5-4864 (Ro5) binding, and crosslinked [3H]-PK14105, we have determined that [3H]Ro5 and [3H]PK11195 (PK) receptors in rat kidney mitochondria (M) reside in the outer membrane. Ten receptor ligands (Ro5, PK, DZ, FLU, MPoIX, DPoIX, DiPy, DiBuPhth, CyA, CL 259,763; IC50's = 20-20000 nM) inhibited M respiratory control without altering ADP:O ratios and their potency correlated with their receptor binding affinity (r = 0.95). Receptors in kidney M photoaffinity labeled with [3H]PK14105 and analyzed by SDS-PAGE, showed specific labeling in only 1 (~17 kDa) or 2 (~17 + ~18 kDa) proteins depending on irradiation time. Specific labeling was reduced by prior treatment of M with diethylpyrocarbonate. Size exclusion HPLC of [3H]labeled proteins in SDS yielded 1 major component (~18 kDa) which was resolved on a reverse phase C8 column in the presence of SDS into several labeled peaks. These results indicate that the M receptor for Ro5 and PK is an outer membrane protein(s) that modulates organelle function. Work is in progress to purify it to homogeneity and establish its identity.

44.1

LOCALIZATION OF ATPase ACTIVITY IN DENDRITIC SPINES OF THE CEREBRAL CORTEX. Virginia Kriho* and Rochelle S. Cohen. Department of Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL 60612.

An ATPase activity has been demonstrated in dendritic spines of the adult rat cerebral cortex using cerium to capture inorganic phosphate that is liberated during the enzymatic hydrolysis of ATP. Small pieces of cerebral cortex tissue were fixed and processed according to the method described by Salama et al. (1987, J. Histochem. Cytochem. 35: 471-482). The tissue was then dehydrated and embedded in Epon or LR White. EM examination of the cerium phosphate reaction product showed an electron dense precipitate localized in the cytoplasm of the spine behind the postsynaptic density (PSD). Whereas the PSD, itself, is not reactive, dense reaction product is seen immediately underneath the PSD and extending into the subsynaptic web. The reaction product is seen to occur along filamentous material in this region. Reaction product is also associated with membranous cisternae within the dendritic spine. No reaction product is seen when the tissue was incubated in a substrate (ATP)-free medium. Similarly, virtually no reaction product is seen when the tissue was incubated in a medium devoid of Ca^{2+} and including 2mM EGTA, suggesting that the reaction depends on the presence of Ca^{2+}. Incubation of the tissue in a medium devoid of Mg^{2+} and containing 10mM EDTA or 10mM CDTA did not inhibit the reaction. Ouabain, a Na^{+}-K^{+}-ATPase inhibitor, and levamisole, an alkaline phosphatase inhibitor, had no effect on the reaction. The presence of an ATPase activity which is dependent on Ca^{2+} in dendritic spines where actin and actin-binding proteins have also been localized, suggests that this activity may be involved in the dynamics of cytoskeletal function leading to shape changes in dendritic spines and synapses, as seen with various behavioral and physiological paradigms.
Supported by NIH Grant NS 15889.

44.2

COLOCALIZATION OF ACTIN AND MAP2 IN DENDRITIC SPINES DEMONSTRATED BY DOUBLE LABELING WITH IMMUNOGOLD PROBE. M. Morales* and E. Fifkova (SPON: J. Werner). Dept. of Psychology, University of Colorado, Boulder, Colorado 80309.

Using immunogold cytochemistry in a postembedding procedure, we have studied localization of MAP2 in dendritic spines of the dentate molecular layer, CA_1 stratum radiatum, visual cortex and Purkinje neurons. We have used monoclonal anti-MAP2 antibody which was the generous gift of Dr. A. Frankfurter (University of Virginia). This antibody recognizes MAP2 in dendritic spines where it is prevailingly associated with actin filaments and endomembranes of the spine apparatus, and to a lesser extent with the postsynaptic density. In all sites MAP2 was colocalized with actin as evidenced by a double labeling procedure. In dendrites, MAP2 was associated with microtubules. However, there was no labeling with this antibody in axon terminals. So far the presence of MAP2 in dendritic spines has been a matter of controversy. While MAP2 in spines has been shown with light and electron microscopy using PAP immunocytochemistry (Binder et al., Ann. N.Y. Acad. Soc., 466:145; Caceres et al., J. Neurosci., 4:394), it was not observed by Burgoyne and Cumming (Eur. J. Cell Biol., 30:154) or by Bernhardt and Matus (J. Comp. Neurol., 226:203). The latter authors considered the spine labeling to be caused by diffusion of the reaction product from dendrites. Our results unequivocally demonstrate the presence of MAP2 in spines with a method that is not beset with the problem of diffusion. Moreover, this method allows for discrete localization of the antigen within the spine compartment and thus for inferences to the possible function. Although MAP2 induces gelation of actin in vitro, its affinity to actin filaments in the presence of microtubules is low (Griffith and Pollard, J. Cell Biol., 78:958). However, in the absence of microtubules, MAP2 will bind to actin filaments. Thus, MAP2 may be one of the actin associated proteins which endows the actin network in spines with the dynamic properties that are necessary for synaptic plasticity.
Supported by the Institute on Aging, grant #AG04804-03.

44.3

TAP-1 EXISTS IN TWO FORMS; AN INTEGRAL MEMBRANE PROTEOGLYCAN OF THE NERVE TERMINAL AND AN EXTRACELLULAR MATRIX PROTEOGLYCAN WHICH LACKS A MEMBRANE INTERCALATING REGION. M. Iwata*, A. Davis* and S.S.Carlson. Dept. of Physiology and Biophysics, University of Washington, Seattle, WA.

Terminal anchorage protein one (TAP-1) is a chondroitin sulfate proteoglycan which has all the properties of a protein that links the nerve terminal to the extracellular matrix (ECM) at the elasmobranch electric organ synapse: 1) It is located on the nerve terminal surface; 2) It behaves as an integral membrane protein; and 3) It is tightly bound to an electric organ ECM fraction (Carlson, et al., J. Cell Biol. 103:509,1986). The molecule purified under denaturing and reducing conditions is a large proteoglycan with $M_r \approx 10^6$. Visualized in the electron microscope it has the typical "bottlebrush" structure characteristic of proteoglycans with an average total length of 345 ±17nm, with about 20 side projections of about 110 nm in length (Carlson and Wight, J.Cell Biol. 105:3075, 1987). The large size of this molecule suggests that it could make a large structural contribution to the synaptic cleft.

Recently we have found that two forms of TAP-1 exist in electric organ. A portion of the isolated TAP-1 molecules behave like integral membrane proteins and the remainder do not. As expected for an integral membrane proteoglycan, about 30-40% have two properties: 1) low buoyant density (< 1.3 g/cc) when sedimented in CsCl density gradients containing 4M guanidine-HCl and non-denaturing detergent, and 2) the ability to be inserted into liposomes. The remainder have a high buoyant density (>1.50 g/cc) on these gradients and do not interact with liposomes. Presumably one form lacks a hydrophobic membrane intercalating domain.

In all other respects these two TAP-1 forms appear the same. They are both chondroitin sulfate proteoglycans of the about the same size which contain 4 disulfide linked subunits with molecule weights of 45Kd, 23Kd, 18Kd, and 12Kd. We recently found these subunits when TAP-1 was purified under denaturing, but non-reducing conditions.

One interesting hypothesis is that the integral membrane form of TAP-1 might be converted to the ECM form on the nerve terminal surface.

44.4

VESIPLEXIN: A PROMINENT PROTEIN COMPLEX ON MAMMALIAN SYNAPTIC VESICLES. E. Floor. Dept. of Anatomy, Univ. of Wisconsin Medical School, Madison, WI 53706.

Peripheral membrane proteins Q, S and V (*J. Neurochem.* **50**: in press, 1988) and the synaptic vesicle-specific, integral membrane protein p65 from rat or cow brain synaptic vesicles co-sediment as an ~10S complex on sucrose gradients after solubilization in the nonionic detergent, CHAPS. Proteins Q, S and V co-migrate as a complex of M_r ~200K during nondenaturing gel electrophoresis or column chromatography in nonionic detergents. These structural parameters are consistent with the presence of one copy each of proteins Q, S, V and p65 in a globular complex. Interaction of proteins S and V (*Ann. N.Y. Acad. Sci.* 493:62, 1987) and Q and S in intact synaptic vesicles was demonstrated by covalent crosslinking. Sensitivity to attack by proteases and reactivity with membrane-impermeant crosslinkers indicate that this protein complex is located on the cytoplasmic surface of synaptic vesicles. I have named this complex vesiplexin to connote its localization and multisubunit structure. Vesiplexin did not appear to contain synaptic vesicle proteins synaptophysin or SV2 or cytoskeletal proteins tubulin or neurofilament protein NF-L in Western immunoblot analyses. The abundance of vesiplexin in purified synaptic vesicles is comparable to that of synaptophysin as estimated from Coomassie Blue-stained SDS gels, which suggests that it is present in several copies per vesicle. The abundance and location of vesiplexin suggest a structural role, possibly in interactions of synaptic vesicles with the neuronal cytoskeleton or presynaptic membrane. The functions of vesiplexin are presumably related to the calcium-sensitive process mediated by p65, a calmodulin-binding protein. (Supported by NIH grant NS24890).

44.5

ACTIN ASSEMBLY and NEUROTRANSMITTER RELEASE. BW Bernstein and JR Bamburg*. Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523

Actin, the major cytoskeletal protein of nerve terminals, assembles, disassembles, and reassembles during a 30sec depolarization of whole mouse brain synaptosomes in a 75mM K^+/ 2mM Ca^{++} buffer. Synaptosomes, loaded with [^{3}H]-noradrenaline (1μCi/ml resting buffer), show rapid release during the first 1-2sec of depolarization (Drapeau, P and MP Blaustein, J. Neurosci.3:703-713, 1983); release recommences 20-30sec later, and after 60sec, total ^{3}H released is twice that of the first 2sec. The abrupt termination of release after the first 2sec occurs during the time of rapid assembly of actin previously described.

Membrane impermeable molecules can be entrapped in synaptosomes during brain homogenization (Akerman, K et al, Biochim. Biophys. Acta 858:275-285, 1986). We are testing whether cycles of actin assembly/disassembly are necessary for the cycles of release: cytoskeletal perturbing agents are entrapped and their effects on release are measured. One such agent, phalloidin (800 kD), binds filamentous actin and stabilizes it against depolymerization. We estimate that micromolar levels of phalloidin were incorporated in synaptosomes, assuming that phalloidin enters them with efficiency equal to that of a fluorescent dextran marker (9,000 kD average). The levels of entrapped phalloidin are sufficient to block 50-60% of radioactivity released during the first 2sec of depolarization.
Supported by NIH GM35126.

44.6

LOCALIZATION OF pp60c-src IN SYNAPTIC VESICLES. D. T. Pang, F. Valtorta*, M. E. DeMarco*(1), J. S. Brugge*(1), F. Benfenati*, P. Greengard. Lab. of Mol. & Cellu. Neurosci., Rockefeller U., New York, NY 10021 and (1) Dept. of Microbiol., SUNY, Stony Brook, NY 11794.

A possible regulatory role of tyrosine phosphorylation in neurotransmission is supported by the recent observations that synaptic vesicle proteins are phosphorylated by endogenous tyrosine kinases in intact vesicles and nerve terminals and that pp60c-src is present in nerve terminals and crude synaptic vesicle fractions. The present study examined pp60c-src in synaptic vesicles purified through the step of chromatography on controlled-pore glass beads. Immunoblot analysis showed that pp60c-src was enriched 4-5-fold in synaptic vesicles compared to the total particulate membrane fraction. To determine the contribution of pp60c-src to the total tyrosine kinase activity, subcellular fractions were solubilized in 1% Triton X-100 and immunoprecipitated with monoclonal antibody 327. With enolase as an exogenous substrate, immunoprecipitates and supernatants were assayed for tyrosine kinase activity. pp60c-src represented 70% and 14% of the tyrosine kinase activity in synaptic vesicles and brain homogenate respectively, indicating that pp60c-src is the major tyrosine kinase in synaptic vesicles. The presence of pp60c-src in synaptic vesicles suggests a possible regulatory role for this tyrosine kinase in neurotransmitter release. Gene regulation of pp60c-src in neurons may be involved in the long term modulation of neural transmission.

44.7

EFFECTS OF PHORBOL ESTER AND ACETYLCHOLINE ON PROTEIN PHOSPHORYLATION IN RAT NEURONS AND GLIAL CELLS. Y.F. Han* and L.A. Dokas (SPON: R.M. Kantner). Depts. of Biochem. and Neurol., Med. Col. of Ohio, Toledo, OH 43699.

Phorbol esters alter several synaptic responses in neurons. Since phorbol esters activate protein kinase C (PKC), it is important to characterize the phosphoproteins which mediate these effects and determine their functions. Hippocampus and cerebral cortex from 19 to 20-day-old rat fetuses or 1-day-old rats were dissociated and cultured to yield neurons or glial cells. Phosphorylation of cell proteins was initiated by addition of [^{32}P]-orthophosphoric acid followed by incubation with the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) or other compounds. Phosphoproteins were characterized by SDS-PAGE, autoradiography and liquid scintillation counting. TPA (10^{-8}-10^{-6}M) stimulated, in a dose-dependent manner, the phosphorylation of a protein with an M_r of 84 kDa both in neurons and glial cells, and a protein with an M_r of 48 KDa in neurons only. 4α-Phorbol 12,13-didecanoate, an inactive derivative of TPA, showed no effects. Stimulatory effects of TPA could be significantly antagonized by 1-(5-isoquinolinyl sulfonyl)-2-methyl-piperazine (H-7), an inhibitor of PKC. Acetylcholine (10^{-3}M) enhanced phosphorylation of both the 84 and 48 kDa proteins. These studies suggest that these proteins may be involved in modulation of neuronal and glial functions. Supported by the Ohio Department of Aging.

44.8

A Ca^{2+}-INDEPENDENT AUTOPHOSPHORYLATION SITE IN NEURONAL TYPE II CaM KINASE SUPPRESSES STIMULATION BY CALMODULIN. B.L. Patton*, S.G. Miller*, and M.B. Kennedy. Division of Biology 216-76, Caltech, Pasadena, CA 91125.

Rapid autophosphorylation of αThr-286/βThr-287 on the α and β subunits of type II Ca^{2+}/calmodulin (CaM)-dependent protein kinase is triggered in the presence of Ca^{2+}/CaM (see accompanying abstract). This produces a partially Ca^{2+}-independent kinase activity and we now find that it also permits the autophosphorylation of additional sites upon removal of Ca^{2+}. The Ca^{2+}-independent autophosphorylation does not reduce Ca^{2+}-independent activity, but is associated with a loss of further stimulation of activity by calmodulin (Hashimoto *et al.*, 1987, JBC 262, 8051; our own observations). We have identified two Ca^{2+}-independent autophosphorylation sites on each of the α and β subunits and have correlated the phosphorylation of one of them with loss of stimulation by calmodulin. The sites were identified following tryptic digestion of ^{32}P-labeled kinase that had been autophosphorylated first in the presence, then in the absence of Ca^{2+}. Tryptic phosphopeptides that appeared in the absence of Ca^{2+} were purified by HPLC and sequenced. Phosphate was found in peptides containing Ser-315 and Thr-306,-307 in the β subunit and in homologous peptides from the α subunit (containing sites Ser-314 and Thr-305,-306). These sites are located in the calmodulin-binding domain. Comparison of the time course of autophosphorylation of these sites with the loss of stimulation by calmodulin, as well as comparison of the rate of dephosphorylation with recovery of calmodulin stimulation, suggests that the threonine sites, rather than the serine sites, are responsible for inhibition of effective binding by calmodulin.

Supported by NIH, the Epilepsy Foundation, and the McKnight Foundation.

44.9

AN EARLY AUTOPHOSPHORYLATION SITE IN NEURONAL TYPE II CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE PRODUCES Ca^{2+}-INDEPENDENT ACTIVITY. S.G. Miller', B.L. Patton', and M.B. Kennedy (Spon: R. Bulleit) Division of Biology 216-76, Caltech, Pasadena, CA 91125.

It is now well-established that the enzymatic activity of neuronal type II Ca^{2+}/calmodulin-dependent protein kinase is regulated by autophosphorylation. Autophosphorylation in the presence of Ca^{2+}/calmodulin (CaM) leads to the generation of a partially Ca^{2+}-independent form of the kinase. This change in activity occurs after phosphorylation of only a few of the 12 subunits in a holoenzyme. We have identified the autophosphorylation sites that are involved in this change in activity using tryptic digestion, followed by reverse phase-HPLC and microsequencing. When autophosphorylation occurs in the presence of Ca^{2+}/CaM a common threonine residue (αThr-286/βThr-287) is rapidly phosphorylated in both the α and β subunits. It is located near the N-terminal boundary of the calmodulin binding domain. The β subunit contains a second rapidly phosphorylated threonine, βThr-382. It is in a region of the β subunit that is not present in the α subunit and that appears to be specifically deleted by mRNA splicing in the β' subunit (Bulleit *et al.* (1988) *Neuron* 1, 63-72).

Purified protein phosphatase 2A dephosphorylates βThr-382 at a higher rate than either αThr-286 or βThr-287. Partial dephosphorylation of the kinase with protein phosphatase 2A completely dephosphorylates βThr-382. The resulting kinase is phosphorylated only at αThr-286/βThr-287 and remains significantly Ca^{2+}-independent. These experiments indicate that βThr-382 is not *required* to maintain Ca^{2+}-independent kinase activity.

Supported by NIH, the Epilepsy Foundation and the McKnight Foundation.

44.10

A POLYCLONAL ANTIBODY THAT SPECIFICALLY RECOGNIZES THE CALMODULIN (CaM) BINDING DOMAIN OF Ca^{++}/CaM-DEPENDENT PROTEIN KINASE II (CK-II). R.Weinberger*, J.Aronowski*, M.N. Waxham*, and P.Kelly. (SPON: M. Mauk). Dept. of Neurobiol. and Anat., and Neurol., Univ. of Tex. Med. Sch., Houston TX 77225.

CK-II is a major neuronal protein kinase in the CNS and has been shown to have both Ca^{++}/CaM -dependent and -independent kinase activity *in vitro*. Based on the primary sequence of the putative CaM binding region of CK-II, a peptide (CBP) has recently been synthesized and shown to display active site directed inhibitory properties (1). In the present study a rabbit polyclonal antibody was raised against CBP and its properties characterised. ELISA and immunoblot analyses indicated that anti-CBP was highly specific, recognizing both alpha and beta subunits of CK-II but sharing no cross reactivity with skeletal muscle myosin light chain kinase (Sk-MLCK), phosphorylase kinase, calcineurin, a 36 kDa CaM binding protein, Ca^{++}/CaM-dependent phosphodiesterase and two synthetic peptides modelled on the CaM binding region of Sk-MLCK. Minor cross reactivity with two unidentified protein bands was observed in crude fractions of rat brain. The only detectable phosphoproteins in brain subcellular fractions immunoprecipitated with anti-CBP were the alpha and beta subunits of CK-II. Anti-CBP inhibited the Ca^{++}/CaM stimulated activity of purified CK-II *in vitro* by 40-90%. This inhibition was dose- and time- dependent and was reversible in the presence of excess CaM. No inhibition of kinase activity was seen for the Ca^{++}/CaM -independent form of CK-II. These results indicate that anti-CBP is a potentially useful reagent with which to investigate the function of CK-II *in vivo* as well as demonstrate that the amino acid sequence contained in CBP is in fact the CaM binding domain of CK-II.

(1) Kelly et al, Proc. Natl. Acad. Sci. (1988), in press.

44.11

ACETYLCHOLINE RECEPTOR AGGREGATION IN CULTURED RAT MYOTUBES: ROLE OF A NEURAL FACTOR AND CA^{++}. E.K. Dutton and A.J. Olek*. Department of Zoology, The University of Maryland, College Park, MD 20742.

A soluble factor from embryonic brain (EBX) induces acetylcholine receptor (AChR) aggregation in cultured rat myotubes within 4 hours at 36° C (Olek et al., Cell, 34:255, 1983). This effect is dependent on extracellular Ca^{++} and increasing extracellular Ca^{++} from 1.8 to 3.6 mM greatly enhances the myotubes response to EBX. It appears that EBX and Ca^{++} are acting synergistically to promote AChR aggregation.

A larger increase in extracellular Ca^{++} to 7.2 mM can, by itself, induce AChR aggregation within 4 hours at 36° C. Increasing the extracellular concentration of other divalents cations does not induce AChR aggregation. Ca^{++}-induced AChR aggregation occurs to the same extent as EBX-induced AChR aggregation but with a clearly different pattern. Like EBX, Ca^{++}-induced AChR aggregation is sensitive to sodium azide and independent of protein synthesis. However, EBX can induce a detectable AChR aggregation response if applied for a 15 minute pulse while increased extracellular Ca^{++} requires a longer application time to induce a detectable AChR aggregation response.

This research was supported by the Muscular Dystrophy Association of America.

44.12

IMMUNOELECTRON MICROSCOPY LOCALIZATION OF SYNAPTIC VESICLE-SPECIFIC PROTEINS AFTER INTENSE RELEASE OF NEUROTRANSMITTER. F.Torri-Tarelli*, A.Villa*, F.Valtorta*, P.De Camilli§, P.Greengard‡ and B.Ceccarelli* (Spon. L.Martini) Dept. of Medical Pharmacology, Univ. of Milan, Italy; § Yale Univ. School of Medicine and ‡ The Rockefeller Univ., New York

In order to follow the movements of the synaptic vesicle (SV) membrane during the exo-endocytotic cycle, we have applied immunogold labeling techniques to ultrathin frozen sections obtained from frog neuromuscular junctions stimulated with α-latrotoxin (α-LTx) using antibodies to an integral protein of the SV membrane, synaptophysin (p38). After one hour treatment with low doses of α-LTx in the presence of extracellular Ca (condition under which an active recycling of both quanta of neurotransmitter and SV occurs), the immunoreactivity pattern resembled that observed in resting preparations. Immunogold particles were found to be selectively associated with the membrane of SVs, whereas the axolemma was completely unlabeled, suggesting that the retrieval process is selective for the SV membrane and no extensive intermixing of membrane components occurs. When the same doses of α-LTx were applied in Ca-free solution (condition under which a block of recycling results in depletion of SVs and swelling of the nerve terminal), the gold particles were found to be uniformly distributed along the axolemma, indicating that SV membrane had been permanently incorporated into it. The same experimental procedures was applied to study the redistribution of synapsin I, a phosphoprotein associated with the cytoplasmic surface of the SV. Preliminary results show that after stimulation with α-LTx in the presence of Ca immunoreactivity for synapsin I is still localized around SVs, suggesting that either synapsin I remains associated with the vesicle membrane throughout the exo-endocytotic cycle, or that it reassociates with the vesicle after retrieval (MDA grant to B.C.).

44.13

QUANTAL RELEASE FROM IDENTIFIED SYNAPTIC BOUTONS IN SNAKE MUSCLE. R. S. Wilkinson. Dept. of Cell Biology and Physiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Snake motor nerve terminals are comprised of discrete boutons, similar to CNS and autonomic terminals in mammals. In the thin transversus abdominis muscle of the garter snake, most tonic muscle fiber endplates are polyneuronally innervated; occassionally, one axon contributes only a few boutons. These small terminal projections have been utilized to investigate quantal release from single synaptic boutons.

Terminals of one tonic motor axon were supravitally labeled using the activity-dependent probe sulforhodamine 101 (200 μg/ml). Endplates having 1-4 boutons from the axon were selected. Spontaneous miniature (mepp's) and evoked endplate potentials (epp's) were recorded in normal or elevated Ca^{++} saline without paralysing agents. Mean mepp amplitudes were 2-10 mV at -80 to -90 mV resting potential. Low frequency stimulation (< 0.2 Hz) evoked epp's of fluctuating amplitudes indicating release of from 1 to 4 quanta/bouton, with mean quantal content, m, of 2-3 quanta/bouton. Higher stimulus frequencies reduced m but did not eliminate fluctuations. These data suggest that snake neuromuscular boutons contain more than one active zone. (Supported by NIH grant NS24752 and the Muscular Dystrophy Association).

44.14

IMMUNOCYTOCHEMICAL LOCALIZATION OF PHOSPHATIDYLINOSITOL-ANCHORED ACETYLCHOLINESTERASE IN EXCITABLE MEMBRANES OF TORPEDO. J. Eichler*, I. Silman*, M.K. Gentry* and L. Anglister. Anatomy Dept., Hebrew Univ. Med. Sch., Jerusalem 91010, Neurobiology Dept., Weizmann Inst., Rehovot 76100, ISRAEL and Walter Reed Army Inst. Res., Washington, DC 20307

In Torpedo electric organ much of the AChE is a hydrophobic dimer (G_2) which copurifies with synaptosomes, suggesting a presynaptic localization. G_2 AChE is anchored to the plasma membrane via covalently attached phosphatidylinositol (PI), and is selectively solubilized, in electric organ homogenates, by a bacterial PI-specific phospholipase C (PIPLC). This suggested use of PIPLC, coupled with immunocytochemistry, to localize G_2 AChE in Torpedo ocellata tissues.

Cryostat sections of electric organ, of electromotor nerve and of muscle were labelled with polyclonal antibodies to Torpedo AChE and then with fluorescein-tagged second antibody. Rhodamine-α-bungarotoxin was used to delineate synapses. Sections pretreated with PIPLC were compared with controls.

The antibodies bound selectively to innervated sites on electrocytes and myofibers. For both, pretreatment with PIPLC markedly reduced immunofluorescence. The strong binding to the electromotor axon surface was also diminished after PIPLC. MCAb 4E7, which binds preferentially to G_2 AChE, labelled both innervated and non-innervated faces of the electrocyte (Howland et al. Soc.Neurosci.Abstr.9:726, 1983) and strongly labelled muscle endplates. PIPLC decreased staining of the electrocyte's innervated face and of muscle endplates, and similarly reduced 4E7 staining of electromotor nerve.

The results support our previous assignment, based on biochemical evidence, for a neuronal and synaptic localization of the PI-linked AChE dimer in Torpedo. We are now using EM to precisely localize the dimer in the synaptic cleft. (Supported by grants from MDA to I.S., BSF to L.A. and Israel Acad.Sci. to L.A. and I.S.).

44.15

SKELETAL MUSCLE ACETYLCHOLINESTERASE A_I & A_{II} FORMS FOLLOWING ORGANOPHOSPHATE (DFP) INACTIVATION.
X. Busquets*, S. Rummel* and H.L. Fernandez. Neuroscience Lab., Veterans Administration Med. Ctr., Kansas City, MO 64128 and Dept. of Physiology, Univ. of Kansas Med. Ctr., Kansas City, KS 66103.

Mammalian skeletal muscles contain multiple molecular forms of AChE [acetylcholine acetylhydrolase, E.C. 3.1.1.7] which have been categorized as globular [low-salt soluble] and asymmetric [high-salt soluble]. The asymmetric [A_{12}] form, which is highly concentrated at the neuromuscular junction, has attracted much attention partly because of its acknowledged usefulness as a sensitive indicator of nerve-target cell interactions. Recently the existence of two pools of A_{12} AChE has been reported: A_I and A_{II}, extractable in high-salt buffer in the absence or presence of divalent cation chelators [e.g., EDTA], respectively. To further define these A_{12} subtypes we studied the time course of recovery of A_I & A_{II} activities following organophosphate [DFP] inactivation.

Anterior gracilis muscles [3 month old Fischer 344 rats] were treated "in situ" with 0.4 mM DFP for 5 min and at various times thereafter they were assayed, along with untreated muscles, for AChE molecular forms. Results showed that the activity of A_I AChE recovered approximately 48h earlier than that of A_{II} AChE. The recovery of A_I began shortly before 24h. In turn, the onset of A_{II} recovery occurred approximately after 72h of treatment. These and other results suggest a possible metabolic association between A_I and A_{II} AChE pools.

44.16

TETANIC AND POST-TETANIC POTENTIATION ARE DECREASED IN DEGENERATING NEUROMUSCULAR JUNCTIONS OF MANDUCA SEXTA.
I. M. Sonea*, C. A. Young* and M. B. Rheuben (SPON: A. E. Kammer), Dept. of Anatomy, Michigan State University, E. Lansing, MI 48824.

The electrophysiological properties of normal larval and degenerating day 1 pupal neuromuscular junctions (NMJ) were compared before, during and after a 6 min train of 20 stimuli/sec. Characteristic differences were observed between the 2 muscles studied, the mesothoracic AB and C muscles, which persisted in degenerating NMJ's. Tetanic potentiation, as measured by single test pulses during a brief interruption of tetanus, was present in normal larval EJP's, but reduced or absent in degenerating ones. Post-tetanic potentiation, measured by test pulses 2.5 sec apart, was also present in normal but not degenerating NMJ's. These alterations of relatively slow processes may reflect metabolic changes within the nerve terminal or secondarily the glial cells surrounding it. Up to 12 hours after pupation a portion of the junctions continued to release transmitter, as judged by intracellular recordings of EJP's. Failure to obtain EJP's was usually abrupt, occurred at high stimulus frequencies, and could be attributed to failure of the nerve to conduct. Morphologically, the glial sheath and the axons within it were undergoing dramatic changes at the same time. (Supported by N.I.H. Grant NS17132)

44.17

EJP PROPERTIES DURING NEUROMUSCULAR DEGENERATION MAY PARTLY BE RELATED TO DECREASING GLIAL FUNCTION. C. A. Young*, I. M. Sonea* and M. B. Rheuben, Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

The properties of the excitatory junction potential (EJP) and ultrastructural features of the larval and early pupal mesothoracic muscle fibers of Manduca sexta were compared during the neuromuscular degeneration associated with metamorphosis. Early in degeneration, lowered (Ca++) became less effective in decreasing EJP amplitudes in both salines with added nutrients (glutamine and sugars) and without. Beyond 4 hrs post-pupation, EJP's measured in added nutrient salines continued to have large amplitudes, while those in low nutrient salines declined. EJP's of pupal fibers in low nutrient saline were inconstantly lengthened (up to 400 msec) in total duration; those measured with nutrients present had statistically significant increases in times to 1/2 fall. Ultrastructurally, the glial cells were withdrawing their processes from the interstices of the neuromuscular junction, even though many nerve terminals continued to have apparently normal pre- and postsynaptic specializations. The observed physiological changes may reflect a decline of energy driven functions of both the nerve terminal and the glial cell, such as uptake and recycling of glutamate and regulation of ion concentrations within and around the nerve terminal. (Supported by N.I.H. Grant NS17132)

44.18

STRUCTURAL AND FUNCTIONAL DEVELOPMENT OF IDENTIFIED ELECTROTONIC SYNAPSES IN CRAYFISH. B. Leitch*, J.L.S. Cobb*, W.J. Heitler, and R.M. Pitman. Gatty Marine Lab., Univ. of St. Andrews, Scotland, KY16 8LB.

The post-embryonic development of the non-rectifying lateral giant septate synapse (LG-LG) and the rectifying giant fibre-motor giant synapse (GF-MoG) were investigated using ultrastructural and electrophysiological techniques.

In adults the LG-LG junction is characterised by windows of close membrane apposition with cross-bridging connexons. On either side of the junction are numerous, large (60-80nm) spherical vesicles. In newly hatched crayfish, vesicles are extremely sparse and much smaller (30-40nm). Occasional large vesicles become evident during the 2-month development period, however, vesicle number remains low. The LG-LG electrotonic junction is functional from day 0, as evidenced by Lucifer yellow dye coupling and the passage of current.

At the adult GF-MoG synapse, contact is made via narrow "bottle-necks". The gap junctional regions are similar to the adult LG-LG synapse except that large spherical vesicles are only present in the presynaptic giant fibre. In hatchlings, GF-MoG contact is made over extended areas of flat membrane apposition. Initially, the junctional region is morphologically indistinguishable from a chemical synapse. During development, gap junctional regions gradually appear bordered by chemical-like synapse areas. However, electrophysiological and dye coupling evidence indicates that these junctions too function as electrotonic synapses from day 0.

44.19

ORGANIZATION AND NEUROCYTOLOGY OF THE BRAIN OF A PARASITIC FLATWORM, *FASCIOLA HEPATICA*. S.C. Sukhdeo and M.V.K. Sukhdeo*. Dept. Zoology, University of Toronto, Toronto, Ontario M5S 1A1.

Flatworms (Platyhelminthes) are the most primitive animals to possess a central nervous system. The organization, architecture and neurocytology of the brain of *Fasciola hepatica* were examined in this study. Three-dimensional reconstructed models of the brain show that paired ganglia situated at the oral sucker/pharynx junction are connected by a dorsal transverse commissure. The ganglia are not surrounded by a capsule or glial sheath. The cell bodies in each ganglion are loosely organized around the periphery and are also found in the neuropile area; no clearly-defined cell rind is present. The neuropile is composed of tightly-packed unmyelinated nerve processes consisting of small nerve fibers (2 μm diameter) and large 'giant' nerve fibers (> 15 μm diameter).

The fine structure of the ganglion cell bodies are typical of cell bodies of higher invertebrates. Nerve processes contain four morphologically-distinct types of vesicles. Simple synapses and wedge-shaped or divergent diad synapses are present in the neuropile, commissure and nerve cords. The unique feature in the nervous system are the 'giant' nerve processes which have highly invaginated cell membranes. "Glial-like" cells interdigitate into the 'giant' processes and cytoplasmic organelles (mitochondria) are frequently found in the invaginations. These 'giant' processes are found in the neuropile and make up the bulk of nerve tissue in the commissure and nerve cords. The functions of these 'giant' nerve processes are unknown at this time but they may fulfill functions similar to those of trophospongium cells of insects.

44.20

SYNAPSES IN THE ANTEROVENTRAL COCHLEAR NUCLEUS PREPARED BY FREEZE-SUBSTITUTION. H.Tatsuoka* and T.S Reese. (SPON: P. Gallant) NINCDS, NIH, at MBL, Woods Hole, MA 02543.

The rostral anteroventral cochlear nucleus (AVCN) of the chinchilla has provided a preparation in which neuronal cell bodies and synapses in the CNS can be examined after direct freezing and freeze-substitution of rapidly excised brain stem slices. Cell bodies in the freeze-substituted AVCN differed from those in perfusion-fixed AVCN in several respects. In spite of these differences, four types of synaptic terminal were distinguished in freeze-substituted AVCN and correlated with the four types of terminals previously reported after chemical fixation. Since the transmitter for each of the four types of terminal is known, the structure of synaptic vesicles and junctions in the four chemical types of synapse could be compared. Synaptic vesicles were uniformly round, but their diameters and deployment near the synaptic junction as well as the detailed structure of the post synaptic density differed in each chemical type of synapse. In particular, the acetylcholine synapse had only a few vesicles clustered at its presynaptic junction-the rest were separated from the junction by a network of fine filaments. Characteristic of the post-synaptic density of glycine terminals were numerous fine filaments parallel to the plasmalemma and suspended between the postsynaptic projections. Thus, freeze-substitution revealed structural differences between chemically different types of synapses which may be related to differences in transmitter storage, release, and reception.

44.21

ULTRASTRUCTURAL CHARACTERISTICS OF THE SYNAPTIC JUNCTION: RELATIONSHIP TO SYNAPTIC SHAPE. E.J. Markus, T.L. Petit & J.B. LeBoutillier. Dept. of Psychology & Program in Neuroscience, Univ. of Toronto, Ont. M1C 1A4, Canada.

Recent research has indicated that synaptic structure & curvature are an important and potentially critical plastic feature of the synapse.

We have divided synapses into 4 types based on their shape: smile, flat, frown and irregular synapses. If one orients a photomicrograph with the presynaptic (axonal) terminal superior and postsynaptic (dendritic) terminal inferior then a protrusion of the presynaptic density into the postsynaptic terminal will form a "smile". Similarly, the protrusion of the postsynaptic density into the presynaptic terminal will form a "frown". Synapses with a parallel pre- and postsynaptic density are designated "flat" synapses, and synapses that are a combination of the above types are categorized as "irregular" synapses.

Alterations in synaptic shape is associated with synaptic function. Specifically, in the occipital cortex, an increase in the proportion of frown to smile synapses is related to maturation, and in adulthood, associated with the level of neuronal activity (see Markus & Petit, in press). The present study examined the ultrastructural characteristics of the different shaped synapses in a group of aged rats (25 month). Our findings indicate that:

1) Both the frown and irregular synapses are longer and have larger pre- and postsynaptic dense elements than smile or flat synapses.
2) Presynaptic dense projections are higher in the frown synapses than in the smile synapses.

A comparison will be made to hippocampal synapses, and functional implications will be discussed.

RECEPTOR MODULATION AND REGULATION I

45.1

THE EFFECTS OF GTP ON [^{3}H]CYCLOHEXYLADENOSINE BINDING TO A_1 ADENOSINE RECEPTORS IN MEMBRANES FROM RAT CORTEX AND HIPPOCAMPUS IN THE PRESENCE OF THEOPHYLLINE, 2-CHLOROADENOSINE, AND CARBAMAZEPINE. S.M. Anderson, R.L. Weir* and J.W. Daly. Dept of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307; Neurotoxicology Section, NINCDS, NIH, Bethesda, MD 20892 and Dept of Neurology, Howard U School of Medicine, Washington, D.C. 20060; Lab of Bioorganic Chemistry, NIDDKD, NIH, Bethesda, MD 20892

High affinity A_1 adenosine receptors mediating the inhibition of adenylate cyclase have been identified in the CNS. The binding of several adenosine agonists to A_1 adenosine receptors in rat brain has been shown to be decreased by guanine nucleotides. We have previously reported inhibition by 6.7 μM GTP of [^{3}H]CHA binding to membranes from rat cortex and cerebellum. In this report we present data showing an enhancement of 0.1 nM and 10 nM [^{3}H]CHA binding to cortical membranes by 0.05-0.80 μM GTP and inhibition of binding at higher GTP concentrations (1-10 μM). Unlike the biphasic actions of GTP on cortical membranes, we found no enhancement of [^{3}H]CHA binding to hippocampal membranes. The actions of GTP on the binding of [^{3}H]CHA in the presence of 2-chloroadenosine, a competitive agonist, theophylline, a competitive antagonist, and carbamazepine (an anticonvulsant which is a mixed competitive/noncompetitive inhibitor) parallel the pattern seen with GTP alone. These data suggest regional variation in GTP effects at A_1 adenosine brain receptors.

45.2

BIVALENT ANALOGS OF ADENOSINE: EVIDENCE FOR BINDING TO THE A1 ADENOSINE RECEPTOR. J.B. Wollack and J.B. Pennywell*. Dept. of Neurology, Div. of Pediatric Neurology, College of Physicians and Surgeons of Columbia University, N.Y., NY 10032

The adenosine receptor system is emerging as an important modulator of brain injury resulting from hypoxic, ischemic and epileptic insults. In seeking to develop new ligands for this receptor, we synthesized a series of bifunctional agents, containing two adenosyl moieties separated by an alkane bridge. This approach has been used previously (Portoghese et. al., J Med. Chem., 29:1855, 1986, Shimohigashi et. al., Molec. Pharmacol., 21:558, 1982) to develop useful agents for the opiate receptor system.

Ethylenediamine, 1,3-propanediamine, and 1,6 hexanediamine were reacted with an excess of 6-chloropurine riboside to produce the corresponding 1,2 bis(N^6-adenosyl)ethane (A-2-A), 1,3 bis (N^6-adenosyl)propane (A-3-A) and 1,6 bis(N^6-adenosyl)hexane (A-6-A), respectively. The affinity of these compounds for the A1 adenosine receptor in whole rat brain membranes was assayed utilizing [^{3}H]cyclohexyladenosine (CHA) as a radioligand. Comparisons were made with N^6 methyladenosine (meADO), a representative monovalent alkyladenosine; CHA, one of the most potent A1 agonists; and theophylline (theo), a commonly used antagonist. The order of binding was found to be CHA>A-6-A>A-2-A>meADO>theo. Limited solubility made evaluation of A-3-A difficult, however, it appeared less potent than A-2-A. These results suggest that bivalent adenosyl compounds represent a new class of compounds to be investigated as ligands for the adenosine receptor.

Supported by the Charles A. Dana Foundation and the Colleen Giblin Foundation.

45.3

PARTIAL PURIFICATION OF RAT BRAIN AND TESTICULAR A_1 ADENOSINE RECEPTOR BY AFFINITY CHROMATOGRAPHY. H. Nakata* (Sponsor: D.M. Jacobowitz), Laboratory of Clinical Science, NIMH, Bethesda, MD 20892.

Adenosine receptors have been classified into two major subclasses, A_1 and A_2. The A_1 receptor is linked to inhibition of adenylate cyclase whereas A_2 receptor is linked to activation of adenylate cyclase. In order to get more information about molecular properties of the A_1 receptor, purification of A_1 receptor by affinity chromatography was performed. The solubilized A_1 receptor from rat brain membranes was applied to a xanthine amine congener (XAC)-linked agarose column and eluted with 8-cyclopentyltheophylline (CPT). The adsorption of receptor to the affinity matrix was biospecific as preincubation of the solubilized preparation with A_1 agonists or antagonists blocked retention of receptor. Elution of the receptor was also biospecific as a potent A_1 agonist (CPA) or antagonists (CPT, XAC) were most effective in eluting the bound receptor. The affinity purification resulted in a recovery $\sim$ 30% of the solubilized A_1 receptor applied with a specific activity of $\sim_3$30 pmol/mg ($\sim$ 50-fold purification) as assayed with [^{3}H]DPCPX. This affinity purification was also applied successfully for the purification of rat testicular A_1 receptor. Chromatography of solubilized rat testes membrane preparations on XAC-agarose gel resulted $\sim$ 50-fold increase in the specific binding activity for [^{3}H]DPCPX. The affinity chromatography procedure described here should prove to be a key tool in the eventual purification of the A_1 receptor.

45.4

PROLACTIN UPTAKE BY THE CHOROID PLEXUS OF HYPERPROLACTINEMIC RATS. L.P. Mangurian*, R.J. Walsh and B.I. Posner*. Anatomy Department, The George Washington University Medical Center, Washington, D.C. 20037 and Department of Medicine, Royal Victoria Hospital, Montreal, Canada.

The choroid plexus (CP) contains prolactin receptors that are involved in transporting prolactin from blood to CSF. Since CSF concentrations of prolactin parallel blood concentrations, up regulation of prolactin receptors at the CP might facilitate transport into CSF during periods of elevated blood prolactin concentrations. In vivo autoradiography was used to assess the ability of the CP to uptake ^{125}I-prolactin during hyperprolactinemia. Ovariectomized rats were made hyperprolactinemic by treatment with haloperidol (2.5 mg/kg b.w./day for 3 consecutive days) and bromocriptine (10 mg/kg b.w., 4 hours prior to perfusion). In vivo administration of ^{125}I-prolactin was followed by vascular perfusion. Paraffin sections were coated with NTB-2 emulsion. Analysis of ^{125}I-prolactin accumulation in the CP was performed with a video grain counting program. A 133% increase in the accumulation of radioactivity was observed in the CP of hyperprolactinemic animals relative to untreated. Experiments are underway to determine whether the increase in ^{125}I-prolactin binding in the CP is due to up regulation of the receptor population or a variation in the occupancy of receptors due to the treatment. NSF Grant BNS-8604760. NIH 2-R01-DK19573-10A1.

45.5

BINDING OF DELTA-9-TETRAHYDROCANNABINOL (THC) TO GLUCOCORTICOID RECEPTORS IN RAT HIPPOCAMPUS. J.C. Eldridge*, D.G. Fleenor*, L.B. Cadwallader* and P.W. Landfield (SPON: M. Levitt), Dept. Physiol. & Pharmacol., Bowman Gray School Med., Winston-Salem, NC 27103

In recent studies (Landfield et al, 1988, Brain Res, vol. 443) chronic treatment of rats with THC, the major psychoactive component of marijuana, was found to induce structural changes in hippocampal neurons similar to normal aging or to the effects of glucocorticoids. Plasma corticosterone and ACTH were also increased by THC treatment. Due to similarities of THC and steroid molecular structure, it seems possible that THC a) binds to corticoid receptors and directly mimics hormonal actions or b) interferes with negative feedback of ACTH or otherwise activates the pituitary-adrenal axis, thereby enhancing endogenous hormonal effects on the hippocampus. To compare these possibilities, we studied competitive interactions of THC with hippocampal corticosteroid receptors (HCSR), and we also examined the functional ability of THC to down-regulate HCSR in adrenalectomized (ADX) rats.

Scatchard-type competition studies on hippocampal cytosol using [^{3}H]-dexamethasone (DEX) showed that 100-fold excess of THC displaced bound tracer in a lesser but parallel fashion to RU-28362 (a specific Type II agonist). Co-incubation of THC and RU failed to displace additional sites, indicating unlikely interaction of THC with Type I HCSR. Cannabadiol, a non-psychoactive cannabinoid, displaced DEX less than did THC. In another study, young ADX male rats were injected subcu. with 10 mg/kg THC daily for 14 days. Hippocampal tissue was analyzed for Type II HCSR by methods previously described (Eldridge et al, 1987, Soc Neurosci Abs). In comparison to controls given vehicle, a 28.3% down-regulation of Type II binding was seen in THC-treated rats ($p < .05$), with no change of K_d.

These results suggest that THC binds to HCSR with a lower affinity than natural corticosteroids, and that this binding can functionally regulate CSR density in hippocampus. (Supported by DA-03637)

45.6

TRANSIENT APPEARANCE OF $GABA_A$ AND BENZODIAZEPINE BINDING SITES IN BRAINSTEM NUCLEI DURING DEVELOPMENT. A. Frostholm, A. Neustadt and A. Rotter. Dept. of Pharmacol., Univ. of Calif., Irvine, CA 92717.

The embryonic and postnatal distribution of [^{3}H]muscimol and [^{3}H]flunitrazepam binding sites was studied autoradiographically in the brainstem of C57BL/6 mice. Although adult brainstem nuclei were almost devoid of [^{3}H]muscimol binding sites, high levels of grain density were distributed throughout the medulla in embryonic and early postnatal animals. The inferior olive and the nucleus of the sensory trigeminal tract were of particularly high grain density. Labeling over all medullary regions increased between embryonic day 18 and postnatal day 7, after which there was a gradual reduction in grain density throughout the brainstem. By postnatal days 14-16, the grain density over all medullary regions, including the inferior olivary and trigeminal nuclei, approached the low adult levels. As with [^{3}H]muscimol binding sites, a moderate to high level of [^{3}H]flunitrazepam labeling was uniformly distributed throughout the brainstem of neonatal mice; there was, however, no similar decrease in the overall grain density during development, but rather a steady increase to adult levels. Again, even higher grain density was observed over the trigeminal nucleus and inferior olive which was reduced to the level of other medullary regions by postnatal days 11-14.

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45.7

N-ACETYL-5-METHOXYKYNURENAMINE AND MELATONIN INTERACTION WITH GABA AND DIAZEPAM RECEPTORS IN MAMMALIAN BRAIN. B.J. Mason and L.P. Niles (SPON: G.K. Smith), Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Pharmacological doses of melatonin enhance γ-aminobutyric acid (GABA) binding in rat brain (1,2). Melatonin's effects on GABA binding in vitro are blocked by the central benzodiazepine (BZ) receptor antagonist, Ro15-1788, indicating involvement of BZ binding sites.

Ring cleavage of melatonin by 2,3-dioxygenase in the CNS followed by the action of formamidase, produces N-acetyl-5-methoxykynurenamine (AMK). Since AMK (IC_{50} = 50-100 µM) is significantly more potent that melatonin (IC_{50} = 500 µM) in inhibiting [^{3}H]diazepam binding in synaptosomal membranes, we have examined this compound's effects on GABA binding in the rat cerebral cortex.

Binding experiments with [^{3}H]GABA ($\sim$1-750 nM) indicate that AMK (100 µM) enhances low-affinity $GABA_A$ binding with a concomitant decrease in binding affinity.

These preliminary findings support our hypothesis that the melatonin derivative, AMK, is a centrally active agent capable of modulating GABAergic activity.

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1. J. Neural Transm. 70, 117 (1987)
2. Biochem. Pharmacol. 37(7), 1271 (1988)

45.8

TESTOSTERONE MODULATES OXYTOCIN RECEPTOR BINDING IN THE VMN OF CASTRATED MALE RATS. A.E.JOHNSON, H.COIRINI*, B.S.McEWEN AND T.R.INSEL. LCS/NIMH, Poolesville, MD 20837 and Department of Neuroendocrinology, Rockefeller University, N.Y., N.Y. 10021.

Oxytocin (OT) receptor binding is modulated by estradiol-17ß in several brain regions including the ventromedial hypothalamic nucleus (VMN) in both male and female rats (Coirini et al, in preparation). To further study steroid regulation of OT receptor binding, we examined the effect of androgen replacement in castrated male rats on OT binding with quantitative autoradiographic methods.

Adult male rats (300g) were castrated, treated one week later with either 250µg testosterone propionate (TP, N=4) or oil (N=3) for two days and killed 48hrs after the last injection. Cryostat cut brain slices (16µm) through the VMN were labeled with 5.0nM [^{3}H]OT± 5.0µM unlabeled OT or 1.0µM [Thr^4,Gly^7]-OT (a specific OT agonist) and apposed to tritium-sensitive film for two months along with plastic tritium standards. Autoradiograms were analyzed with a computer assisted densitometer (DUMAS) that converted gray levels to moles/mg tissue equivalents using the standard curve derived from tritium standards.

Results of this study show that TP increased [^{3}H]OT binding 3 to 5 fold in the ventrolateral VMN. In addition, [Thr^4,Gly^7]-OT completely displaced [^{3}H]OT binding in the VMN indicating that binding in this brain region was specific to OT receptors. Because E_2 also increases OT receptor binding in male rats (Coirini et al), it is possible that TP affects OT receptors after being converted by aromatase to E_2. Supported in part by grant numbers TW03617 (HC) and MH41256 (BMc).

45.9

EFFECTS OF DORSAL RHIZOTOMY ON NEUROKININ RECEPTOR DENSITIES IN THE RAT SPINAL CORD. K. Yashpal[1]*, T.V. Dam[1,2] and R. Quirion[1] (SPON: S. Gauthier) [1]Douglas Hospital Research Centre and Department of Psychiatry, McGill Univ., Verdun, Canada, [2]Dept. de Pharmacologie, Univ. Sherbrooke, Sherbrooke, Canada

Previous studies have indicated a loss of substance P/neurokinin 1 (NK-1) binding sites following chronic deafferentation (Brain Res. 343:268, 1985). This suggests that SP receptors are located on terminals of primary afferents in the spinal cord. However, it is not known if other NK receptor classes (NK-2, NK-3) are similarly located. In the present study, we determined the effects of unilateral dorsal rhizotomy on binding of ^{125}I-BH substance P, (2-(^{125}I)-iodohistidyl^{1})-neurokinin A and (^{125}I)-BH-eledoisin as respective radioligands for NK-1, NK-2 and NK-3 receptor subtypes. 7, 14, 21, and 28 days after a unilateral lumbosacral dorsal rhizotomy, receptor binding for the above neurokinins was evaluated using an autoradiographic method. Sections were prepared and incubated with 50pM of the different radioligands as described before (J. Neurosci. 6:2187, 1986). Preliminary results indicate a significant decrease of NK-1 sites at 7 and 14 days after rhizotomy. This is specifically seen in the dorsal horn of the spinal cord. This supports the hypothesis of a presynaptic location of certain NK receptor sites on primary afferents. (Supported by the Canadian Heart Foundation)

45.10

BINDING OF ^{125}I-SUBSTANCE P IN THE MEDIAL NUCLEUS OF THE AMYGDALA IS NOT AFFECTED BY GONADECTOMY. T.R. Akesson, P. Popper, and P. E Micevych. Dept. of Anatomy, Lab. of Neuroendocrinology, and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The medial nucleus of the amygdala (MeA) has a well established role in the processing of olfactory and endocrine information essential to reproductive function in the rat. This nucleus is a major target of gonadal steroids and contains a large population of substance P immunoreactive (sPir) cells as well as a dense plexus of sPir fiber terminals. Sex differences in the number of sPir fibers and, in males, a loss of sPir elements following castration suggest that gonadal steroids interact with sP in the MeA. The finding that this nucleus also contains a rich field of sP binding sites prompted us to test the possibility that gonadal steroids regulate sP binding. 30 µm sections through the MeA from intact and castrated males, ovariectomized females, and females receiving 50 µg estradiol benzoate 24 hours before death were used to generate autoradiographical images. Highest levels of ^{125}I-sP binding were found to be specifically localized in the posterior third of the MeA and ventral to the MeA. While the region that exhibited intense sP binding was slightly larger in males, gonadectomy in both males and females did not appear to affect either the extent or intensity of sP binding. Thus while immunohistochemical evidence suggests steroidal interaction with sP, in the MeA this interaction does not appear to involve modulation of sP binding. (Supported by NS 21220 to PEM and HD 22869 to TRA).

45.11

EVIDENCE FOR THE INVOLVEMENT OF PROTEIN KINASE C IN NEURONAL ANGIOTENSIN II RECEPTOR EXPRESSION. C.J. Kalberg*, M.K.Raizada* and C. Sumners. Dept. of Physiology, University of Florida, Gainesville, FL 32610.

Previously, we demonstrated that protein kinase C (PKC) activating phorbol esters increase [125I] Angiotensin II (Ang II) specific binding in neuronal cultures prepared from 1-day-old-rats (Sumners, C. et al., J. Neurochem. 48:1954-1961, 1987). This suggests PKC is involved in the expression of central Ang II receptors. However, phorbols have many nonspecific effects. Therefore, we used three distinct experimental approaches to substantiate the involvement of PKC in Ang II receptor expression in neuronal cultures. First, mezerein and teleocidin, two PKC activators that are chemically unrelated to phorbols, were shown to increase [125I] Ang II specific binding in a dose and time dependent manner. ED50 values for stimulation of [125I] Ang II specific binding by mezerein and teleocidin were 32 nM and 72 nM respectively. Second, the PKC antagonist H-7 was shown to significantly inhibit 0.8 uM phorbol-12-myristirate-13-acetate (TPA) stimulated increases in [125I] Ang II specific binding at doses of 50 uM, 75 uM, 100 uM, 150 uM, and 300 uM. The latter three doses of H-7 produced [125I] Ang II specific binding levels not significantly different from control levels (1% level Newman-Keuls). Thirdly, PKC downregulation by chronic phorbol ester incubations for 24 and 48 hrs prevented 0.8 uM TPA stimulation of [125I] Ang II specific binding. These results substantiate the involvement of PKC in Ang II receptor expression and suggest that certain neurotransmitters or hormones may affect central Ang II receptor numbers through modulation of PKC activity. (Supported by NIH grant NS-19441).

45.12

NEURONAL SPECIFIC SUBSTRATES FOR A GTP-PREFERRING PROTEIN-KINASE. A. Babinska*, R. McCullum* and Y.H. Ehrlich. Dept. of Psychiatry, Univ. of Vermont and The Ctr. Dev. Neurosci., CUNY/CSI, Staten Island, N.Y. 10301

GTP is a critical regulator of receptor function. Here we have characterized the endogenous substrates of a GTP-preferring protein kinase from rat brain membranes (BBRC 107:669-706). Using two-dimensional polyacrylamide gel electrophoresis it was found that in the M.W. range of 52Kd to 59Kd there are four distinct proteins (pI range 4.6-5.0) phosphorylated preferentially by GTP. Among them a protein with apparent M.W. of 53.1±0.6Kd and pI of 4.95 was phosphorylated almost exclusively by GTP (in the presence of Mn^{2+}). It was determined that this protein is not tubulin, it is enriched in synaptic plasma membranes, and present in brain but NOT in other tissues. This neuronal specific phosphoprotein was isolated from Triton-X-100 extract of brain membranes and characterized by phosphopeptide mapping. Using various concentrations of chymotrypsin, trypsin and V8-protease we identified three fragments of this protein (47.5K, 26.3K and 14.8K) that are phosphorylated by GTP greater than by ATP, and two fragments (21K and 32K) with preference for ATP. Raising antibodies which recognize specifically the sites phosphorylated preferentially by GTP will provide the tools for investigating their role in neuronal function.
Supported by NSF grant BNS85-07238.

45.13

REGULATION OF NEURONAL RECEPTOR EXPRESSION: THE TARGET CELL HYPOTHESIS. A. Rotter and A. Frostholm. Dept. of Pharmacol., Univ. of Calif., Irvine, CA 92717.

Cellular interactions regulating the expression of transmitter receptors are of considerable importance in the establishment of synaptic transmission. Based on results obtained on $GABA_A$/benzodiazepine receptors in the cerebellum of developing and neurologically mutant mice, we propose a hypothesis of neuronal receptor induction, stabilization and maintenance. We postulate that actively proliferating neuroblasts do not express transmitter receptors; these are acquired only after the cessation of cell division, during the period of cell migration. Since migrating neurons have not at this stage formed either afferent or efferent synapses, we propose that the initial induction of neurotransmitter receptor synthesis precedes, and is independent of, synaptogenesis. It is generally accepted that receptors actively turn over, thus, in order for them to be continuously expressed, the initial induction of neurotransmitter receptor synthesis must be stabilized.We suggest that the stabilization of receptor synthesis is dependent on the formation of efferent synaptic contacts with target neurons. If synapse formation with target cells fails to occur during development, the initial induction of neurotransmitter receptor synthesis is not stabilized, receptor expression remains labile and receptors are lost. Following receptor stabilization, continuous presence of the target cell is required for maintenance of receptor expression. In contrast to the role of the efferent connection, the afferent synaptic input plays no role in receptor induction, stabilization or maintenance; in most cases, in fact, it tends to down-regulate receptor number. The above hypothesis has features which may provide explanations for the phenomena of mismatches in receptor/neurotransmitter localizations and for the transient expression of receptors during CNS development. Supported by USPHS grants NS18089 and HL34472 to A.R.

45.14

SYNERGISM BETWEEN ACTIVATED MEMBRANE RECEPTORS THAT TRIGGER THE SAME EFFECTOR MECHANISM. G.J. Christ[1,3]*, J. Goldfarb[2], A. Melman[3]*, R. Osman[4]*, and S. Maayani[1]. Departments of 1. Anesthesiology, 2. Pharmacology, 3. Physiology and Biophysics, Mount Sinai School of Medicine, CUNY, NY, NY 10029 and 4. Urology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY.

Simultaneous activation of distinct membrane receptors which are linked to the same physiological effect may result in either simple additivity, synergism, or antagonism. A steady-state model designed to predict simple additivity throughout the concentration-response curves for two agonists sharing the same effector systems was derived using the Black and Leff equations for operational agonism; equations which were originally utilized to predict the concentration-response relationship for agonists assuming a ternary (agonist-receptor-effector) complex model. Simultaneous activation of the 5-HT_2 receptor and alpha-1 adrenoceptor in the isolated rabbit aorta was assessed, and the observed dose response curves were compared to those predicted by the model for simple additivity. A robust synergism was detected at low occupancy levels. Supported by UPSH G5-34852.

45.15

A NON-LINEAR ONE-PARAMETER MODEL FOR RECEPTOR REGULATION: EQUILIBRIUM EQUATIONS AND COMPARISON WITH EXPERIMENTAL BINDING DATA. Eugene Somoza. Psychiatry Service, V.A. Medical Ctr, Cincinnati, Ohio 45220; and Lab. of Behavioral Neuroscience, Psychiatry Dept., Univ. of Cincinnati, OH 45267.

A simple non-linear one-parameter mathematical model is postulated which allows for receptor regulation. This is done by defining the association rate "constant" ka of receptor-ligand systems to be a monotonically decreasing function of receptor occupancy. The value of the parameter which governs the functional dependence of ka, to be called the regulation index, δ, is a characteristic property of a given receptor-ligand system. The value of δ can be calculated from experimental binding data along with the two classical parameters: the receptor density R and the dissociation constant K_D. Thus, according to the present model, three parameters (R, K_D, and δ) are always required to describe the interactions between receptors and their ligands. It will be shown that non-linear Scatchard plots can generally be fitted as well with this three-parameter model as they can be with the currently used model, which needs to invoke the ad hoc assumption that such non-linear plots represent the existence of several independent sites.

INTERACTIONS BETWEEN NEUROTRANSMITTERS I

46.1

STRIATAL DOPAMINE-ADENOSINE INTERACTIONS IN VIVO. P.J.Knott, D.M.Togasaki and K.L.Davis. Dept. of Psychiatry and Pharmacology, The Mount Sinai School of Medicine, New York, N.Y. 10029.

Previous work has revealed that L-DOPA increases uric acid in striatum and that there is a linear relationship between the induced striatal uric acid asymmetry and turning behavior in unilateral nigral-lesioned rats. We believe that uric acid may result from adenosine release in response to dopamine receptor interaction (Togasaki et al., Life Sci., 41, 1361, 1987).In the present study we find that uricase infusion close to the working electrode completely prevents the increase of Peak 2 in response to L-DOPA (25mg/Kg i.p.) administration. Evidence that striatal changes of uric acid result from increased adenosine release is suggested by the observation that local infusion of adenosine (1-10mM) also produced large increases in the uric acid peak. Our present data indicates that adenosine may indeed be released as a result of dopamine receptor interaction and may subserve a neuromodulatory role linked to regulation of dopaminergic activity. Accordingly, its production is greater in lesioned striatum where receptor numbers would be increased. (Supported by the Parkinson's Disease Foundation.)

46.2

MODIFIED SENSITIVITY OF RAT CORTICAL NEURONS TO BIOGENIC AMINES AFTER ABOLITION OF CORTICAL NORADRENERGIC TRANSMISSION. Jean Goyer and André Ferron. Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Canada.

We previously showed that 2 to 3 weeks following bilateral destruction of noradrenergic afferents with 6-hydroxydopamine (6-OHDA), the sensitivity of fronto-parietal neurons to microiontophoretic applications of the three biogenic amines (MA), noradrenaline (NA), dopamine (DA), and serotonin (5-HT), was increased in urethane-anesthetized rats (Soc. Neurosci. Abstr. 13: 1654, 1987). To verify whether these changes were due to the absence of NA or to other factors, we assessed the sensitivity of cortical neurons to iontophoretically applied MA after NA depletion with U-14,624 a dopamine-beta-hydroxylase inhibitor (200 mg/kg i.p. x 2 days). The responsiveness to MA was tested by measuring the charge (in nC) which induced a 50% decrease in the spontaneous firing rate. After U-14,624, neurons proved hypersensitive to both NA (1087 vs 1502 nC) and 5-HT (130 vs 974 nC), but not to DA (1197 vs 1265 nC). Thus, whereas NA and 5-HT hypersensitivities were probably due to the absence of NA, the DA hypersensitivity observed after 6-OHDA appeared imputable to other causes. In a second set of experiments, we sought to determine which types of receptors might be involved in the MA hypersensitivities. Preliminary results on NA receptors using amidephrine (AMI; α_1), clonidine (CLO; α_2) and isoproterenol (ISO; β) as specific agonists show increased responsiveness to α_1 agonist (1994 vs 2415 nC), while the responsiveness to α_2 and β receptors remains unchanged (1543 vs 1368 nC; and 661 vs 708 nC). These results suggest a synergism of action of the NA and 5-HT cortical systems that should be taken into account in the analysis of cortical monoaminergic function.

46.3

THE ROLE OF NOREPINEPHRINE IN THE SHORT-TERM DECREASE IN HIPPOCAMPAL TRYPTOPHAN HYDROXYLASE ACTIVITY INDUCED BY METHAMPHETAMINE. M. Johnson, G.R. Hanson and J.W.Gibb. Dept. Pharmacol. and Toxicol., University of Utah, Salt Lake City, UT 84112.

A single dose of methamphetamine (METH) induces a rapid and reversible decrease in brain tryptophan hydroxylase (TPH) activity. The diminution in neostriatal TPH activity can be prevented by depleting dopamine (DA) concentrations. Hippocampal TPH activity is as sensitive to the METH effect as the neostriatal enzyme although its DA content represents a fraction of the neostriatal concentration. In contrast, a significant amount of norepinephrine (NE) is stored in the hippocampus. The purpose of this study was to determine if NE participates in the short-term decline of hippocampal TPH activity induced by METH. Male Sprague-Dawley rats (200-240 g) received two doses of the dopamine-ß-hydroxylase inhibitor, U14,624 (200 mg/kg, i.p.), 18-h apart. The rats received METH (15 mg/kg, s.c.) 6 h after the second U14,624 injection, and killed 3 h later. TPH activity was determined using $^{14}CO_2$ release while NE concentrations were determined by HPLC-EC. The U14,624 treatment induced a 80% reduction in hippocampal NE concentration. Hippocampal TPH activity was unaffected by the decline in NE concentration while METH reduced the enzyme activity to 43% of control. The inhibition of NE synthesis did not alter the METH-induced decrease in TPH activity, thus suggesting that NE does not participate significantly to this decline. (Supported by USPHS DA 00869 and DA 04220)

46.4

RELEASE OF ENDOGENOUS ACETYLCHOLINE AND DOPAMINE FROM NEOSTRIATAL SLICES FROM SALINE- AND 6-HYDROXYDOPAMINE-TREATED RATS. L.M. Alcorn and M.H. Weiler. School of Pharmacy and Env. Tox. Center, Univ. of Wis., Madison, WI. 53706.

Neostriatal dopaminergic/cholinergic systems have been extensively studied (Lehmann and Langer, Neurosci., 10:1105,1983). For many of these studies, neostriatal neurotransmitter stores were prelabelled with radioisotope precursors and radioactive outflow subsequently monitored. In this study, release of endogenous acetylcholine (ACh) and dopamine (DA) was monitored in neostriatal slices from saline- and 6-hydroxydopamine (6-HDA)-treated rats. Each slice served as its own control by stimulating release of DA and ACh twice (K1 and K2) with 25mM potassium. Spontaneous release before each stimulation period and tissue content following K2 were also monitored.

ACh release (nmol/mg protein) during K1 was 0.79±0.14 and 0.84±0.15 and during K2 was 0.73±0.24 and 0.76±0.12 in control (n=5) and 6-HDA slices (n=5), respectively. ACh tissue content was also similar between both groups. In contrast, release of DA from slices of saline-treated rats was similar for both stimulation periods (K2/K1=0.95), but DA release during K1 from slices of 6-HDA-treated rats was 31% that of control, and during K2 was undetectable. The DA tissue content (n=4) was 2.35±0.62 and 0.12±0.09 nmol/mg protein (saline- and 6-HDA-treated slices, respectively). The data show that endogenous ACh release from neostriatal slice preparations is not affected by 6-HDA pretreatment. (Supported by NIH Grant AG05953).

46.5

OPPOSITE EFFECTS OF FORSKOLIN ON DOPAMINE VERSUS NOREPINEPHRINE RELEASE FROM RABBIT CAROTID BODY IN VITRO. G.-F. Cheng*, B. Dinger and S. Fidone, Dept. Physiol., Univ. of Utah, Salt Lake City, UT 84108

Previous experiments in our laboratory demonstrated that hypoxia preferentially releases dopamine (DA) over norepinephrine (NE) in the carotid body, and conversely, that nicotine preferentially releases NE over DA. The present study evaluated the effects of the adenylate cyclase activator forskolin on the stimulus evoked release (stimulus minus basal release in 100% O_2 media) of ^{3}H-DA and ^{3}H-NE (synthesized from ^{3}H-tyrosine) in superfusion media equilibrated with either 10% O_2 or media containing nicotine (50-100 uM). Evoked release was expressed as a fraction of the ^{3}H-DA or ^{3}H-NE content, respectively, and the data compared for carotid bodies incubated in the presence or absence of forskolin (10 uM). The basal release of ^{3}H-DA and ^{3}H-NE (in 100% O_2-media) was unaffected by forskolin, but the release of ^{3}H-DA evoked by 10% O_2-media was increased by 287% ($p<0.0025$). In contrast, forskolin depressed the nicotine evoked release of ^{3}H-NE by 91% ($p<0.005$). The data suggest that cyclic AMP has opposite effects on hypoxia vs. nicotine evoked release of DA and NE from the carotid body.

Supported by USPHS grants NS12636 and NS07938.

46.6

COEXISTENCE OF TYROSINE-HYDROXYLASE AND DOPAMINE-β-HYDROXYLASE IMMUNOREACTIVITIES IN TYPE I GLOMUS CELLS OF THE CAT CAROTID BODY. Z.-Z. Wang*, B. Dinger, S. Fidone and L.J. Stensaas (SPON: J.W. Woodbury), Dept. of Physiol., Univ. of Utah, Salt Lake City, UT 84108

We have recently shown that hypoxia evokes the preferential release of dopamine (DA) from rabbit carotid body and conversely, that nicotine primarily releases norepinephrine (NE), suggesting different storage sites of these substances in the chemosensory tissue. The present study was undertaken to determine whether DA and NE are synthesized (and stored) in different subpopulations of type I glomus cells. Double immunocytochemical staining was accomplished with a combination of a monoclonal antibody to tyrosine hydroxylase (TH; APAAP method; red stain) and a rabbit polyclonal antibody to dopamine-β-hydroxylase (DBH; PAP method; blue-black granules). The resulting sequence revealed red-stained cells containing blue-black granules. We observed that 98.9% of TH-stained glomus cells contained DBH, while 98.2% of DBH immunoreactive glomus cells contained TH. In view of the differential release of DA and NE, the data suggest that these two catecholamines may coexist in type I cells. Whether they are stored in different subcellular compartments (granular vesicles) will be addressed in future studies at the EM level (Supported by USPHS grants NS12636 and NS07938)

46.7

SPECIES-DEPENDENT AUGMENTATION OF RECEPTOR-MEDIATED cAMP PRODUCTION BY BACLOFEN. R.W. Scherer*, J.W. Ferkany and S.J. Enna, Nova Pharmaceutical Corp., Baltimore, Maryland 21224.

A neuromodulatory role for $GABA_B$ receptors has been suggested since the $GABA_B$ agonist baclofen (BAC) augments receptor-mediated cAMP production in rat brain slices but inhibits forskolin-stimulated second messenger production. The present study examined whether a similar role for $GABA_B$ receptors exists in other animal species.

For this study, the effects of BAC (100 μM) on cAMP production in the presence of isoproterenol (ISO; 10 μM), histamine (HIST; 100 μM), VIP (1 μM), 2-CADO (100 μM) or forskolin (5 μM) was examined in slices of guinea pig, rat and rabbit cortex. Whereas BAC alone had little or no effect on cAMP production in any of the species examined, it augmented cAMP production in response to ISO, HIST, VIP and 2-CADO in rat and rabbit but only to HIST and VIP in guinea pig. Forskolin-stimulated cAMP accumulation was inhibited in all species by BAC, although the extent of the inhibitory response varied among species from 20% for guinea pigs to 53% for rat.

These findings suggest that $GABA_B$ receptors regulate brain neurotransmitter activity in a variety of animal species. However, this interaction varies both quantitatively and qualitatively among species indicating phylogenetic differences in function of the $GABA_B$ receptor system.

46.8

PERTUSSIS TOXIN PRETREATMENT BLOCKS POSTSYNAPTIC BUT NOT PRESYNAPTIC EFFECTS OF ADENOSINE IN THE RAT HIPPOCAMPUS. Thomas V. Dunwiddie, William R. Proctor, and Bertil B. Fredholm, Dept. of Pharmacol., Univ. of Colo. Hlth. Sci. Cntr. and Veterans Administration Med. Center, Denver, CO, and Karolinska Institutet, Stockholm, Sweden

Adenosine has a variety of biological effects upon the rat hippocampus which are exerted via at least two distinct adenosine receptors. Adenosine can stimulate and inhibit adenylate cyclase activity via the A2 and A1 receptors respectively, but it has proven difficult to link either of these biochemical effects with electrophysiological responses to adenosine. In this study, we have compared the biochemical and electrophysiological responses in terms of their sensitivity to an A1 receptor selective antagonist (8-cyclopentyltheophylline; CPT), and to pertussis toxin (PTx), which can ADP-ribosylate (and inactivate) the GTP-binding protein that mediates the inhibition of adenylate cyclase activity by various receptors, including the adenosine A1 receptor.

Schild plots were used to estimate the affinity of CPT for the adenosine receptors mediating 1) inhibition of excitatory transmission in the CA1 region, 2) the inhibition of repetitive spiking following antidromic stimulation of the CA1 pyramidal neurons in low calcium medium, 3) inhibition of [^{3}H]-cAMP formation, and 4) increases in [^{3}H]-cAMP, all in the *in vitro* hippocampus. The pA_2 values were 42 nM, 45 nM, 60 nM, and 2200 nM respectively, suggesting that the first three responses are all mediated via A1 receptors, and accumulation of cAMP via an A2 receptor. Although both electrophysiological responses had A1-like pharmacology, only the effect of adenosine on low-calcium bursting (postsynaptic) response was blocked in animals pretreated with PTx. PTx pretreatment also reduced the adenosine-induced hyperpolarization of pyramidal neurons by about 95%, and depressed postsynaptic $GABA_B$-mediated responses to an equivalent extent. Since the inhibition of adenylate cyclase and the activation of a potassium conductance by adenosine are both inhibited by PTx, this suggests that the presynaptic modulatory effects of adenosine are exerted through another mechanism.

Supported by the Veterans Administration and DA02702.

46.9

L-DOPA INDUCED CHANGES IN ENKEPHALIN AND SUBSTANCE P LEVEL IN RAT SUBJECTED TO NEONATAL DOPAMINERGIC DENERVATION. S. P. Sivam, Dept. Pharmacol. & Toxicol., Northwest Ctr. Med. Ed., Indiana Univ. Sch. Med., Gary IN 46408

Previous studies showed that lesion of dopaminergic neurons with 6-hydroxydopamine (6OHDA) during neonatal period led to an enhanced and a retarded development of Met-enkephalin (ME) and substance P (SP) systems in striatum respectively. Lesioned animals exhibited self mutilation behavior (SMB) when challenged with L-DOPA. The present study examined whether L-DOPA would modify the changes in SP and ME sytems established following neonatal lesion. Sprague-Dawley rat pups were lesioned with 6-OHDA and tested when they reached adulthood. One h after L-DOPA treatment, the animals were sacrificed; ME and SP levels were determined by radioimmunoassay. Lesioned group had an increase (+45%) in ME in striatum and a decrease (-22%) in SP level in striatum and substantia nigra. L-DOPA treatment to lesioned animals induced SMB; produced a greater decrease (-61%) in SP level than that observed with lesion alone; failed to modify the increased ME level. These results suggest that L-DOPA induced SMB is associated with a marked reduction of SP content; this has two components namely, a retarded development of SP system per se as a result of 6OHDA lesion and a depletion of remaining SP by L-DOPA presumably by enhanced release. Supported by USPHS grants S07 RR 5371 and NS 26063

46.10

DRAMATIC RESPONSES BY DYNORPHIN SYSTEMS OF THE BASAL GANGLIA TO COCAINE TREATMENT. G.R. Hanson, M. Johnson, L. Bush*, P. Smiley*, and J.W. Gibb. Dept. Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112

The effects of cocaine administration on neurochemical markers of the extrapyramidal monoaminergic systems are relatively small and of short duration. However, cocaine-induced changes in some neuropeptidergic pathways are dramatic, relatively long-lasting and likely mediated by dopaminergic mechanisms. This study reports the response of the dynorphin (Dyn) systems of the basal ganglia to multiple cocaine doses. Dyn is an opioid-related peptide associated with a striatal-nigral projection which is involved in regulation of locomotor activity. Five doses of cocaine (6-h interval) resulted in dose-dependent increases in striatal and nigral levels of Dyn A_{1-17}-like immunoreactivity (DLI). At 1 h following treatment, the maximum increases were 200-300% of control with 30 mg/kg-dose. The striatal effect was diminished by 8 h and no longer detectable by 24 h after cocaine administration. In contrast, nigral DLI levels increased to 500% of control by 8 h and were still elevated to 200% of control as long as 48 hr following treatment. The striatal effects were totally blocked by either D-1 or D-2 DA receptor blockade. In contrast, the cocaine-induced increases in nigral DLI concentration were only attenuated by either D-1 or D-2 antagonism. (This research was supported by USPHS Grant DA 00869).

46.11

RECEPTOR-SPECIFIC INTERACTIONS OF THE NIGRO-STRIATAL DOPAMINE PROJECTIONS WITH STRIATAL NEUROTENSIN SYSTEMS IN RAT BRAIN. K.M. Merchant, L. Bush, J.W. Gibb and G.R. Hanson, University of Utah, Salt Lake City, Utah 84112.

We have previously demonstrated that changes in dopaminergic activity causes dramatic alterations in the level of striatal neurotensin-like immunoreactivity (NTLI). In order to investigate the mechanisms underlying these observations, the nigro-striatal DA projections were lesioned with intra-striatal injections of 6-hydroxydopamine. Interestingly, when the pathway was destroyed by more than 85 to 90% (as judged by the residual tyrosine hydroxylase activity), the striatal NTLI level was significantly elevated. This suggests that basal activity of the nigro-striatal DA projections exerts chronic control over the activity of NT terminals in the striatum. Based on this and our earlier observations that blockade of D2 but not D1 receptors increase the striatal NTLI content, we propose that D2 receptors mediate the regulation of striatal NT systems by basal DA activity. To explore this role of D2 receptors, effects of treatments with LY 171555 (a D2-selective agonist) or SKF 38393 (a D1-selective agonist) on striatal NTLI concentrations were examined. Activation of D2 receptors decreased the striatal NTLI content whereas that of D1 receptors increased the same and when the two were combined their individual effects were nullified. Thus, D1 and D2 receptors regulate the striatal NT systems in an antagonistic manner with only the D2 receptors exerting basal control over them. (Supported by USPHS Grant 00869).

46.12

NEUROTENSIN COMPLEXES WITH DOPAMINE. D.K. Adachi*, P.W. Kalivas and J.O. Schenk (SPON: R. Abhold). Dept. of VCAPP, Wash. St. Univ., Pullman, WA 99164

The effects of neurotensin (NT) on mesolimbic dopamine (DA) transmission are paradoxical. NT appears to activate DA neurons at the level of the cell bodies, but blocks the behavioral effect of DA release in the accumbens, an axonal terminal field. Neuromedin N (NN), a peptide in the NT precursor, produced the activation of DA transmission in the cell bodies, but was ineffective at blocking the behavioral effect of DA in the accumbens. These data caused us to evaluate the possibility that a non-receptor mechanism may be responsible for the anti-dopaminergic effect of NT in the accumbens. Using molecular modeling designed to predict conformations of peptides in water, we found the structure of NT forms a highly basic pocket containing -Pro^8-Arg^9-Arg^9-Pro^{10}-, while NN did not. The basic -Arg-Arg- moiety of NT may be forming an acid-base complex with the hydroxyl groups in the catechol moiety of DA. Complexation of NT to DA in solution was measured using UV/visible spectroscopy and potential sweep voltammetry. Both techniques indicated that a complex was formed and from analysis of the shift in half-wave potential obtained from the voltammograms, the stoichiometry of complexation was 1: 1 (K_D = 3.7 X 10^{-7}). No complexation between NN and DA was observed. Thus, the complexation between DA and NT may partly underly the fact that NT has antidopaminergic qualities in vivo.

46.13

ANTICHOLINERGIC DRUGS DO NOT ALTER NEUROTENSIN CONCENTRATION IN THE NUCLEUS ACCUMBENS OR STRIATUM. B. Levant, C. Bissette, and C.B. Nemeroff, Depts. of Pharmacology and Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

Chronic treatment with typical antipsychotic drugs increases neurotensin (NT) concentration in the nucleus accumbens and striatum. In contrast, the atypical antipsychotic clozapine increases NT concentration only in the nucleus accumbens but not in the striatum. This study sought to determine whether the failure of clozapine to increase NT concentration in the striatum is due to its anticholinergic effects. Adult, male, Sprague-Dawley rats (175-200g) were injected (IP) daily for 21 days with haloperidol (1 mg/kg), atropine (20 mg/kg), atropine methyl bromide (20 mg/kg), scopolamine (2 mg/kg), or haloperidol (1 mg/kg) + atropine (20 mg/kg). NT concentrations in 7 brain regions were measured by a sensitive and specific radioimmunoassay. Haloperidol produced significant increases in NT concentration in the nucleus accumbens and anterior and posterior caudate. In contrast, atropine, atropine MeBr, and scopolamine failed to alter NT concentration in these regions. Concomitant administration of atropine with haloperidol attenuated but did not abolish the increases in NT concentration observed after haloperidol treatment. Because anticholinergic drugs alone do not alter NT concentrations, it is unlikely that the effects of clozapine on NT neurons are due to its anticholinergic properties. Supported by NIMH MH-39415.

46.14

NEUROTENSIN EVOKES THE PRODUCTION OF ENDOGENOUS DIHYDROXY PHENYLACETIC ACID (DOPAC) FROM SUPERFUSED RAT STRIATAL SLICES. L.P. Dwoskin. Dept. Pharmacol. Toxicol., Univ. Kentucky Col. Pharm., Lexington, KY 40536.

Neurotensin (NT) has been suggested to be an endogenous neuroleptic. Neuroleptics increase evoked dopamine (DA) release via blockade of D-2 DA receptors. Prior to determining if NT similarly modulates evoked DA release, it was necessary to examine the effect of NT itself. Single slices (6 mg) were superfused (1 ml/min) with Krebs' buffer. After 1 hr, 2 one-ml samples were taken to determine basal outflow. Then, NT (30 nM -10 uM) was added to the buffer and 15 samples collected. Samples received ascorbic acid oxidase and 50 ul was injected into the HPLC-EC. Detection limit was 10 pg/ml superfusate. NT, in a dose-related manner, increased the amount of DOPAC from 44 to 3800 pg/ml/mg tissue. DA was not detected. This response was not altered by adding nomifensine (10 uM), a DA uptake blocker, to the buffer from the start of superfusion. Pargyline (10 uM), a monoamine oxidase (MAO) inhibitor, included from start of superfusion decreased the basal outflow of DOPAC 86%, but surprisingly, enhanced the amount of DOPAC collected in response to NT by shifting the dose-response curve for NT to the left. One interpretation of these results is that NT releases DA from a (storage) pool which then is metabolized to DOPAC by extracellular MAO. Supported by NIMH Grant MH42934.

46.15

CHOLECYSTOKININ MODIFIES THE NEUROMODULATORY ACTION OF DOPAMINE IN NUCLEUS ACCUMBENS: ELECTROPHYSIOLOGICAL EVIDENCE. C.Y.Yim and G.J.Mogenson, Dept. of Physiology, Univ. of Western Ont., London, Ontario, Canada, N6A 5C1.

Dopamine (DA) has been shown to modulate responses of accumbens (NAcc) neurons to excitatory inputs from the amygdala (AMY). The present study investigates whether or not cholecystokinin (CCK-8), recently shown to co-exist and appears to be co-released with DA in the NAcc, modifies the modulatory action of DA in their sites of co-existence in the NAcc. Single unit recordings were made in the medial and caudal NAcc of urethan anesthetized male Wistar rats. These neurons were found to be strongly excited by AMY stimulation, as was observed before, and concurrent stimulation of the ventral tegmental area (VTA) at 10 Hz attenuated the responses, presumably due to DA release. Iontophoretic application of proglumide (PRG) at 30 nA enhanced the attenuating effect of VTA stimulation by 32-126% in 23 out of 37 neurons tested. Iontophoretic application of DA produced similar attenuation of responses to AMY stimulation in 12 NAcc neurons. The attenuation was, however, reduced by concurrent iontophoresis of CCK-8 at 20 nA (7 cells). CCK-8 by itself at 20 nA did not produce significant changes in the spontaneous activity of the neuron nor its response to AMY stimulation. These results demonstrate that exogenous as well as endogenous CCK-8 may modify the postsynaptic action of DA in the NAcc in addition to modulating its release shown in other studies. (Supported by MRC)

46.16

DOPAMINERGIC MODULATION OF IN VIVO GLUTAMATE RELEASE IN STRIATUM. B.K. Yamamoto and B.A. Donzanti. Dept. of Pharmacology, Northeastern Ohio Univs. Col. of Med., Rootstown, OH, 44272.

Previous in vitro evidence has shown that glutamatergic corticostriatal transmission is under presynaptic control by dopamine (DA). To further investigate the mechanism of this interaction, we have measured potassium-induced striatal glutamate release in vivo and the manner in which this is modulated by DA. Male Sprague Dawley rats (300-350 g) were anesthetized with urethane and implanted with microdialysis probes into the medial caudate. The probes were perfused with Krebs/Ringers bicarbonate buffer (pH 7.4) at a rate of 1.5 ul/min. The dialysates, containing extracellular glutamate and other amino acids, were collected at 20 min intervals and analyzed by pre-column derivatization and HPLC with electrochemical detection. After a 1 hr stable baseline period, rats were injected i.p. with the dopamine D2 agonist LY171555 (2 mg/kg) or saline and then perfused for 20 min with 80 mM KCl directly through the probe. Sampling continued for a 2 hr period. KCl increased glutamate release by 3024% above basal levels. This increase was maximal within 40 min and returned to baseline by 2 hr. Pre-treatment with LY171555 decreased KCl-induced glutamate release by 80%, i.e. to 595% above basal levels. This diminished glutamate response returned to baseline within 1 hr. Therefore, this study provides in vivo evidence that DA inhibits KCl-induced glutamate release in striatum and suggests that corticostriatal glutamate release is at least, in part, modulated by DA and the D2 receptor.

46.17

DOPAMINE MODULATION OF THE GABA PATHWAY BETWEEN THE NUCLEUS ACCUMBENS AND THE VENTRAL PALLIDUM. A.J. Bourdelais* and P.W. Kalivas (SPON: R.T. Kuczenski), Dept. of VCAPP, Washington State Univ., Pullman, WA 99164

It has been established that GABAergic neurons in the nucleus accumbens (NA) project to the ventral pallidum (VP). Also an increase in spontaneous motor activity produced by injection of dopamine (DA) agonists into the NA is prevented by pretreatment with a GABA agonist injection into the VP. This has led to the postulate that DA may be acting in the NA to inhibit GABA modulation of VP neurons. To investigate DA modulation of GABAergic pathway between the NA and VP, intracranial dialysis probes were used to simultaneously measure DA levels in the NA and GABA levels in the VP following amphetamine (AMP) administration. Rats were acutely implanted with dialysis probes, one in the NA and the other in the VP. Artificial CSF was used for dialysis. HPLC-EC was used to measure the neurotransmitter levels. By 60 min. a stable baseline was reached for both DA and GABA, 2 mg/kg AMP was then given. Samples were collected for another 120 min at 20 min intervals. The average baseline concentration of DA and GABA in the dialysate, based on four rats was 0.2 pg/ul DA in the NA and 0.06 pg/ul GABA in the VP. After AMP administration there was a maximal increase in DA, 1000% of baseline. GABA was found to be maximally decreased, 48% of baseline, by 40 min. The increase of DA in the NA and simultaneous decrease of GABA in the VP may indicate modulation of the GABA neurons in the NA by DA.

46.18

MULTIPLE EFFECTS OF DOPAMINE ON GABA EFFLUX FROM RAT STRIATUM: ROLE OF D1 AND D2 RECEPTORS AND GABA UPTAKE. S. Bernath,* D. Jackson and M. J. Zigmond, Dept. of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

We have examined the effects of dopamine (DA) on the overflow of [^{3}H]GABA from rat striatal slices elicited by electrical field stimulation (2 Hz, 3 min). SCH 23390 (1 uM), sulpiride (1 uM), and nipecotic acid (100 uM) were used to examine the role of D1 and D2 receptors and the GABA transporter, respectively. We observed:

DA conc.	GABA overflow	blocked by
0.01 uM	+58%	SCH 23390
0.10	-28	sulpiride
10.0	+74	nipecotic acid

Amphetamine (100 uM) increased spontaneous GABA efflux (+30%); this was was not blocked by D1 or D2 antagonists, but was blocked by nipecotic acid. These results suggest that D1 and D2 receptors mediate opposing effects of DA on GABA release, that DA also can facilitate GABA release by a third mechanism, and that the high affinity GABA transport mechanism is involved in some of these processes. (Supported by NS 19608. S.B. on leave from Institute for Experimental Medicine, Hungarian Academy of Science, Budapest, Hungary.)

46.19

SELECTIVE DEPRESSION OF GABA-MEDIATED IPSPS BY SOMATOSTATIN IN AREA CA1 OF RABBIT HIPPOCAMPAL SLICES. **H.E. Scharfman and P.A. Schwartzkroin. Dept. Neurol. Surg., Univ. WA, Seattle, WA 98195.**

A subpopulation of interneurons in area CA1 of hippocampus, that contacts pyramidal cells (PCs), colocalizes the neuropeptide somatostatin(SS) and the inhibitory transmitter GABA. To investigate the significance of colocalized SS and GABA, we tested the effects of SS and GABA on 76 PCs recorded intracellularly.

SS had a potent effect on GABA-mediated IPSPs. GABAergic cells that synapse on PC somata were activated antidromically to elicit IPSPs in PCs. Pressure-ejection of SS(<1uM) at somatic synapses caused a large decrease in IPSPs (mean IPSP amplitude=41.8% of pre-SS amplitude; n=18). The effective dose of SS was less than that required to elicit changes in PC membrane potential(MP) and input resistance(R_{in}). Reversal potential of the IPSP did not change and vehicle had no effect. Stimulation of excitatory afferents that synapse on PC dendrites evoked an EPSP followed by a biphasic IPSP; when SS was ejected in the area of these dendritic synapses only the IPSP decreased(n=21). Coapplication of SS(1uM) and GABA(10mM) to PCs from double barrelled pipettes did not reveal any post-synaptic interactions of SS and GABA. The changes in MP and R_{in} produced by coapplication of SS and GABA appeared to reflect a summation of the effects elicited by separate application(n=23).

The results suggest that SS depresses release of GABA without affecting release of excitatory neurotransmitters. Since the dose required to elicit this effect is less than that necessary to change MP or R_{in} of PCs, the effect of SS on IPSPs may be more "physiologic" than direct actions of SS on PCs. Supported by NINCDS grant NS-07144.

46.20

COEXISTENCE OF GABA AND SOMATOSTATIN IN CULTURED RAT HIPPOCAMPAL NEURONS. A Legido*, J Buchhalter, S Reichlin^, M Dichter, Dept. Neurology, Graduate Hospital and the U of PA, Phila., PA 19146 and ^Dept. Medicine, Tufts Univ School of Medicine, Boston, MA.

We have examined the development of SOM in neurons in dissociated rat HC cultures and the degree with which SOM coexists with GABA. SOM was measured in culture media and cell extracts from 1 day to 8 weeks in culture. For SOM IHC, cultures were fixed with 4% paraformaldehyde (PF) and 5% DMSO, blocked with undiluted goat serum, treated with 0.2% Triton X-100, incubated with rabbit anti-SOM (1:200 in HBS) for 60 min, rinsed and incubated for 20 min with rhodamine-conjugated goat antirabbit IG (R-GARIG, 1:100 in HBS). For co-localization studies, cultures were fixed with 2% PF/0.1% glutaraldehyde, and then incubated with monoclonal mouse anti-SOM (1:100 in HBS) (MRC Regulatory Peptide Group of Canada) for 60 min, fluorescein-conjugated goat anti-mouse IG (1:100 in HBS) for 20 min, rabbit anti-GABA (1:50 in HBS) (Chemicon) for 60 min, and R-GARIG (1:100 in HBS) for 20 min. No staining occurred if the first antibody was omitted or adsorbed with antigen, or, during costaining, if the "opposite" second antibody was used. Staining patterns for SOM and GABA were quite different.

Levels of SOM progressively increased from undetectable to over 2000 pg per 35 mm dish in cells and media by 3-4 weeks in culture. At 3 weeks, approximately 11% of the neurons stained for SOM; staining was prominant in a perinuclear distribution, but could also be localized to processes with varicosities. After treatment with cochicine (100nM for 15 hours), the whole soma and proximal processes were stained. SOM was noted in pyramidal, bipolar and multipolar neurons. When neurons were stained for both SOM and GABA, 62 of 99 neurons staining for SOM also stained positively for GABA. These included bipolar, pyramidal, and multipolar neurons. Of 180 neurons staining for GABA, 62 (34%) were also positive for SOM.

Thus, a subpopulation of HC neurons in culture synthesize, store and secrete SOM and many of these also contain GABA. The coexistence of SOM and GABA suggests a functional relationship, but the nature of this has yet to be determined.

SPROUTING AND SPROUTING MECHANISMS I

47.1

NMDA PROMOTES BRANCHING, MK801 STIMULATES ELONGATION OF DENTATE GRANULE NEURONS. G.J. Brewer and C.W. Cotman†, Dept. of Med. Microbiology/Immunology, Southern Illinois Univ. Sch. of Med., Springfield, IL 62794-9230. †Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

During development in the central nervous system, recent data suggests a modulatory role of the NMDA receptor. The voltage-dependence of the NMDA receptor makes it activity dependent. As glutamate acts directly on this receptor, neuronal fiber elongation and synaptogenesis could both be regulated by synaptic transmission involving NMDA receptors. A direct test of these concepts is possible with the development of a serum-free defined culture medium that supports the growth of central neurons at low density. The dentate gyrus was dissected from 4 day old neonatal rats. Isolated cells were plated at low density (15,000 cells/cm^2) onto glass coverslips. In the continuous presence of 20 µM NMDA or 25 µM glutamate, abundant fine branches were apparent after 6 days in culture. Branching appeared to be specifically stimulated by the action of glutamate on the NMDA receptor because the high levels of branching were not observed upon depolarization with 25 mM KCl. The specific inhibitor of the NMDA receptor, MK801 dramatically reduced branch points almost 75%, and increased the longest neurite mean length to 350 µm, 2.5 times that for neurons treated with NMDA alone. Thus, neurite extension and branching appear to be reciprocally controlled by the NMDA receptor on dentate granule neurons in culture. This suggests that the NMDA receptor may provide the signal to induce localized branching, initiate synaptogenesis and thereby establish experience-dependent circuitries. Supported by NIH MH19691 to C.W.C. and Central Research Committee of SIUSM to G.J.B.

47.2

INTRAVENTRICULAR INFUSION OF NGF IN THE RAT RESULTS IN REDUCED SYMPATHOHIPPOCAMPAL SPROUTING. B.N. Saffran* and K.A. Crutcher. (SPON: G.C. Schoenwolf) Department of Neurosurgery, University of Cincinnati School of Medicine, Cincinnati, OH 45267

Intraventricular administration of NGF does not elicit sympathohippocampal sprouting, although it enhances the innervation of the extracerebral vasculature (Anat. Rec. 220:4, 1988). Since NGF increases the sympathetic innervation of normal targets, we asked whether the anomalous sympathetic projection to the hippocampus, which arises following septal denervation, would be similarly affected by intraventricular NGF infusions. Animals with a medial septal lesion received either ß NGF or cytochrome C in the right lateral ventricle through cannulae connected to an osmotic minipump. Solution was delivered at an approximate rate of 0.5 µl per hour for two weeks beginning two or four weeks after the lesion. NGF was measured using a two-site ELISA (reagents kindly provided by W.C. Mobley, UCSF), and the sprouting quantified by counting sympathetic axons between the blades of the dentate gyrus. The intensity of catecholamine fluorescence around the middle cerebral artery was quantified using a microspectrophotometer in 9 vehicle and 19 NGF-injected animals. The average fluorescence intensity in NGF-injected animals was twice that of vehicle-injected rats ($p<.01$); however, animals which received NGF for two weeks beginning two weeks after a septal lesion did not exhibit sympathetic ingrowth (7 of 7), whereas all vehicle-injected animals exhibited normal sprouting (6 of 6). Animals in which NGF was administered for two weeks, beginning four weeks after a septal lesion, did exhibit sprouting (7 of 7), albeit much less than controls. Interestingly, animals receiving NGF for two weeks, beginning four weeks after a septal lesion showed less sprouting than four-week controls suggesting that the sympathohippocampal fibers are retracting in response to NGF infusion. (Supported by NIH # NS 17131)

47.3

DEGENERATION AND REGENERATION IN THE OUTER MOLECULAR LAYER OF THE DENTATE GYRUS AFTER PERFORANT PATH LESIONS: ROLE OF ASTROCYTES, MICROGLIA AND INTERLEUKIN-1. A.M. Fagan*, R. Robertson* and F.H. Gage (SPON: A. MILLER). Dept. of Neurosciences, UCSD School of Medicine, La Jolla, CA 92093.

Damage to the perforant path (PP) results in sprouting of cholinergic fibers from the inner molecular zone of the dentate gyrus (DG) to replace those in the deafferented outer molecular zone. Concommitant with this sprouting is a dramatic astroglial response within this same region, suggesting a role of astrocytes in mediating the sprouting, perhaps via secretion of trophic factors. The present study is concerned with identifying the stimuli responsible for eliciting the astroglial response.

In vitro studies have shown that reactive microglia produce Interleukin-1 (IL-1), and that this molecule stimulates astrocyte proliferation. In the present experiment adult rats were sacrificed at various time points after PP lesions. Brain tissue was stained with antibodies recognizing astrocytes (GFAP), microglia (OX-42), and IL-1, as well as acetylcholinesterase (AChE) histochemistry. Cells immunoreactive with OX-42 and exhibiting the morphology of microglia are observed in the outer molecular zone of the DG within the first few days post-lesion. Cells similar in morphology and immunopositive for IL-1 are also seen in this same region at these times. However, GFAP immunoreactivity in the outer zone does not reach its maximum until a later time point. This time course of events supports the hypothesis that microglia and their ability to secrete IL-1 are important stimuli for the astroglial response observed in the DG after PP lesions.

47.4

INFLUENCE OF SUSPENSION HYPODYNAMIA ON THE FUNCTIONAL RESPONSES OF RAT PLANTARIS TO PARTIAL DENERVATION. R.N. Michel and P.F. Gardiner, Sciences de l'activité physique, Université de Montréal, Québec, Canada, H3C 3J7.

A functional index of neural adaptability is the capacity of motoneurons to extend and establish supernumerary contacts with neighbouring denervated muscle fibers. This study was conducted to gauge this response in rat plantaris muscles (P) subjected to decreased mechanical loading (suspension hypodynamia) resulting from hindlimb suspension. Three weeks of unweighting decreased P relative weight (26%), X-sectional area (19%), and absolute (32%) and relative (17%) tetanic tension (Po). In a separate group of animals which had undergone 2 weeks of unweighting, 3/4 of the P fibers were denervated by cutting radicular nerve L4. Contractile responses were obtained via sciatic nerve stimulation (sprouting motor units residing in L5) 7 days post-lesion, and the results compared to a weightbearing group which had undergone this same procedure. Partial denervation decreased P relative weight (15%), X-sectional area (19%), Pt (53%), and Po N/cm^2 (58%), in weightbearing (PD) and suspended (PDSUS) muscles to a similar extent. Decreases in Po as a result of partial denervation were smaller in PDSUS muscles. Our results suggest that neuromuscular adaptations occurring in response to mechanical unloading favor the functional recovery from a partial denervation lesion. This condition may be more conducive to optimal motoneuron sprouting. (Supported by NSERC Canada)

47.5

A SINGLE LOCUS MUTATION INCREASES CHOLINERGIC AXON TERMINALS INNERVATING MOUSE HIPPOCAMPAL DENTATE NEURONS. J.L. Noebels and N.W. Kowall[1] Developmental Neurogenetics Laboratory, Section of Neurophysiology, Dept. of Neurology and Inst. of Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030, and [1]Dept. of Neurology, Massachusetts General Hospital, Boston, Massachusetts 02114.

Mutant mouse mocha (recessive, chr. 10) expresses an abnormally persistent synchronization of hippocampal and neocortical neurons, resulting in prolongation of the usual brief bursts of 6-7 Hz theta rhythms into virtually exclusive theta wave discharge in the behaving mouse. Electrogenesis of this specific frequency of atropine-sensitive hippocampal activity is modulated by a cholinergic synaptic projection to dentate granule cells and CA pyramidal neurons from the medial septal nucleus. We now find a gene-linked cholinergic hyperinnervation of the dentate granule cells in the mocha hippocampus that may directly account for the electrophysiological expression of the hypersynchronous mutant EEG phenotype.

Brains of 4 adult mh/mh mice stained for acetylcholinesterase were compared with 4 age-matched homozygous mice wild type at the mocha locus. Regional survey of major forebrain targets of limbic and ascending tegmental cholinesterase-positive pathways revealed uniformly increased laminar staining density of terminals bordering the dentate granule cell layer. No intensification of fiber tracts in the parent diagonal band and neurons of the medial septal nucleus was discerned. The potential of the septal Ach projection for reactive overgrowth following injury is known, however no pathology which might provoke an axonal sprouting reaction has yet been observed. This inherited structural error in hippocampal synaptic organization may represent a direct gene-linked action on cholinergic terminal neurogenesis.

Supported by a Pew Foundation Scholars Award, NS 11535, and J.P. Kennedy Foundation.

47.6

Unilateral Hypothalamic Lesion Results in Changes in Size and Vasopressin Immunoreactivity in Rat Neural Lobe. J.A. Watt* and C.M. Paden, Dept. of Biology, Montana State University, Bozeman, Montana 59717.

Sprouting of vasopressinergic axons has been shown to occur following transection of the pituitary stalk or lesion of the paraventricular nucleus in adrenalectomized animals. In order to further investigate the plasticity of magnocellular neurosecretory efferents of both the supraoptic and paraventricular nuclei, we have induced partial denervation of the neural lobe (NL) using unilateral knife-cuts of the hypothalamo-neurohypophysial tract. Animals were sacrificed 10, 20 and 30 days post-surgery, and coronal sectons of NL were prepared for immunohistochemical localization of vasopressin. NL size and staining densities were measured by digital image analysis. A significant change in the size of the NL was observed, with the greatest reduction (47%) occurring in the 10 day post-surgical group. A trend toward recovery of NL cross-sectional area was apparent at later times, with the 20 and 30 day groups showing 30% and 17% reductions from control values, respectively. A decrease in the density of vasopressin immunoreactivity in the NL was also observed in all groups, but this was statistically significant only at 10 days post-surgery. Further immunocytochemical and ultrastructural studies are in progress. Rabbit anti-vasopressin sera was the generous gift of Dr. G. Nilaver. This work was supported by NIH grant NS23642.

47.7

CHANGES IN THE IMMUNOLOCALIZATION OF THE GROWTH-AND PLASTICITY-ASSOCIATED PROTEIN GAP-43 DURING LESION-INDUCED SPROUTING IN THE RAT DENTATE GYRUS. J.J. Norden, R. Woltjer* and O. Steward. Dept. of Cell Biology, Vanderbilt School of Medicine, Nashville, TN 37232, and Dept. of Neuroscience, U. of Virginia, Charlottesville, VA 22908.

Growth-associated protein-43 (GAP-43) is a fast-axonally transported phosphoprotein whose synthesis and transport appear to be selectively enhanced during periods of axon growth. The phosphorylation state of this protein has also been linked to axon growth and to synaptic plasticity during long-term potentiation in the hippocampus. In an effort to determine if GAP-43 is associated with other forms of synaptic plasticity, we have used LM immunocytochemical methods to examine any change in GAP-43 immunolabeling which occurs during lesion-induced sprouting in the dentate gyrus. To induce collateral sprouting, the entorhinal cortex on one side was lesioned electrolytically or by aspiration in adult male Sprague-Dawley rats, and the ABC immunoperoxidase method was used to localize GAP-43 antigenic sites. In normal animals, GAP-43 immunolabeling is laminar within the dentate molecular layer, with the inner one-third the most intensely immunoreactive and the outer two-thirds moderately immunoreactive. By 6-8 days following entorhinal cortex lesions, a dense band of GAP-43 immunoreactivity appears in the outer denervated zone, indicating that sprouting terminals, which are known to be proliferating at this time, are rich in GAP-43. This is the first identification of a specific presynaptic protein whose expression is correlated with collateral sprouting. Supported by Grant RR05424 to J.J.N.

47.8

PROGESTERONE INFLUENCES HIPPOCAMPAL PLASTICITY: Morse, J.K., Scheff, S.W., and DeKosky, S.T. Depts. Anatomy and Neurology, Lexington VA and Sanders-Brown Research Center on Aging, Univ. of Kentucky, Lexington, KY 40536

We have previously reported that there is a sexually dimorphic effect of glucocorticoids on Hippocampal lesion-induced fiber outgrowth. Ovariectomy leads to a decrease in fiber outgrowth while adrenalectomy leads to an increase in the reactive response in female rats. The present study has extended our research of steroid interaction with the mechanisms of reactive outgrowth.

Sixty day female Sprague-Dawely rats were ovariectomized and were exposed to replacement therapy of estrogen and/or progesterone via silastic capsule implants. Unilateral entorhinal cortex ablation was performed on all animals 7 days following surgery. Capsules were implanted on day of entorhinal cortex lesion. Hippocampal commissural-associational reactive outgrowtyh was assessed 15 days later.

The results suggest that progesterone was able to ameliorate the decrease in reactive fiber outgrowth seen following ovariectomy.

(Supported by the NIH (NS 21541) and the V.A.)

47.9

THE RELATIONSHIP OF BEHAVIOUR AND MORPHOLOGY FOLLOWING EARLY LESIONS IN RATS. R. L. Ladowsky, B. Kolb, and R. Gibb* University of Lethbridge, Alberta, Canada.

In infant rats neocortical lesions produce decreases in cortical thickness and changes in cell morphology that may be related to behaviour. It has previously been reported that recovery of function may be due to increased dendritic arborization. The present study supports these results and suggests that concomitant decreases in cortical thickness may also affect behaviour. Rats were given bilateral lesions of either temporal (T) or visual (V) cortex at 5 or 90 days. Several behaviours, including grooming and spatial navigation, were studied and after sacrifice the brains were processed for Golgi-Cox. The thickness of frontal and parietal cortex was measured and layer II/III pyramidal cells were drawn from these areas. Group V had significantly more basilar dendrites than group T ($p<.01$). Since group V showed recovery on a test of spatial navigation and group T did not, this recovery may be related to increased arborization of basilar dendrites. Both lesion groups showed significant reductions in the thickness of parietal cortex ($p<.05$), and many rats also showed reductions of frontal cortex. As the thickness of frontal cortex decreased, the length of grooming sequences decreased significantly in both group T ($r=.85$) and group V ($r=.83$). It therefore appears that both dendritic arborization and cortical thickness affect behaviour in rats with neonatal lesions.

47.10

PARACHLORAMPHETAMINE LESIONS OF THE DORSAL RAPHE PROJECTION TO THE AREA DENTATA DO NOT INDUCE SEROTONERGIC SPROUTING. J. Daugherty, B. Majidi* and J.H. Haring. Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Dorsal (DRN) and median (MRN) raphe nuclei provide serotonergic (5HT) innervation to the molecular layer (ML) and hilar region (HR), respectively, of the area dentata (AD). We have reported (Soc. Neurosci. Abstr. 13:1595) that lesions of the MRN induce a proliferation and migration of remaining DRN axons from the ML to the HR. The present study asked whether selective lesions of DRN afferents to the ML would elicit sprouting by MRN fibers. Systemic injections of parachloroamphetamine (PCA; Soc. Neurosci. Abstr. 13:907) were used to selectively ablate DRN projections to the AD. Animals were studied using 5HT immunocytochemistry. Two wks after PCA treatment, the 5HT plexus of the ML appeared to be diminished. Hyperchromic, swollen 5HT axons were seen suggesting degeneration of 5HT fibers in the ML. The hilar 5HT plexus was essentially normal. Six wks after PCA injection, the 5HT plexus was unchanged except that presumptive degenerating 5HT axons were not present. Unlike the proliferation of 5HT fibers 6 wks after MRN lesion, the morphology of the remaining 5HT axons appeared normal. Thus removal of the 5HT projection to the ML was not sufficient to induce sprouting of the hilar 5HT plexus. Biochemical studies are being conducted to verify this result. Support: USPHS grant NS25752.

47.11

ASTROCYTIC RESPONSE TO A SMALL LOSS OF SYNAPSES. J. Wells, D.G. Wells*, and B.P. Vietje*. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

In response to extensive denervation astrocytes typically become reactive, change morphology, multiply and migrate into the area of degeneration. A different astrocytic response is described following lesions in the dorsal column nuclei (DCN) which led to a loss of only 3% of the total number of synapses in the ventral posterolateral nucleus (VPL). In order to describe the reaction of astrocytes over the time course of recovery, antibodies to the astrocytic proteins S-100 and glial fibrillary acidic protein (GFAP) were immunocytochemically localized in VPL. Since the projection of the DCN to VPL is entirely contralateral, the VPL containing the degeneration could be compared to the normal VPL on the opposite side in the same animal. When GFAP was present, it was colocalized with S-100. GFAP immunoreactivity began to increase in the first week after the lesion and was greatly increased by 10 days which is consistant with existing observations. However, there was no increase in the number of astrocytes containing S-100 at any time point, nor was there a change in astrocytic morphology. The increase in GFAP lasted for as long as 10 months even though the neuropil in VPL had recovered by 50 days indicating that the GFAP increase is not correlated in time with the re-establishment of synapses. We suggest that recovery after quantitatively small denervation may have different characteristics than those previously described.

47.12

AGE-RELATED REMODELING OF GAD-POSITIVE ELEMENTS IN DEAFFERENTED PIRIFORM CORTEX. N.A. Nousek-Goebl,* L.E. Westrum, and J.-Y. Wu[1] (SPON: A. Bunt-Milam). Depts. Neurol. Surg. and Biol. Struct., Univ. of Washington, Seattle, WA 98195; [1]Dept. Physiol., Penn State Univ., Hershey, PA 17033.

Olfactory bulb (OB) removal has been shown to result in plasticity in piriform cortex that is age-dependent. We are studying this phenomenon using immuno-electron microscopy of GAD reactivity at selected postnatal ages and in adults with emphasis here on short survival times. Normally GAD-labeled terminals form type II, symmetric contacts onto unlabeled dendrites and GAD labeled dendrites receive type I, asymmetric contacts from unlabeled terminals. The OB lesion results in degenerating terminals with type I contacts onto unlabeled and GAD-labeled dendrites. Type I postsynaptic sites are frequently seen partially or entirely devoid of degenerated terminals and may be apposed by normal unlabeled and GAD-positive terminals. GAD-labeled terminals now form asymmetric type I contacts with unlabeled and with GAD-labeled dendrites. The findings are most common in the younger subjects. The results provide new information about the connectivity of the GABA system in this area and also suggest a major role for this system in lesion-induced, age-related remodeling. (Supported by NIH Grants NS09678, DE04942. LEW is an affiliate of CDMRC.)

47.13

ROBUST PROLIFERATION OF SYMPATHETIC INGROWTH AXONS IN DEVELOPING RAT HIPPOCAMPUS, R. M. Booze, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

The capacity of the developing CNS to exhibit axonal migration and growth is great in comparison to the mature CNS. A model of neural plasticity in the adult and aging hippocampus, sympathetic ingrowth, was studied to determine the extent of axonal growth in early development.

Five-day-old Long-Evans rat pups were anesthetized and the septal region removed by aspiration. At 30 days of age, the deafferented hippocampus was processed for identification of sympathetic axons and cholinergic markers to verify the extent of the lesion. Quantitation of sympathetic axons in the hippocampus revealed extensive axonal proliferation. The sympathetic axons were localized to the dentate hilus subgranular layer and in the mossy fiber layer of hippocampal area CA3.

These results indicate (1) sympathetic ingrowth is a robust phenomenon in early hippocampal development, (2) CNS maturation modulates sympathetic ingrowth, and (3) sympathetic ingrowth is a useful model for studying CNS maturation and neural plasticity.

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48.1

ALTERED SLOW AXONAL TRANSPORT IN OPTIC NERVE OF SHIVERER MUTANT MICE. S. de Waegh* and S.T.Brady. Dept. of Cell Biol., Univ. of Texas Southwestern Med. Ctr.,Dallas, TX 75235.

Previous studies in slow axonal transport in the myelin deficient Trembler mouse revealed significant differences in the composition and rates of transport of the cytoskeleton proteins. In Trembler peripheral nerve, reduced axonal caliber and poor myelination correlates with a decrease in the rate of transport of neurofilament proteins and brain spectrin. The amount of tubulin transported as part of slow component b is increased by 40 % in the Trembler compared to the normal. Differences exist between myelination in the central and in the peripheral nervous system including the existence of two types of myelin producing cells, oligodendrocytes in the CNS and Schwann cells in the PNS and differences in the structure of the node of Ranvier. In light of these differences, we evaluated the effects of profound abnormalities in the myelin on slow axonal transport of cytoskeleton proteins in the CNS. In the Shiverer mutation the lack of myelin basic protein affects myelin compaction in the central nervous system but not in the peripheral nervous system, resulting in distinctive tremors, convulsions and early death. We analyzed slow axonal transport in the optic nerve of the Shiverer mice (provided by C.Readhead and L.Hood), by injecting 0.4 mCi of ^{35}S-methionine in the eye. Seven to 18 days after the injection, the transport was then analyzed by SDS-PAGE, fluorography and segmental analysis. Interestingly, our preliminary results indicated an increase in the rates of transport of neurofilament and tubulin when compared to the rates in control C57Bl/6N mice. It thus appears that a defect in the myelin producing cells affects slow axonal transport in the CNS in a different manner than in the PNS. The availability of transgenic Shiverer mice homozygous for MBP should permit us to analyze if restoring a normal compacted myelin in the CNS will reestablish normal rates for slow axonal transport of cytoskeleton proteins in the CNS.

48.2

PATCH-CLAMP STUDIES ON MYELIN-DEFICIENT SCHWANN CELLS FROM MUTANT TREMBLER MICE. S.Y. Chiu. Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Recent studies in our laboratories have shown a relation between ion channels and myelinogenic behavior of Schwann cells in the normal adult mammalian PNS; neuronal-like ion channels are detectable only on the non-myelin type, but not on the myelinating type (Chiu, 1987, J. Physiol. 386, 181-203). We have now extended these studies to Schwann cells with genetically abnormal myelinogenic behavior. In the mutant Trembler mice, the myelinating Schwann cells fail to produce sufficient myelin in the presence of apparently normal axons. Axon-associated myelinating Schwann cells were acutely isolated from normal and Trembler mice (age 1-3 months) as described previously (Chiu, 1987). With KCl-filled pipettes, whole-cell recordings made at the cell body of Trembler Schwann cells revealed a large outward non-inactivating current (range 609-1781 pA at 105 mV depolarizations, mean 1157 $\pm$ 191 pA (n=13)) which resembled the delayed rectifying potassium current seen in excitable membranes. This outward current appeared to be normally "down-regulated"; whole-cell recordings in normal myelinating cells showed little or no outward currents. Cell-attached recordings in Trembler with KCl-filled pipettes revealed single *inward* current activities (single channel conductance about 34 pS, n=2) at potentials hyperpolarized relative to the resting potential.

The Trembler mice offer a unique system to examine dysregulation of ion channels and of myelinogenic behavior in genetically abnormal Schwann cells.

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48.3

DO ASTROCYTES CAUSE THE MYELIN DEFICIENCY IN THE mdr MUTANT? J. Rosenbluth, M. Hasegawa* and R. Schiff*. Depts. of Physiology & Rehab. Medicine, N.Y.U. School of Medicine, New York, N.Y. 10016.

The CNS of myelin-deficient rats (mdr) contains small amounts of rudimentary myelin, which is often infiltrated by astrocyte processes. Immunocytochemical studies using monoclonal antibodies to GFAP show increased astrocyte staining in the lateral columns of the mdr spinal cord. These observations suggest the possibility of a defect in the astrocytes of this mutant that leads to their proliferation and also causes myelin deficiency by either inhibiting its formation or breaking it down after formation. To test this hypothesis, fragments of spinal cord from normal rat embryos (E15-E18) were transplanted into the dorsal columns of 7-13 d. mdr pups. 11 d. later, foci of thin, normal-looking myelin were found in host fiber tracts, in some cases remote from the transplant. At 16 d.,we found foci of thicker myelin (up to 19 layers) with no signs of breakdown or infiltration by astrocyte processes. These results show that normal myelin can form in the environment of the mdr CNS after transplantation of normal spinal cord fragments and do not support a primary role for astrocytes in the myelin deficiency. Supported by the NIH and NMSS.

48.4

A TRANSGENIC MOUSE WITH A MYELIN DEFECT. A. Privat*[1], M. Mondain*[1], F. Sandillon*[1] and M. Rassoulzadegan*[2] (SPON: M.C. Calvet) [1]Neurobiologie du Développement, LP8402 CNRS U.249 INSERM. Institut de Biologie, 34060 Montpellier France. [2]Centre de Biochimie, LP 7300 CNRS, 06034 Nice, France.

Transgenic mice were obtained by injection of the plasmid pP$_4$LT$_1$ coding for the polyoma T antigen (Nature 300, 713-18.1982). Upon clinical inspection, these mice appeared to develop a tremor during the first post-natal month. Microscopic analysis disclosed a paucity of myelin in the central nervous system, whereas the peripheral nervous system appeared normal. Electron microscopic examination of spinal cord, cerebellum and forebrain has shown in the white matter of adult animals a drastic reduction of the thickness of myelin sheaths, and even the absence of compact myelin around large axons, which are thus invested by one or two layers of oligodendrocyte cytoplasm. Oligodendrocytes appeared generally immature, with a low nuclear and cytoplasmic density, and a paucity of organelles. Conversely, myelinated tracts coursing in the grey matter appeared less affected. Myelin thickness was normal in dorsal and ventral roots of the spinal cord. Immunocytochemical detection of CNPase disclosed a marked increase of white matter immunoreactivity versus controls due to loose myelin, whereas IR oligodendrocytes of the grey matter appeared immature. GFAP immunocytochemistry detected an intense gliotic reaction in both white and grey matter of CNS. (supported by IRME)

48.5

DEVELOPMENT OF MYELIN AND OLIGODENDROCYTE ABNORMALITIES IN CAPRINE β-MANNOSIDOSIS. **K. L. Lovell. Dept. Pathology, Mich. State Univ., East Lansing, MI 48824.**

β-Mannosidosis, a genetic dysmyelinating disorder in goats, is associated with a deficiency of lysosomal β-mannosidase activity and tissue accumulation of oligosaccharides in lysosomal storage vacuoles. The myelin deficit shows substantial variation, consistent among all animals, throughout the nervous system. The present study compares the development of myelin and oligodendrocyte abnormalities in optic nerve and corpus callosum, regions which show different extents of deficits and which undergo myelination at different time periods. The diameters and density of myelinated axons and the density and appearance of glial cells were analyzed in affected and control animals at the following ages: 115/150 days gestation, 124/150 days gestation, 2-4 days postnatal and 3-4 weeks postnatal. In optic nerve, the density of myelinated axons in affected goats was 20-25% of that in control goats at all ages, while in corpus callosum, the myelinated axon density in affected animals was about 5% of the control value. In all postnatal animals in both regions, the mean diameter of myelinated axons in affected animals was substantially larger than the corresponding control value. The number of oligodendrocytes was decreased in affected animals, and dark vacuolated cytoplasm was common. These results help define the nature and timing of defects associated with myelin deficits in caprine β-mannosidosis. Supported by NS20254 to K.L.L. and by NS16886 to M.Z. Jones.

48.6

IMPAIRED EXPRESSION OF GLYCEROL PHOSPHATE DEHYDROGENASE AND OTHER OLIGODENDROCYTE MARKERS IN THE MYELIN DEFICIENT MUTANT RAT. M.N. Gordon, M.A. Espinosa de los Monteros*, S. Kumar* and J. de Vellis, Mental Retardation Research Center and Dept. of Anatomy, UCLA Medical School, Los Angeles, CA 90024-1759, USA

Oligodendrocyte differentiation and myelinogenesis are characterized by the sequential and coordinate expression of marker proteins. We have explored the temporal pattern of development of oligodendrocyte-specific marker proteins using immunohistochemistry in the sex-linked myelin deficient (md) rat mutant, which is characterized by a lack of CNS myelin. The number of oligodendrocytes containing glycerol phosphate dehydrogenase (GPDH)-like immunoreactivity is reduced in mutant rats, and in general these cells appear morphologically less complex with shorter processes. Reduced expression of transferrin, galactocerebroside, myelin basic protein and proteolipid protein are also observed. Expression of neuronal and astrocyte markers are largely unaffected. We have also observed reduced expression of these oligodendrocyte markers at the mRNA level by RNA blot hybridization (Kumar et. al., J Neurosci Res, in press). Although an abnormality in the PLP gene analogous to the mutation in the jimpy mouse is suspected in the md rat, these findings document profound deficits in many oligodendrocyte gene products. Supported by HD06576, HD07228, HD07032 and DOE Contract DE-FC03-87-ER60615.

48.7

DEVELOPMENT OF OLIGODENDROCYTE SPECIFIC MARKERS IN CELL CULTURES FROM THE MYELIN DEFICIENT MUTANT RAT. M.A. Espinosa de los Monteros*, M.N. Gordon, S. Kumar* and J. de Vellis, Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Oligodendroglial cell development proceeds through distinct stages before myelin formation. The myelin deficient (md) rat mutant lacks myelin in the CNS and fails to express the developmental increase in galactocerebroside (GC), glycerol phosphate dehydrogenase (GPDH), myelin basic protein (MBP) and proteolipid protein (PLP) as previously detected by immunohistochemistry of tissue sections and by RNA blot hybridization. Newborn male rat brains from md carrier mothers were cultured individually on glass coverslips in Waymouth medium supplemented with 10% calf serum. Immunostaining of cultures prepared from affected md rats revealed a decreased number of oligodendroglial cells at all time points ranging from 5-19 days in vitro, compared to control cultures. However, the sequential pattern of marker appearance was preserved, resulting in delayed, but demonstrable, expression of oligodendrocyte markers, including transferrin, GC, MBP and PLP. These observations suggest that at early stages of oligodendrocyte differentiation in vivo, the progenitor cells or young oligodendrocytes are not able to survive, resulting in a diminished number of oligodendrocytes. In tissue culture, these cells are able to survive, giving rise to a new population of healthy oligodendrocytes which express several myelin markers. Supported by NICHD and DOE.

48.8

LOCALIZATION OF NILE GP AND MBP IN ADULT BRAIN OF NORMAL AND HYPOMYELINATING MOUSE MUTANTS.
E. Trenkner. Dept. of Pathology., Columbia University College of Physicians and Surgeons, New York, NY 10032.

Several glycoproteins have been attributed with the ability to modulate and regulate cell contacts during development of the nervous system. This study analyzes the expression of one of these molecules, the NILE GP/L1/Ng CAM epitope, in its relation to myelin basic protein (MBP) in normal and myelin deficient mutants.

The immunochemical localization of NILE-GP epitope in adult brain appears to vary with fixation and processing procedures and thus in its proposed functional roles. We demonstrate here, using immunochemical localization in Bouin's fixed paraffin sections of adult mouse brain and sciatic nerve of normal and myelin deficient mutants, that: 1. NILE GP is confined to presumptive myelinated fiber tracts in adult brain and sciatic nerve, but is expressed independently of MBP at early developmental stages; 2. NILE GP and MBP are coexpressed in normal mice and show the same abberant pattern in the myelin deficient mutants jimpy, quaking and shiverer (shi); 3. When the MBP gene is introduced into shi[1] (kindly provided by Dr. R.L. Sidman) not only MBP but also NILE GP is expressed in the normal pattern on myelinated axons, suggesting a coregulation of NILE GP and MBP and thus a role of NILE GP during myelination.
(1) C. Readhead et al. (1987) Cell 48: 703-712.
Supported by NS 20073 to E.T. and NS 16839 to M.L. Shelanski.

48.9

MYELIN CONTENT INCREASED IN TRANSGENIC MICE PRODUCING ELEVATED LEVELS OF INSULIN-LIKE GROWTH FACTOR-1 (IGF-1). M. J. Carson +*, R. R. Behringer §*, L. S. Mathews#*, R. D. Palmiter#*, R. L. Brinster §*, and F. A. McMorris+. +Wistar Institute, Phila.,PA 19104; §School of Vet. Med., U. of PA, Phila.,PA 19104; & #Dept Biochem. and Howard Hughes Inst, U. of WA, Seattle, WA 98195.

Transgenic (Tg) mice carrying a mouse metallothionein I promoter/human IGF-1 cDNA (MT/IGF-1) gene construct have higher levels of IGF-1 in serum and in brain than normal mice (1.5 and 2-fold respectively) (L.Mathews, ms. in prep). Because IGF-1 induces the development of oligodendrocytes *in vitro* (McMorris et al., PNAS *83* :822), we compared brain weight and myelin content of Tg mice with that of non-Tg littermate controls. Brain weights of Tg mice were 10-14% higher than their non-Tg littermates by postnatal day 15, and 55-65% higher by day 55. This increase in brain weight is due to both hypertrophy and hyperplasia. At day 15, the relative myelin content (mg myelin /g brain wt.)was not appreciably different from control; but by day 55, the relative myelin content was 25-30% higher in Tg mice than in non-Tg littermates, and the total myelin content of the Tg mice was 90-100% higher. These Tg mice were bred to a mouse line which was growth hormone (GH) deficient and hypomyelinated in the cerebrum. As GH regulates postnatal levels of IGF-1 in the brain, we examined if the observed hypomyelination could be reversed in GH-deficient mice carrying the MT/IGF-1 gene. The relative myelin content in the cerebrum of GH-deficient progeny carrying the MT/IGF-1 gene was 90% higher than their GH-deficient littermates. These data suggest that IGF-1 can enhance oligodendrocyte development and myelination *in vivo*. Supported by NMSS RG 1767-A-1, NSF BNS 85-18023, NIH NS11036, and NS 26119.

48.10

A 31.5kD PROTEIN UNIQUE TO CNS: EXPRESSION IN NORMAL AND JIMPY ANIMALS. D.M. Studzinski*, J.A. Benjamins, and R.P. Skoff, Depts. of Neurology and Anatomy & Cell Biology, Wayne State Univ. Sch. of Med., Detroit, MI 48201

We have identified a unique 31.5kD protein in mouse CNS, first detected in a rabbit antiserum raised against a synthetic peptide corresponding to amino acids 109-128 of PLP (Hudson et al., Am. Soc. Cell Biol., 101, 435a, 1985). The antibodies are not anti-PLP, as they are not absorbed by PLP. Palmitic acid labeling suggests that the protein is acylated in both normal and jp mice. It is present in brain and cord of normal mice from 2-200 days, and in jp mice until their death. It is not present in normal or jp sciatic nerve, liver, kidney, or muscle. Scanning of immunoblots shows the protein increases with age in brain and cord of both normal and jp and appears prior to PLP in normal brains. The level in jp increases from 8 to 14d and peaks at 17d, being 1.25 times normal levels. Myelin from 17d mice shows higher levels of the protein than that isolated at 200d. Levels are reduced in highly purified myelin, suggesting it is not a myelin component. Since the 31.5kD is present in jp mice, it is probably not related to PLP, which is absent in jp. The protein is slightly elevated in 17d jp but is not related to GFAP, which is increased at this age. Immunocytochemical and biochemical studies are in progress to determine its localization, function, and relationship to PLP.
Supported by NIH grants NS18883, NS15338.

48.11

GLIAL CELL DEATH IS NOT INCREASED IN FEMALE CARRIERS OF THE JIMPY GENE. P.E.Knapp, S.Dutta* and R.P.Skoff. Dept. of Anat. & Cell Biol., Wayne State Univ. School Med., Detroit MI 48201

Previous ultrastructural studies have shown extensive death of oligodendrocytes (OLs) in jimpy (jp) male mice. This OL death precludes the formation and maintenance of normal myelin sheaths in jp CNS. To clarify whether this defect is primary or if it is a secondary effect, glial death in female carriers (mosaics) of the jp gene was examined. Due to random X-chromosome inactivation, roughly half of the OLs in mosaic CNS should express the jp gene while the other half should express a normal phenotype. If OL death is a primary effect of the jp mutation, the jp OLs in the mosaic should die prematurely. Numbers of total and dying glia were counted in 1um plastic sections of spinal cords (white and gray matter) in 5 and 15D mosaics and controls. In 5D mosaics and controls the percentages of total glia which were dying were 0.56 and 0.40, respectively. At 15D, the percentages were 1.2 in both mosaics and controls. Thus, OLs in mosaic cords are not dying at greater rates than those in control cords of the same age. We conclude that OL death is not a primary effect of the jp mutation and that jp OLs are probably able to survive in the mosaic CNS. It is unknown if jp OLs in the mosaic are able to make and/or maintain myelin sheaths. It is unlikely that the defect in the PLP protein itself is the cause of OL death since jp OLs do not die in mosaic CNS. Supported by NS 18883.

48.12

THE S PHASE OF THE CELL CYCLE OF NEUROGLIA IS LENGTHENED IN THE MURINE MUTANT JIMPY. L.A. Furicchia* and R.P. Skoff. Dept. Anat. & Cell Biol., Wayne State Univ. School Med., Detroit MI 48201

Our previous study of the myelin deficient mutant jimpy (jp) revealed a defect in the cell cycle of neuroglia (Knapp & Skoff, Dev. Br. Res.,35:301, 1987). This defect was revealed by determining the ratio of the no. of {^{3}H} thymidine labelled cells to the no. of mitotic cells. The ratio was always 4:1 in normal mice regardless of the age or labelling index but the ratio was always 8:1 in jp mice. The cause for the different ratios was unknown but was most likely due to a shortened M phase or a lengthened S phase. To measure the cell cycle of glia, we utilized the "percent of labelled mitoses" method. 14 day old mice were injected with {^{3}H} thymidine, sacrificed at different time points ranging from 1 to 35 hrs and spinal cords processed for autoradiography. Between 50-100 meta- and anaphases at each time point were scored for the presence or absence of silver grains. We find the duration of the S phase to be about 6.5 hrs in normals and 9.5 hrs in jp. The length of the duration of the cell cycle is about 16 hrs in normals and about 19.5 hrs in jp. The 3.5 hr difference in the jp cell cycle length is attributed to the 3 hr increase in the S phase. Since the proliferating cells in jp are mostly oligodendrocytes (OL) at this time, this study shows the OL cell cycle is lengthened. This finding shows the differentiation of the jp OL is compromised in its earliest stage of development. SUPPORTED BY NS 15338.

48.13

MYELINATION IN VITRO: GLIA FROM ADULT CNS NEED UNKNOWN NUTRIENTS NOT REQUIRED BY IMMATURE GLIA. G.B. Stanhope*, W.J. Hendelman+, S. Billings-Gagliardi and M.K. Wolf, Dept. Cell Biol., U.Mass. Med. Sch., Worcester, MA 01655 and +Dept. Anat., U. Ottawa Fac. Hlth. Sci., Ottawa, Ont. K1H 8M5

In co-cultures, glia from both immature and adult mouse optic nerve will invade mouse cerebellum and myelinate its axons. We now find that the nutritional requirements for myelination are different for glia from immature and adult tissue. Cerebellar cultures were treated with cytosine arabinoside for 8 DIV to eliminate native myelinating glia, and then received fragments of immature (P8-9) or adult (P85-100) optic nerve in direct contact with cerebellum. Cultures were fed 25% each serum and embryo extract (SEE) or Hendelman's semisynthetic medium (HM). After 12 to 20 more DIV, myelination was estimated in living cultures or by counting myelin profiles in serial semithin sections. Abundant myelin (over 2000 profiles/culture) formed in over 50% of cultures with immature nerve (either SEE or HM) or with adult nerve fed SEE. However, no culture with adult nerve fed HM formed more than traces of myelin (70 profiles/culture). This suggests that glia from immature nerve can synthesize or store unknown nutrients, which glia from adult nerve must receive from SEE. The need for such nutrients may limit myelin regeneration in adult CNS. Grant support: NINCDS (Javits Award) and American Paralysis Association.

48.14

INCREASED MYELINATION IN HYPOMYELINATED MUTANT MOUSE CEREBELLUM CO-CULTURED WITH NORMAL RAT OPTIC NERVE. X.Y. Shen and S.C. Chang*, Dept. of Anatomy, Shanghai Medical University, Shanghai 200032, People's Republic of China.

When mouse optic nerve is co-cultured with hypomyelinated mouse cerebellum, the optic nerve glia invade the cerebellum and myelinate its axons. The experiment succeeds with genetically or chemically hypomyelinated cerebellum and with normal or mutant, immature or adult optic nerve. A similar experiment succeeds with rat glia and rat axons. We now find that rat glia can myelinate mutant mouse axons. Fragments of newborn shi^{mld}/shi^{mld} cerebellum were cultured on coverslips. On the 7th DIV, fragments of optic nerve from Wistar rats aged 7 to 82 D were placed in direct contact with the cerebellar explants. After 2 to 3 additional weeks in vitro, examination of living cultures showed that 11 of 24 cultures with added rat optic nerve had more myelin than any control, a success rate comparable to that obtained with mouse optic nerve. shi^{mld} myelin is not normally compacted, and is difficult to see in cultures. However, the additional myelin in successful cultures was concentrated near the optic nerve, and looked thicker and better defined than shi^{mld} myelin. Studies are underway to determine its ultrastructure and thus confirm its rat origin. This result suggests that at least part of the cell recognition system for CNS myelin formation is the same in closely related species.

PAIN PATHWAYS: CENTRAL MECHANISMS

49.1

NATURAL GROUPINGS OF SPINOCERVICAL TRACT CELLS BASED ON RESPONSES TO CUTANEOUS STIMULI, C.M. Owens, D. Zhang and W.D. Willis, Marine Biomed. Inst. and Dept. of Anat. & Neurosci., Univ. Texas Medical Br., Galveston, TX 77550.

Neurons with different degrees of convergence of noxious and innocuous inputs are often classified as low threshold, high threshold or wide dynamic range cells. A classification scheme that is sensitive to variations in responses to innocuous and graded noxious stimuli was applied to spinocervical (SCT) cells. Single units were recorded from the lumbar cord of nembutal and chloralose anesthetized cats. Antidromic activation of the unit from C_3 but not C_1 (or with a >50% reduction in conduction velocity), frequency following (333 Hz) and collision identified SCT cells. The receptive fields (r.f.) of isolated units were mapped and four mechanical stimuli were applied for 10 s to the center of the r.f. The 4 cutaneous stimuli included innocuous brushing and three graded reproducible noxious pressures. The activity evoked by each stimulus was quantified and the values expressed as a percentage of the sum of activity to all 4 responses. In a population of 88 SCT cells studied so far, there have been units which have responded to noxious and innocuous input from glabrous and non-glabrous skin. One unit had no cutaneous input and responded only to deep input. At this point there appear to be at least 4 major classes of cell types based on a k-means cluster analysis of the percent response to cutaneous stimuli. (Supported by NS 09743 and NS 11255.)

49.2

RESPONSE PROPERTIES OF CONTRALATERALLY PROJECTING SPINOTHALAMIC NEURONS IN THE SECOND CERVICAL SEGMENT OF CAT, M.V. Smith*, A.V. Apkarian and C.J.Hodge, Dept Neurosurg, SUNY Hlth. Sci. Ctr., Syracuse, NY 13210.

Anatomic studies have shown that the upper cervical spinal cord contains the highest density of spinothalamic tract (STT) cells. The purpose of this study was to investigate somatic response properties of contralateral STT cells in the second cervical segment (C2).

Single unit recordings were made in the gray matter of C2 in chloralose anesthetized cats. Cells projecting to the contralateral ventrobasal complex of the thalamus were identified by antidromic stimulation and collision techniques. The response properties of these cells to cutaneous stimuli were studied. Stimulation and recording sites were determined by electrolytic lesions.

Typical response properties of C2 STT units were: 1) large receptive fields (RFs), at times involving both trigeminal and hindlimb distributions; 2) bilateral RFs; 3) responses to a wide variety of stimuli including touch, pressure, tap, deep muscle squeeze, and at times noxious pinch; 4) relatively small areas responsive to noxious stimuli compared to the areas responsive to low threshold stimuli. Widespread inhibitory RFs were occasionally seen.

The properties of STT cells projecting to the contralateral lateral thalamus from C2 differ from those reported in the spinal cord enlargements.

49.3

THE LOCATION OF SPINOTHALAMIC AXONS WITHIN THE DORSOLATERAL FUNICULUS OF CAT, R.T.Stevens, A.V.Apkarian and C.J.Hodge, Dept Neurosurg., SUNY Hlth Sci Ctr., Syracuse, NY 13210

Previous studies in cat have shown that ascending axons of lamina I spinothalamic (STT) neurons are located in the contralateral DLF. The purpose of this study was to determine the location within the DLF of projecting DSTT fibers by direct visualization of HRP-filled axons.

In this study, a constricting ligature was placed on the spinal cord at the high lumbar level of cat. Injections of 4% WGA-HRP were then made into the thalamus and animals were then allowed to survive for 5 to 7 days before sacrifice. The ligature caused a backup within axons of retrogradely transported HRP resulting in the ability to visualize and localize HRP-labeled fibers of fine caliber.

Large numbers of very course HRP-filled axons were seen in the ventral and ventrolateral funiculi. The DLF contained a moderate number of very fine HRP-labeled fibers. The dorsal most aspect of the DLF contained the fewest labeled axons while the labeled axons in the ventral portion of the DLF were evenly distributed from the periphery to the border of the dorsal horn.

49.4

CERVICAL LAMINA I SPINOTHALAMIC PROJECTIONS IN THE CAT. A. D. Craig, Jr. Divisions of Neurobiology and Neurosurgery, Barrow Neurological Institute, Phoenix AZ 85013.

Lamina I neurons constitute half the spinothalamic tract in the cat and convey nociceptive- and thermoreceptive-specific activity in anatomically-selective projections. The PHA-L anterograde transport method is being used to identify the terminations and pathways of ascending spinal lamina I projections. One or more iontophoretic injections are made at recording sites in C5-7 in anesthetized, young (8-16 wks) cats and 3-4 wks later the PHA-L is immunohistochemically identified. Transport to the DCN by passing fibers is usually present but little labeling has been found in the LCN. Ascending axons of lamina I cells can be seen in the lateral funiculus in oblique C3-4 sections. Terminations are present in the parabrachial, Kolliker-Fuse, subcoeruleus, cuneiform and periaqueductal gray regions of the brainstem and in the dorsal hypothalamus. Thalamic targets include caudal PO, Pf, the ventral paralaminar periphery of VPLm, the ventral margin of VMb, and Sm. Comparison with trigeminal lamina I projections indicates that terminations in VP and VMb are coarsely topographic, whereas there is a point-to-point projection to Sm. The distinct morphological characteristics of terminations in each site support possible functional/neurochemical segregation within this distributed projection.

Supported by NS 25616 and the Barrow Neurological Foundation.

49.5

EFFECTS OF FRONTAL CORTEX STIMULATION ON NOCICEPTIVE NEURONS IN NUCLEUS SUBMEDIUS OF THE ANESTHETIZED RAT. J.O. Dostrovsky and K. Kawakita. Dept. of Physiology, Univ. of Toronto, Toronto, Ontario M5S 1A8, Canada.

We have recently reported the existence of many nociceptive neurons in nucleus submedius (Sm) of the rat thalamus (Dostrovsky & Guilbaud, Brain Res. 1988). Since Sm has reciprocal connections with the ventrolateral orbital cortex (VLO) we have now investigated the effects of electrical stimulation of VLO on Sm neurons. The majority (70%) of the 77 Sm neurons recorded in urethane-anesthetized rats responded only to noxious stimuli and these responses could be frequently (50%) evoked by stimuli applied to all body regions. No neurons responding to non-noxious mechanical stimuli were found. Most of the responsive neurons were located in the dorsal part of Sm and usually had larger responses than those located in ventral Sm. VLO stimulation induced long lasting inhibition of the spontaneous activity of most Sm neurons (lasting up to 200ms). However, when the rate of stimulation was increased to 10Hz excitation was also frequently observed. This wind-up phenomenon occurred in 69% of the nociceptive neurons; the mean minimum latency of this excitatory response was 7.3ms. Some short latency antidromic responses were also evoked. These results provide further support for the possible role of dorsal Sm in nociception and indicate the existence of complex interactions between Sm and frontal cortex. (supported by NIH DE05404).

49.6

DISTRIBUTION IN THE HYPOTHALAMUS AND TELENCEPHALON OF FIBERS ORIGINATING IN THE SPINAL CORD. K. D. Cliffer, R. Burstein and G. J. Giesler, Jr. Dept. of Cell Biology and Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455.

We previously reported that cells in nociceptive areas of the spinal cord project to the hypothalamus and telencephalon. The present report provides a more complete description of the locations of the afferent fibers. PHA-L was applied iontophoretically with multiple injections into the spinal cord. After immunohistochemical processing, labeled fibers were found in medial hypothalamus and medial preoptic area, including the suprachiasmatic nuclei and the vicinity of the paraventricular nuclei. Fibers were also labeled in the lateral hypothalamus and lateral preoptic area. Labeled fibers were seen in the supraoptic decussation, crossing the midline in the most posterior portion of the optic chiasm. Further anteriorly, numerous labeled fibers were seen in ventral pallidum, nuclei of the diagonal band of Broca, bed nucleus of the stria terminalis and globus pallidus. Some labeled fibers were embedded in the internal capsule, in nucleus basalis of Meynert. Some of the labeled fibers approached the surface of the olfactory tubercle, presumably as part of a projection to ventral striatum. Fibers were labeled in both medial and lateral septal nuclei and nucleus accumbens, and extended to the cortex medial and dorsomedial to nucleus accumbens. The wide distribution of spinal afferents to these hypothalamic and telencephalic areas provides a direct route from the spinal cord for somatosensory information to reach areas involved in autonomic, neuroendocrine and emotional responses to somatosensory stimuli. Supported by DA03981 and BNS84187878.

49.7

PHYSIOLOGICAL CHARACTERIZATION OF SPINOHYPOTHALAMIC TRACT (SHT) NEURONS IN LUMBAR CORD OF THE RAT. R. Burstein, K. D. Cliffer, R. J. Dado and G. J. Giesler, Jr. Dept. of Cell Biol. and Neuroanat. Univ. of Minn., Minneapolis, MN 55455.

The hypothalamus participates in autonomic, neuroendocrine and emotional responses to somatosensory and nociceptive stimuli. Spinal projections to the hypothalamus have been thought to be multi-synaptic. Recently, we reported that in rats a large number of spinal cord neurons project directly to the hypothalamus. The present report describes our continuing studies of these projections. To date, we have recorded 33 neurons in lumbar cord that were antidromically activated (< 50 uA) from electrodes in the contralateral and ipsilateral hypothalamus of urethane- anesthetized rats. Recording points were found within the marginal zone (10 neurons), nucleus proprius (8), lateral reticulated area (4), ventromedial dorsal horn (2) and the lateral spinal nucleus (1). Approximately 60% of characterized SHT neurons responded to innocuous mechanical stimuli but responded at higher frequencies to noxious mechanical and thermal stimuli. Roughly 20% responded only to noxious stimuli. Other neurons responded to joint movements. In 11 cases, the SHT axon was found to be located in the upper cervical cord using systematic tracking with an additional stimulating microelectrode. Lesions marking the locations of these SHT axons were within the dorsal (5) or ventral (6) lateral funiculus contralateral to the recording point. The mean conduction velocity between the recording point and cervical cord was 28 M/S (range=10-38; n=17). Conduction in each axon slowed considerably between the cervical cord and the hypothalamus (mean=12 M/S; range=4-24; n=17). In 3 cases, SHT neurons were backfired from low-threshold points within the hypothalamus bilaterally. Using electrophysiological tracking techniques, the axons of these neurons were found to cross from contralateral to ipsilateral hypothalamus within the supraoptic decussation. These findings indicate that spinal cord neurons that project to the hypothalamus carry somatosensory and nociceptive information. Supported by DA03981 and BNS84187878.

49.8

SPINOTHALAMIC CELL RESPONSES TO RENAL PELVIC DISTENTION W.S. Ammons, Dept. of Physiology, Thomas Jefferson University, Philadelphia, PA 19107.

Twenty-seven spinothalamic tract (STT) neurons in the T_{11}-L_2 segments were studied in 15 anesthetized monkeys (Macaca fascicularis). All cells were antidromically activated from the VPL nucleus of the thalamus. Each cell was excited by renal nerve stimulation and was tested for responses to distention of the renal pelvis with saline or a balloon-tipped catheter. Pelvic distention to a pressure of 50 mm Hg excited 20 of 27 STT cells. Responses were characterized by an early dynamic phase followed by some degree of adaptation. Average activity increased from 11 $\pm$ 3 to 29 $\pm$ 9 spikes/s. The stimulus-response relation was determined for 9 cells. Response thresholds averaged 30 $\pm$ 4 mm Hg. Responses increased linearly up to 80 mm Hg. Greatest responses were observed from cells in the L_1 and T_{12} segments. All cells had somatic receptive fields located in the ipsilateral flank, abdomen, or upper hindlimb. One hundred percent of the wide dynamic range cells responded while 63% of high threshold cells responded. The results show that STT cells are excited by a noxious renal stimulus. Response characteristics and somatic receptive field location suggest that these cells are important in causing renal pain and its referral to somatic structures. Supported by NIH Grant HL-36378.

49.9

DIFFERENCES IN VISCEROSOMATIC INPUT TO SPINOTHALAMIC (ST) CELLS PROJECTING TO VPLc AND VPLo THALAMUS IN MONKEYS. **RD Foreman, SF Hobbs, MJ Chandler, and DC Bolser. Dept. Physiol., Univ. of Okla., HSC, Okla. City, OK 73190.**

VPLc and VPLo thalamic nuclei respond best to skin and muscle afferent input, respectively, and receive input from ST cells. Thus, we hypothesized that skin but not muscle input would excite VPLc-ST cells, and muscle but not skin would excite VPLo-ST cells. Also, since visceral pain is referred to muscle, we hypothesized that urinary bladder distension and electrical stimulation of hypogastric nerves would activate VPLo- and not VPLc-ST cells. In 7 anesthetized monkeys L_2 to S_3 segments were searched for ST cells that were antidromically activated from VPLc but not VPLo (n=26), and VPLo but not VPLc (n=13). VPLc- and VPLo-ST cells (n=29) with somatic fields that included thigh, abdomen, or tail received similar viscerosomatic input i.e. 60% of the cells were excited by both skin and muscle input, 23% by muscle only, 17% by skin only, and of those 40% received visceral input. In contrast, only VPLc-ST cells had somatic fields confined to foot and calf. Of 10 VPLc cells with distal somatic fields 6 (60%) were excited by skin only, 4 (40%) by skin and muscle, and 0 by visceral input. Thus, VPLc-ST and VPLo-ST cells with proximal/axial somatic fields were more likely to be excited by visceral input ($P<0.02$) or by both skin and muscle input ($P<0.04$) then were VPLc-ST cells with only distal somatic fields. (NIH grants HL22732, HL07930, NS08150, and HL07304)

49.10

SOMATO- AND VISCEROTOPIC ORGANIZATION OF SPINOTHALAMIC TRACT (STT) CELLS IN PRIMATE LUMBOSACRAL CORD. M.J. Chandler, S.F. Hobbs, D.C. Bolser, and R.D. Foreman. Univ. of Okla. HSC, Dept. of Physiol., Okla. City, OK 73190.

Visceral pain is referred to proximal body areas, and probably involves activation of the STT. Thus, we hypothesized that visceral afferent input would excite STT cells with somatic input from proximal body areas. Seventy lumbosacral STT cells from 31 chloralose-anesthetized monkeys (Macaca fascicularis) were identified by antidromic activation from lateral thalamus. The somatic field of each cell was mapped by brushing hair and pinching skin and muscle of the limb (foot, calf, thigh), and proximal areas (tail, groin, and abdomen). Urinary bladder distension and electrical stimulation of hypogastric nerves produced visceral afferent input. Each cell was lesioned and its spinal segmental and laminar location was identified. A somatotopic map was constructed from somatic fields and spinal locations of the cells. A viscerosomatic map was constructed from visceral and somatotopic data. STT cells with input from foot and calf were located in the medial dorsal horn in segments L_4-S_1. STT cells with input from the thigh, groin, abdomen, or tail were located in the medial and lateral parts of the L_3-L_4 and S_1-S_2 segments but only the lateral part of the L_5-L_7 segments. Visceral input excited only 1 of 23 STT cells with somatic fields confined to the limb but excited 12 (63%) of 19 STT cells with proximal somatic fields ($P<0.001$). These data can explain why visceral pain is referred to proximal and not distal somatic areas. (NIH grants HL22732, HL07930, NS08150, and HL07304)

49.11

A THERMAL SOMATOSENSORY EVOKED POTENTIAL STIMULATOR FOR ANALGESIA STUDIES. G.M. Strain, B.L. Tedford,* and S.G. Kamerling. Vet. Physiol., Pharmacol. and Toxicol., Sch. Vet. Med., Louisiana State Univ., Baton Rouge, LA 70803.

In analgesic drug assessment the rat hot plate and tail flick tests do not adequately assess drugs producing motor depressant effects and give poor evaluations of some drugs with proven analgesic effects. Somatosensory evoked potentials (SEPs) elicited by nociceptive stimuli have been used for drug assessment, but non-nociceptive fibers are also usually activated, resulting in a contaminated signal. A stimulator for SEP studies of analgesics has been designed that produces a pure thermal nociceptive stimulus with temporal characteristics adequate for EP averager activation. Thermal stimuli are produced by the ceramic elements of a thermal printhead; current to the printhead is controlled by a relay and multiplexing DIP switches. Timing control of stimulus frequency and duration may be provided by any lab stimulator, which also provides the EP averager trigger. The area of skin heated is determined by the number of elements activated through the DIP switches. Temperatures able to activate thermal nociceptors (> 45°C) are produced with durations below 100 ms, so that the stimulus has ended before pain perception can occur, minimizing pain and discomfort.

Calibration curves of temperature vs. duration at both the skin surface and intradermally are presented, and the typical thermal SEP produced over somatosensory cortex in the rat is characterized. Preliminary results of drug studies with prototype analgesics are reported.

49.12

PERCEPTION OF PAIN IN MAN AFTER TOTAL TRANSECTION OF THE CORPUS CALLOSUM. B.E. Stein, D.D. Price* and M.S.Gazzaniga, Depts. Physiology & Anesthesiology, Medical College of Virginia, Richmond, VA 23298 and Dept. Neurology, Cornell University School of Medicine, New York, NY 10021

Although theories emphasize that crossed pathways subserve pain, the role of uncrossed input is uncertain. We sought to determine the contributions of ascending ipsilateral inputs to pain, and the possibility that some dimensions of pain perception might be lateralized. This was possible in a patient with a total transection of the corpus callosum. Quantitative ratings of innocuous tactile, and noxious and innocuous thermal stimuli were accomplished using verbal (left hemisphere) and visual analogue scale (both hemispheres tested independently) methodologies. Surprisingly, noxious ipsilateral stimuli evoked all components of pain experience (sensory and affective) but only at intensities well above normal (49-51°C). Apparently, neural activity via inputs other than the classical crossed spinothalamic pathway (that synapses in VPLc and projects to somatosensory cortex) is sufficient to evoke the complex of responses associated with pain. These alternate pathways contribute to perceived pain intensity, unpleasantness, and location of the stimulus. However, they do not contribute much to these aspects of pain in the lower noxious ranges (<47°C), where they often fail to subserve detection and identification of stimuli, functions normally subserved by contralateral and interhemispheric (corpus callosum) pathways.

49.13

A PSYCHOPHYSICAL ANALYSIS OF SPATIAL SUMMATION MECHANISMS IN PAIN. D.D. Price, J.G. McHaffie & M.L. Larson, Depts. of Anesthesiology & Physiology, Medical College of Virginia, Richmond, VA 23298 and Dept. of Physical Therapy, Univ. Southern Calif., Los Angeles, CA 90033.

Psychophysical experiments were initiated to determine the influence of spatial summation on perceived pain intensity. Six trained human subjects (5 male, 1 female) made visual analogue scale ratings for sensory intensity and unpleasantness to noxious thermal heat stimuli (45° to 50°C) delivered by 1,2 or 3 contact thermodes (1 cm^2 each) applied within a single dermatome of the anterior median forearm. Stimulus size was varied from 1-3 cm^2 by varying the number of thermodes activated, and stimulus size and temperature were randomly interspersed. Spatial separation between thermodes was systematically varied (0, 5, 10 cm) across 3 experimental sessions. Considerable spatial summation occurred in both sensory intensity and unpleasantness dimensions. Unlike spatial summation of warmth (Stevens, J.C. & Marks, L.E., Percep. & Psychophys. 9:291, 1971), that of pain was not characterized by systematic changes in power function exponents but as parallel upward displacements in double logarithmic coordinates. Increasing thermode separation also significantly enhanced spatial summation. The results indicate that powerful spatial summation mechanisms exist for pain and that such mechanisms are likely to depend on numbers of neurons recruited at some central level. The spinal cord dorsal horn is a likely site of action.

49.14

DIFFERENTIAL EFFECTS OF HETEROTOPIC CONDITIONING STIMULI ON FIRST AND SECOND PAIN PERCEPTION IN HUMANS. J.G. McHaffie and D.D. Price, Depts. Physiol. & Anesthesiol., Medical College of Virginia, Richmond, VA 23298.

Psychophysical experiments were initiated to determine if experimentally produced first and second pain is reduced by concomitant heterotopic nociceptive stimuli, a form of pain modulation termed diffuse noxious inhibitory controls (DNIC- LeBars et al., Pain 6:283, 1979). The magnitude of electrically evoked first and second pain (9ms, 6-15mA pulses delivered subepithelially to the ankle) conditioned by 10 sec thermal heat pulses (43, 47, 50°C applied to either the contralateral foot or abdomen) was rated with visual analog scales by 7 male subjects. Frankly noxious thermal conditioning stimuli (47, 51°C), but not innocuous stimuli (43°C), applied to the contralateral foot or abdomen reliably inhibited both first and second pain. However, the degree of inhibition was not equal but was significantly greater for second pain. In addition, the inhibitory effects did not outlast the duration of conditioning stimuli. All of these results closely parallel electrophysiological observations about DNIC in primates. Since the magnitude of first pain reduction was relatively weak and the duration of inhibition brief, it is unlikely that DNIC subserves a major role in either relieving pain or providing a mechanism of coding pain. The spatial and temporal pattern of DNIC suggests that it may be a phenomenon more closely associated with the elaboration of withdrawal reflexes.

49.15

QUANTITATIVE EVALUATION OF NOCICEPTIVE NEURONS IN RAT TRIGEMINAL PARS CAUDALIS. M.A. Larson, J.G. McHaffie and B.E. Stein. Dept. Physical Therapy, USC., Los Angeles, CA. 90033 & Dept. Physiol., Med. Col. Va, Richmond, VA 23298.

Somatosensory neurons (n=135) were studied in pars caudalis of urethan anesthetized rats: 38% were low threshold (LT), 29% wide dynamic range (WDR), and 39% nociceptive specific (NS). WDR receptive fields (RFs) were restricted to the face but usually included 2 or 3 nerve divisions, had thermal thresholds of 42°-50°C, mechanical thresholds <100mg, and some responded to noxious cold. NS RFs were smaller and restricted to one nerve division. WDR and NS neurons had RFs on face, cornea, and in the oral cavity and rarely had responses outlasting the stimulus durations. They had positively accelerating power functions for thermal stimuli (3.9 WDR, 4.4 NS) but negatively accelerating power functions for LT mechanical stimuli (0.7). Superior colliculus (SC) nociceptive neurons with facial RFs reflect some of these properties (Larson et al. J. Neurosci. 7:547,1987) but lack oral and corneal representations, exhibit longlasting afterdischarges, lower thresholds (42-44°C), and lower exponents. This suggests that SC properties are derived from a select subpopulation of trigeminal nociceptive neurons: this finding is consistent with our anatomical studies (Bruce et al. J. Comp. Neurol. 262:315,1987) showing that trigeminotectal and other ascending trigeminofugal projections arise from two largely segregated populations. Supported by A.D. Williams Foundation and NIH grant EY05554.

50.1

PROJECTIONS OF LEMNISCAL AND SPINAL AFFERENTS TO RELAY AND LOCAL CIRCUIT NEURONS OF THE PRIMATE VENTROBASAL THALAMUS. H.J. Ralston, III, D.D. Ralston and P.T. Ohara. The Department of Anatomy, University of California, San Francisco, California 94143.

The dorsal column-medial lemniscal (ML) system carries information arising from nonnoxious mechanical stimuli, and terminates throughout VPLc - that portion of the ventrobasal complex (VB) which exhibits a somatotopic map of the contralateral body. The spinothalamic tract (STT) carries both nonnoxious and noxious mechanical and thermal information; its projections arborize as cylindrical arrays running rostrocaudally through VPLc, thus partially overlapping the ML distribution. Both the ML and STT axons project to thalamocortical relay (TCR) cells and to GABAergic local circuit neurons (LCN) of VB. The present study compares the synaptic organization of the ML and STT terminations, particularly in regard to the contacts made by these afferents with TCRs and LCNs, and whether the interactions are different in these two systems which mediate different types of information.

Macaque monkeys were anesthetized and, using sterile neurosurgical techniques, the ML or STT projections to VPLc were labeled by the orthograde axonal transport of WGA-HRP. After appropriate survival times, the animals were reanesthetized, perfused, and thalamic slices processed to demonstrate HRP reaction product in the ML or STT terminals. Serial thin sections of identified terminals were taken, examined by EM, and the images reconstructed by computer to permit a precise analysis of the synaptic interrelationships of ML or STT terminals with dendritic processes of TCRs and LCNs. Serial reconstruction reveals that a single ML or STT afferent axon contacts numerous branches of LCN presynaptic dendrites (PSDs), which have been shown to contain GABA, and that the afferent axon and the multiple PSDs also contact an extensive region of the dendritic arbor of a single TCR. Thus, the "triadic" relationship between afferent axons and VB neurons is extraordinarily complex, having a morphological organization which is far more elaborate than that shown by previous studies. These complex synaptic arrays serve both excitatory and feed-forward inhibitory synaptic functions in VB. (Supported by NS-23347 from NIH.)

50.2

SOMATOSENSORY RESPONSES OF PEDUNCULO-PONTINE NUCLEUS UNITS PROJECTING TO THE MEDIAL THALAMUS. B. Grunwerg*, J. Krol* and G. M. Krauthamer. Department of Anatomy, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

The parabrachial-pedunculopontine system is one of the major cholinergic cell groups in the brainstem. The pedunculopontine tegmental nucleus (PPTN) is located in the lateral aspect of the most rostral portions of this complex and projects to medial thalamic nuclei, subthalamus, hypothalamus and superior colliculus. Reciprocal projections link it with several extrapyramidal structures including the globus pallidus and substantia nigra pars reticulata. Extracellular recordings were made from single PPTN neurons in anesthetized hooded rats. Units activated antidromically from the medial thalamus responded to stroking, tapping and pinching. Small to large receptive fields were located on the contralateral paws, shoulder and neck. Other neurons in this area could not be activated antidromically from the medial thalamus but showed a similar receptive field organization. These findings suggest that the PPTN may be involved in the processing of sensory input to the medial thalamus.

50.3

COMPARISON OF THE MORPHOLOGY OF TRIGEMINOTHALAMIC AFFERENT TERMINATIONS IN THE VENTRAL POSTERIOR NUCLEUS OF THE RAT. H.P. Killackey, N.L. Chiaia and R.W. Rhoades. (SPON: M. Rayport) Dept. of Psychobio., Univ. of Calif., Irvine, CA 92717 and Dept. of Anat., Medical College of Ohio, Toledo OH 43699.

Both the principal sensory nucleus (PrV) and the subnucleus interpolaris (SpI) of the brainstem trigeminal complex project to the medial portion of the contralateral ventral posterior nucleus (VPM) in a topographic fashion. The functional correlates of these two projections differ greatly. In the present study, we examined the morphology of both PrV and SpI afferent terminations labeled with either phaseolous vulgaris leucoagglutinin (Phal) or intracellularly injected horseradish peroxidase (HRP).

A group of adult rats received iontophoretic injections of Phal (5 uA for 30 min) into either PrV or SpI. In a second group of rats trigeminal lemniscus fibers were isolated, functionally characterized and injected with HRP. Following appropriate histological procedures, labeled fibers in VPM were morphologically characterized.

Both procedures result in excellent labeling of trigeminothalamic terminations. The distribution of both PrV and SpI terminations in the medial to lateral dimension is quite restricted. The distribution of the SpI terminations along both the dorsoventral and rostrocaudal axis is more extensive than that of the PrV terminations. Qualitatively, PrV terminations appear denser than SpI terminations.

Support: BNS 87-19311 (HPK), BNS 85-17537 & DE07734 (RWR)

50.4

CHARACTERIZATION OF NEURONS IN THE VENTRAL POSTERIOR (VP) AND POSTERIOR (PO) NUCLEI OF THE RAT, USING PHYSIOLOGICAL, LIGHT AND ELECTRON MICROSCOPIC TECHNIQUES. G.R. Belford*, H.P.Killackey, N. L. Chiaia and R.W. Rhoades (SPON: M. Le Gare). Dept. of Psychobio., Univ. of Calif., Irvine, CA and Dept. of Anat., Medical College of Ohio, Toledo, OH.

Neurons in both VP and PO are responsive to vibrissae stimulation. We have used intracellular recording to physiologically characterize these neurons, combined with injection of horseradish peroxidase (HRP) for anatomical characterization with light and electron microscopes.

Neurons in VP were responsive to deflection of only one or two vibrissae, whereas PO neurons were typically responsive to 5 or more vibrissae. The cross-sectional areas of the dendritic fields of VP neurons did not overlap with those of PO neurons (VP range = 24,437-54,027 um^2; PO range = 58,832-110,381 um^2).

Examination with the electron microscope also showed differences. HRP-filled dendrites of VP neurons coursed through neuropil containing fewer profiles of myelinated axons than did dendrites of labelled PO neurons (8.5 per 100 um^2 for VP vs. 23.7 per 100 um^2 for PO). There were more profiles of large dendrites (cross-sectional area > 1 um^2) in VP than in PO (VP: 4.26 per 100 um^2; PO: 2.04 per 100 um^2). Finally, there were more large axonal terminals containing round vesicles in VP than in PO (VP: 1.64 per 100 um^2; PO: 0.41 per 100 um^2). This terminal type has been correlated with the ascending lemniscal input.

Support: BNS 87-19295 (GRB), BNS 85-17537 & DE07734 (RWR)

50.5

NEURONS IN THE DORSAL COLUMN NUCLEI OF RAT WHICH PROJECT TO THE IPSILATERAL THALAMUS. J.R. Norris and H.P. Killackey (Spon: R. Josephson) Dept. of Psychobiology, University of California, Irvine, CA. 92717

A recent study in cat reported that a small population of DCN neurons projected to the **ipsilateral** thalamus (Berkley, 1984). In the present study, retrograde tracing methods were used to determine if a similar population of cells exists in the DCN of rat.

Adult rats received large injections of 10% WGA-HRP into the lateral thalamus unilaterally, or thalamic injections of two fluorescent tracers into opposite sides of the thalamus. The animals were sacrificed and the tissue processed according to standard methods.

The rat DCN contains a small (<5%) but consistently labeled population of neurons which project to the ipsilateral thalamus. Labeled cells were observed in both the gracile and cuneate nuclei, though generally more gracile cells were labeled than cuneate cells. The cells occur singly and their positions within the cross-sections of the DCN are highly variable and follow no apparent pattern. Labeled neurons are most numerous in the middle and, especially, the caudal portions of both nuclei, although labeled cells are present throughout the entire rostral-caudal extent of the DCN. Based on the double labeling studies, the thalamic projection from these cells appears to be primarily, if not exclusively, ipsilateral.

Supported by BNS 87-19311.

50.6

AUDITORY INFLUENCES THROUGH THE COCHLEAR NUCLEUS ON CUNEATE NEURONS IN DECEREBRATE-DECEREBELLATE CATS. S.F. Atweh, Y. Bassim*, S.J. Jabbur and N.E. Saadé. Fac. of Med., American University of Beirut, Beirut, Lebanon.

In a previous study, we have demonstrated visual and auditory interactions in the cuneate nucleus (J. Physiol., 238:343, 1974). Recently, direct and reciprocal interconnections between the cuneate and cochlear nuclei have been anatomically demonstrated. We now report the effects of click and tone stimuli on cuneate nuclear activities in decerebellate cats with brainstem transected either below the inferior colliculi or at mid-collicular level with bilateral suction of the inferior colliculi. Conditioning clicks or tones produced negative and positive waves on the surface of the cuneate nuclei. The same conditioning stimuli modulated the activities of 15 cuneate neurons but had no influence on another 30 cuneate and 6 gracile neurons. One neuron was activated, 8 neurons were inhibited, 3 neurons were facilitated and the remaining 3 neurons showed cycling of facilitation-inhibition. Direct projection as well as interneurons were involved. Both gross potentials and effects on single neurons were more pronounced from the ipsilateral stimuli.

We have earlier demonstrated reciprocal intermodulatory loops involving the DC nuclei with trigeminal, raphe, periaqueductal and reticular nuclei. This study completes the description of another loop and illustrates heterosensory interactions between two primary sensory relays. (Supported from Lebanese N.R.C. and D.T. Sabbagh Fund).

50.7

A DORSAL COLUMN INPUT INTO COCHLEAR NEURONS IN DECEREBRATE-DECEREBELLATE CATS. S.J. Jabbur, A. Franjieh*, S.F. Atweh and N.E. Saadé. Fac. of Med., American University of Beirut, Beirut, Lebanon.

Direct projections from the dorsal column nuclei have been recently described in cats (Brain Res., 400:145, 1987). We now demonstrate that ascending activation of the dorsal columns (DCs) can modulate cochlear neuronal discharges evoked by sound clicks and tones. Seven cats were anesthetized, decerebrated (mid-collicular) and decerebellated. The C_1-C_4 dorsal roots were cut and the ventral and lateral funiculi were bilaterally lesioned at both C_1 and C_4 levels leaving the DCs intact. DCs were activated either directly through localized electrical stimulation or indirectly by stimulating the paws. The extracellular activity of 50 neurons in dorsal and ventral cochlear nuclei were recorded in response to peripheral clicks or tones. Conditioning activation of DCs modulated the activity of 23 neurons and had no influence on 27 others. Fourteen neurons were discharged from DC input with a latency of 4 to 10 ms. Conditioning DC activation produced weak facilitation in 3 neurons and inhibition in 6 neurons. The inhibition peaked at conditioning-testing interval of 10-25 ms and lasted for about 150 msec. DC effects were bilateral.

This report provides evidence for another extralemniscal output of the DC nuclei which plays a role in modulation auditory input at its entry nucleus in the brain.
(Supported from Lebanese N.R. Council and D.T. Sabbagh Fund).

50.8

SOMATOTOPIC ORGANIZATION OF FORELIMB AND HINDLIMB CUTANEOUS AFFERENTS TO THE DORSAL COLUMN NUCLEI (DCN) IN THE RAT. S. Maslany*, D.P. Crockett and M.D. Egger. Dept. of Anatomy, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ. 08854

Recent studies in the monkey and racoon have demonstrated that cutaneous afferents from specific digits of the hand project to discrete regions in the cuneate nucleus. We have now carried out a detailed analysis of projections from cutaneous afferents of the limbs to the DCN in the rat. A mixture of 25% free HRP and 2.5% WGA-HRP was intracutaneously injected into different limited regions of the forelimbs and hindlimbs. The rats were perfused transcardially after three days. Horizontal sections 60-µm thick were cut. The HRP was reacted using the TMB method. Data from 15 rats were analyzed. DCN labeling from different regions of the limbs was precisely restricted mediolaterally, with little or no overlap into adjacent areas. In the gracile nucleus (GN) more variability in labeling was found for the rostrocaudal direction than in the cuneate nucleus (CN). The detailed projection patterns demonstrated that primary cutaneous afferents from the limbs in the rat are strictly segregated within the CN and GN according to the origin of their peripheral fibers.

50.9

INTRA-AXONAL INJECTIONS OF HORSERADISH PEROXIDASE (HRP) INTO PRIMARY AFFERENTS TO THE MAIN (CN) AND EXTERNAL CUNEATE (ECN) NUCLEI IN THE RAT. D.P. Crockett, S.L. Harris* AND M.D. Egger. Dept. of Anatomy, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ. 08854

We have begun a structure-function analysis of the axonal arbors of primary afferents to the CN and ECN in the rat by intra-axonally injecting HRP into physiologically identified primary afferents. Two proprioceptive afferents (one responding to bending of the wrist and the other to movement of the elbow) were traced throughout much of the CN, ending in the ECN. Their arbors were simpler in the CN than in the ECN. Also, the density of boutons was greater in the ECN than in the CN. In the ECN, boutons were concentrated rostrocaudally within 400 µm. In contrast, within the CN, boutons were distributed rostrocaudally over 1.7 mm, extending from 1.6 mm caudal to the obex to 0.1 mm rostral to it. A rapidly adapting fiber with a receptive field on the second digit of the forepaw had a complex distribution of terminals confined to the CN, with boutons extending over 3.1 mm caudal to the obex. This distribution of rapidly adapting boutons agreed precisely with the overall distribution of transganglionically labelled primary afferent fibers we observed following intracutaneous injections of WGA-HRP into the forepaw (Maslany et al., 1988).

50.10

POSTSYNAPTIC DORSAL COLUMN (PSDC) AFFERENTS TO THE DORSAL COLUMN NUCLEI (DCN) OF RATS REVEALED BY *PHASEOLUS VULGARIS* LEUCOAGGLUTININ (PHA-L). M.D. Egger, S.L. Harris* and D.P. Crockett (SPON: S. Rosner). Dept. of Anatomy, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

The PSDC cells of the spinal dorsal horn provide an important source of ascending input to the DCN. Although the locations of many PSDC cell bodies have been described, the terminal projection patterns of PSDC cells are not well known. We have begun an examination of these terminal projections of PSDC cells in the DCN by injecting the anterograde tracer, PHA-L, at various segmental levels in the spinal cords of rats. Injections within the cervical (C5; n=1) and lumbar (L4; n=1) enlargements produced dense projections throughout much of the cuneate (CN) and gracile nuclei (GN), respectively, with the densest projections in the rostral and ventral regions of these nuclei. On the other hand, injections within the C8 (n=2) or the S1-S2 (n=1) segments labelled only a few fibers. The cervical injections produced no labelling in GN, confirming that the PHA-L is taken up only by cell bodies, but not by fibers of passage. The wide distribution of labelled afferents within CN or GN following an injection confined to a single segment suggests that the PSDC afferents may not provide detailed spatial information.

50.11

IMMUNOHISTOCHEMISTRY OF PUTATIVE NEUROTRANSMITTERS IN THE LATERAL CERVICAL NUCLEUS OF THE MONKEY. J. Broman* and A. Blomqvist (SPON: M. Bull). Dept. of Anatomy, Uppsala University, Box 571, S-751 23 Uppsala, Sweden.

The immunohistochemical localization of GABA in the lateral cervical nucleus (LCN) was investigated in cynomolgus monkeys. Light microscopy revealed the presence of a small population of GABA-positive neurons located within all parts of the LCN. Ultrstructural investigation showed that the labeled neurons were small and were contacted by few somatic boutons. GABA-positive terminals were numerous and scattered throughout the LCN. Most labeled terminals were presynaptic to dendrites, but axosomatic synapses were also seen.

The distribution of substance P (SP) and serotonin (5-HT) was investigated in owl monkeys. SP-like immunoreactivity was densest in the areas immediately surrounding the LCN, but densely labeled puncta were also seen within the nucleus. Electron microscopy showed that most labeled terminals were large and made synaptic contacts with dendrites and nerve cell bodies.

5-HT immunohistochemistry revealed the presence in the LCN of a dense network of thin fibers with bouton-like swellings. Most labeled profiles were identified as thin, unmyelinated fibers which often were surrounded by astroglial processes. Somewhat larger profiles containing densely packed synaptic vesicles were also seen, sometimes in apposition with dendrites or nerve cell bodies.

50.12

LIGHT AND ELECTRON MICROSCOPIC DEMONSTRATION OF SEROTONIN IMMUNOREACTIVITY IN THE DORSAL COLUMN NUCLEI OF CATS AND MONKEYS. A. Blomqvist and J. Broman*. Dept. of Anatomy, University of Uppsala, Box 571, S-751 23 Uppsala, Sweden.

The distribution of serotonin (5-HT) in the dorsal column nuclei (DCN) of cats and monkeys was studied by light and electron microscopic immunohistochemistry. A network of thin immunoreactive fibers with bouton-like swellings was seen throughout the DCN. However, in both species, regional differences in the density of the labeling was observed. In the cat, dense 5-HT immunoreactivity was seen in the rostral and middle-ventral "reticular" regions of the DCN, whereas the middle-dorsal "cell nest" regions were more sparsely labeled. In the monkey, the densest labeling was seen in the gracile nucleus and in the pars triangularis of the cuneate nucleus, with sparser labeling in the pars rotunda. In the neuropil of the DCN, electron microscopy showed the presence of thin, unmyelinated immunoreactive fibers as well as larger, labeled bouton-like swellings. The latter were often in contact with dendrites and nerve cell bodies.

The findings suggest that the 5-HT innervation of the DCN is related mainly to the regions of the nuclei that have been implicated in nociceptive and/or motor related functions.

50.13

THEORY OF IMPULSE PROPAGATION IN CAT CUNEATE NUCLEUS AXONS WITH SEROTONIN-LIKE IMMUNOREACTIVITY. M.D. Goldfinger, V.R. Roettger, & J.C. Pearson. Depts. of Physiology & Biophysics and Anatomy, Wright State University, Dayton, OH 45401-0927.

The cat Cuneate Nucleus is innervated by axons with serotonin-like immunoreactivity (5HT-LI: Pearson & Goldfinger, Neurosci. Lett. 74:125, 1987). The present work used 5HT-LI morphological data with cable theory to construct a model for impulse propagation in 5HT-LI axons. The assumptions were: (a) 1-dimensional unmyelinated core-conductor had uniform geometry-independent cable constants; (b) fiber varicosities [Diameter: D=2-3μm] and intervaricosities [D=1-2μm] differed only in diameter and length assessed from high-power sagittal photos of individual 5HT-LI axons; (c) both varicosity and intervaricosity membranes contained voltage-and-time dependent Na^+ and K^+ conductances represented by the Hodgkin-Huxley equations (J. Physiol. 117:500, 1952). The computational method [modified Euler integration; dT=0.1 us; dX/λ=0.0165; PDP-11/34] was tested for uniform-geometry axons [D=1-3μm] with no varicosities and gave CV (Conduction Velocity) =0.370*. Solutions were stable, requiring appr. 10 hr-cpu/4 ms-simulated, for 200 3μm-compartments; propagated impulse duration and amplitude were 2.0 ms and 100. mV, respectively.

Propagation (CV=0.37m/s) past a single varicosity [length = integr. step = 3μm] centered in a 1μm-D 600μm-length axon was unchanged by varicosity diameters [1-3μm] observed in stained axons. Incorporation of additional 3μm-long x 2μm-D varicosities to a 1μm-D 600μm-long axon (integr. step = 3μm) slightly increased CV towards that of a uniform 2μm-D axon. When varicosity number and spacing approximated those observed in stained axons: (i) doubling axial resistivity (110 to 220 ohm·cm) decreased CV from 0.39 to 0.29 m/s; (ii) speeding conductance kinetics (3X) increased CV (39%), shortened impulse duration (59%), and decreased impulse amplitude (11%).

These computations indicated that single impulse propagation: (a) is not compromised by varicosity-type geometrical inhomogeneities; (b) is influenced predictably by cable parameters; and (c) can occur at slow CVs (< 1 m/s) over long distances without failure.

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50.14

RAT CUNEATE NUCLEUS CONTAINS GAMMA-AMINOBUTYRIC ACID-LIKE IMMUNOREACTIVE NEURONS. V.R. Roettger and J.C. Pearson. Depts. of Physiology and Biophysics and Anatomy, Wright State University, Dayton, Ohio 45401-0927

A neurotransmitter role for gamma-aminobutyric acid (GABA) in rat cuneate nucleus is supported experimentally. Immunohistochemistry reveals the presence of glutamic acid decarboxylase (GAD) in small cells within this nucleus [Barbaresi, et al., Brain Res. 382:305 1986]. Antiserum, raised against GABA-glutaraldehyde-keyhole limpet hemocyanin in guinea pigs, was specificity-tested by preabsorption with 100 or 500 μM concentrations of GABA-, glutamine (GLN)-, glutamate (GLU)-, or taurine (TAU)-glutaraldehyde conjugations. Cerebellar tissue staining was inhibited by 100μM GABA-glutaraldehyde whereas normal staining was seen with 500μM GLU-, GLN-, and Tau-glutaraldehyde. GABA-stained rats were intraventricularly colchicinized and transcardially perfused with 4% paraformaldehyde, 0.5% glutaraldehyde, 0.5% $K_2Cr_2O_7$ in 0.05M phosphate-buffered saline (pH=6.5). Visualization of cells was with avidin-biotin-peroxidase for GABA-LI staining and with cresyl violet acetate for Nissl staining. GABA-LI and Nissl-stained neurons from cuneate nucleus were compared in terms of cell body size, shape, and distribution using camera lucida tracings. GABA-LI cell bodies were distributed randomly throughout the cross-sectional area of the nucleus at all rostral-caudal levels except around the obex where they were limited to the peripheral border. GABA-LI cell bodies comprised 21.5% of the total population of cuneate neurons and had a mean cross-sectional area which was significantly smaller than that of the total neuronal population. These results closely approximate results obtained using anti-GAD antiserum [ibid.]. GABA-LI processes were seen throughout the cross-sectional area of the nucleus at all rostral-caudal levels, often surrounding empty spaces which appeared to be large, unstained neuronal cell bodies. Similarities between GABA-LI cells and previously reported GAD-LI cells suggest that GABA is synthesized and present within small cell-bodied rat cuneate neurons. These results are consistent with the hypothesis that GABA is a neurotransmitter involved in somatosensory information processing in this nucleus.

50.15

EFFECTS OF CHOLINERGIC AGONISTS ON THE VENTRO-BASAL COMPLEX OF THE RAT. G.A. Marks, A.M. Hoelscher*, H.P. Roffwarg, Dept. of Psychiatry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX 75235-9070.

The influence of cholinergic agonists on thalamic relay cells was investigated by iontophoretic application to 71 identified ventro-basal neurons from 28 chloral hydrate anesthetized rats. Carb was applied to 63 cells eliciting either: no effect (64%), inhibition (8%), inhibition followed by facilitation (I/F) (3%), and facilitation (25%). Onset of facilitation was always later than onset of inhibition. Glutamate (glu) ejection produced a short latency facilitatory response in every cell. The simultaneous ejection of glu and carb to 36 cells was used to detect the short latency effects of carb ejection. Three response types detected were: no effect (8%), antagonism of glu (84%), and potentiation of glu (8%). Low-current continuous ejection of glu was also used to produce a single spike pattern in 24 cells. Ejections of carb now produced responses in every cell: inhibition (54%), I/F (33%), and facilitation (13%). The M_1 selective agonist McN-A-343 was applied to 27 cells. The major difference between McN and carb was McN inability to produce facilitation. Two response types were observed: no effect (74%) and inhibition (26%). Short-duration simultaneous ejections of McN and glu antagonized glu in all cases. Continuous application of glu lead to McN inhibition in 94% of cases. In cells receiving both carb and McN, it was observed that carb could facilitate and McN inhibit.

50.16

BRAINSTEM PROJECTIONS TO THALAMUS AND HYPOTHALAMUS. E. Carstens, J. Leah*, J. Lechner* and M. Zimmermann. II. Physiol. Inst., Univ. Heidelberg, D-69 Heidelberg (FRG).

A paucity of information on brainstem projections to medial thalamus and hypothalamus prompted us to map them using the retrograde transport of colloidal gold-WGA-HRP with silver intensification in the rat. Injections of <1 ul were restricted to centrum medianum (CM), centralis lateralis (CL), medialis dorsalis (MD), zona incerta (ZI), or medial or lateral hypothalamus (MH, LH).

Injection of CL/MD labeled numerous (>100/section) neurons bilaterally throughout the rostro-caudal extent of midbrain periaqueductal gray (PAG), dorsal raphe, reticular formation (RF) and substantia nigra, and in pontomedullary raphe (eg., raphe magnus), RF, locus coeruleus and dorsolateral pontine n., lateral reticular n. (LRN), n. of solitary tract (NST), and trigeminal n. caudalis (V). Many were labeled ipsilaterally in superior colliculus (SC) and ant. pretectal n. ZI similarly labeled numerous neurons except that there were many more in contralateral V and the dorsal column n. (DCN). CM or CL alone labeled relatively fewer midbrain neurons in a similar pattern, but with few or no pontomedullary cells. LH labeled numerous neurons in a distribution similar to CL/MD and ZI, but with fewer in SC or DCN and almost none in V. A small MH injection labeled scattered cells in midbrain PAG/LF, but revealed a strikingly dense bilateral projection from the ventral and laterodorsal tegmental n.

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50.17

EXAMINATION OF RECEPTIVE FIELD SURROUNDS IN SOMATOSENSORY NEURONS IN CAT SUPERIOR COLLICULUS. H. R. Clemo and B. E. Stein. Dept. Physiol., MCV/VCU, Richmond, VA 23298.

Somatosensory receptive fields (RF) of superior colliculus (SC) neurons contain best areas where stimulation elicits the greatest response. As the distance from the best area increases, the number of spikes elicited and response probability decrease. We now examined RFs to determine the influence of surrounding regions. Two methods were used: a) some cells were studied by comparing the response to stimulation of the RF center to that evoked by simultaneously stimulating the RF center and (individually) sites outside the excitatory borders; b) in some cells, the response to a stimulus applied inside the RF border was compared to the response to a stimulus that was elongated and straddled the border. In most cells, stimulation at the majority of sites outside the RF had no significant effect on the within-field response. However, in almost one-half of the cells, the within-field response was influenced from at least one site outside the RF: 70% showed some response facilitation and 30% response inhibition. These results suggest that for many somatosensory SC cells the border defining the area within which impulses can be elicited does not define the extent of the excitatory region. Furthermore, in contrast to other sensory systems here, surround inhibition is of comparatively minor importance.

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51.1

SIGNIFICANCE OF SPATIAL LOCATION DETERMINES THE FIRING CHARACTERISTICS OF RAT HIPPOCAMPAL PLACE CELLS. C.R.Breese*, R.E.Hampson, and S.A.Deadwyler. [*SPON: J.H. Ryu*], Dept. of Phys. and Pharm., Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27103.

Previous work from this laboratory demonstrated that control of hippocampal place cell firing was dependent upon several factors, including direction of travel, specific boundaries on the platform, off platform cues, distance from the center of the place field, and the significance of a spatial location (Breese *et al.*, Soc. Neurosci. Abstr. 13:173.6, 1987). We report here additional aspects of place cell plasticity determined by a comparison of firing before and after instituting a restrictive baiting procedure which was shown to alter the location of place cell firing. Place fields were defined as clusters of cell discharges within 0.75 cm of each other which constituted at least 20% of the total number of spikes. In control sessions, place fields emerged near regions of the platform where water was randomly available, but such fields could not be predicted on the basis of either traversal or time-in-location maps. Following a 20-30 min period in which baiting was restricted to only one location on the platform, a majority of the place fields (19 out of 21, X^2=20.5-225.2; $p<0.01$) shifted to that location. Analysis of variance showed no difference between control and restricted baiting fields in either total number of spikes, spike density, or total number of pixels containing at least one spike. Significant decreases were found in the distance of individual cell spikes from the region of highest firing density in the field ($F(41)=9.58$; $p<0.003$), total area included in the place field ($F(41)=11.503$; $p<.002$) and the number of fields meeting the field size criteria ($F(39)=6.896$; $p<.02$) in the baiting protocol. These results indicate that nonspatial attributes of the environment can change the location in which place cells fire and that such changes appear to affect all place cells regardless of field size, location, or density. *[Supported by NIDA grants DA 04441, DA 03502 and DA 00119 to S.A.D.]*

51.2

HIPPOCAMPAL PLACE CELL TO SILENT CELL RATIOS IN FREELY-BEHAVING RATS. L.T. Thompson & P.J. Best, Neuroscience Program and Department of Psychology, University of Virginia, Charlottesville, VA 22903.

A frequent criterion used for inclusion of units in both intra- and extracellular studies of neuronal function is spontaneous activity. In any given hippocampal electrode track, however, more neurons can be visualized proximal to the track than are recorded. Our interest in place cell activity led to the present study, which determines the relative proportions of place cells to silent cells in the dorsal CA1 area of rat hippocampus.

Well-isolated complex-spike cells were located using driveable chronic microwire electrodes in anesthetized rats. Unit activity was recorded under anesthesia, during exploration of a number of spatial environments consquent to place cell studies, and during later re-anesthetization. Only units demonstrating similar physiological characteristics during both periods of anesthetization were included in this study. Some units were also electrically driven by chronic stimulating electrodes in the conditions listed, to verify that anomolous changes in electrode position or other variables were not responsible for lack of detectable unit activity on many electrodes in waking rats.

Both barbiturate and benzodiazepine anesthetics increase the firing activity of hippocampal complex-spike neurons isolated in waking rats. Use of these agents during unit isolation therefore increases the likelihood that a unit is included in our sample. Over 60% of all units isolated in dorsal hippocampus under anesthesia have no detectable spontaneous activity, even when exposed to numerous spatial environments. Spontaneously active units in the waking rat generally exhibited place field activity, although the characteristics vary from cell to cell and from environment to environment. Within a given environment, such activity is extremely stable.
Supported by NSF grant BNS-81119030.

51.3

TWO FUNCTIONAL CATEGORIES OF HIPPOCAMPAL COMPLEX SPIKE CELLS. **S.I.Wiener & H.Eichenbaum. Dept. Biology, Wellesley College, Wellesley MA 02181.**

Several investigators have reported that most hippocampal complex spike cells fire in relation to significant behavioral events in learning tasks, but others have reported that most of these cells fire primarily in relation to the animal's location in its environment. To directly compare behavioral versus spatial correlates, we recorded from 100 neurons while rats performed an odor discrimination and a spatial navigation task in the same environment. In the odor task, 23 cells were characterized as cue-sampling (CS) cells that fire when the rat is investigating the odor cues, 22 as goal-approach (GA) cells that fire when the rat advances towards the odors or reward, and 55 as having no identifiable correlate (NC). In the spatial navigation task, 81 cells had a significant place field, that is, locus of elevated firing.

65% of CS cells had place fields in the spatial task compared to 86% of GA cells and 87% of NC cells. Place fields of CS and GA cells in the spatial task were not necessarily where odors or rewards were delivered, although more GA cells than CS or NC cells had fields encroaching on that locus. Directly comparing the correlates, 72% of CS cells increased firing more during odor sampling than when the rat was in the place field in the spatial task. Conversely, 74% of GA cells increased firing more when the rat was in the place field than when the rat was performing the approach behavior (X^2=8.35, p<.01). Hippocampal complex spike cells can have seemingly unrelated behavioral and place correlates in the same environment. However, there appear to be two functional subtypes: one type of cell that fires maximally during stimulus analysis and another that fires maximally during spatial navigation.
Supported by NS18744, BNS8507677 & BNS8810095.

51.4

THE ORGANIZATION OF PLACE FIELDS IN THE HIPPOCAMPUS. **H.Eichenbaum, S.I. Wiener & N.J. Cohen. Dept. Biology, Wellesley College, Wellesley MA 02181.**

Since the discovery that most hippocampal cells have "place fields", that is, they fire selectively when a rat is in a particular locus in its environment, investigators have wondered how the representations of places are anatomically organized in the hippocampus. We recorded simultaneously from groups of neighboring (0.5mm radius) CA1 neurons in rats performing a spatial navigation task; over 80% of the cells had significant single place fields or multiple subfields. In this preliminary study, we asked whether neighboring cells represent a restricted part of space with close and overlapping place fields (like the organization of receptive fields in visual cortical column), represent all places (like the organization of trigger features in a column), or are not related. For each of 62 groups of 4-13 cells, the mean distance between nearest-neighbor subfields and field overlap were compared to similar measures on 1000 "simulated" groups with place fields matching those in the real group but located and oriented randomly. As a control, we made the same comparisons on groups of cells selected randomly from different animals.

Place fields of 76% of real groups were closer than the average for simulated groups (z=3.93, p<.001; 19% were above the 95th %ile) and 77% had more overlap (z=4.19, p<.001; 42% were above the 95th %ile). These signs of "clustering" were not seen in random-selection groups (real vs random-selection groups: X^2=5.13, p<.02 for closeness; X^2=8.93, p<.01 for overlap). Further analyses revealed that real groups usually have multiple clusters of place fields covering a total of up to 75% of the environment. Hence the mapping of space in the hippocampus involves a combination of overlapping place fields and representation of widespread loci.
Supported by NS18744, BNS8507677 & BNS8810095.

51.5

EFFECTS OF ENVIRONMENTAL MANIPULATIONS ON THE DISCHARGE OF HEAD-DIRECTION CELLS IN THE POST-SUBICULUM. J.S. Taube, R.U. Muller*, J.B. Ranck, Jr. Dept. of Physiology, SUNY Health Sciences Center, Brooklyn, NY 11203.

Many neurons in the postsubiculum discharge as a function of a rat's head direction in the horizontal plane, independent of the rat's behavior and place in the environment. The present study examined the effects on head-direction cell firing of 1) removing the salient visual cue or 2) changing the shape of the environment. Recordings were made from head-direction cells while rats moved freely in a 76 cm diameter gray cylinder. The orienting visual cue was a white card taped to the inside wall of the cylinder. Following an initial session, an 8 min session was run with the cue card removed, followed by another control session. An 8 min session was then run with the rat in either a rectangular or square enclosure; each alternative enclosure contained a white cue card in a similar position as in the cylinder.

Results from both environmental manipulations were similar. The rate of cell firing remained unchanged, but the head direction for maximal firing rotated more than 36° in 9/15 cells when the cue card was removed, and in 9/11 and 3/8 cells in the rectangular or square enclosure, respectively. Some cells showed no change in the preferred head direction in one enclosure, but showed a change in the other. On 2 occasions, 2 head direction cells were recorded simultaneously. The effects of an environmental manipulation on the preferred head direction for one cell were nearly identical to the observed effects in the second cell. These results demonstrate that head-direction cell firing is not simply a sensory response to a visual cue. Furthermore, changes in the shape of the environment can influence the preferred head direction for maximal firing.

51.6

HIPPOCAMPAL PLACE CELLS CAN FIRE DIFFERENTLY IN TWO VISUALLY IDENTICAL LOCATIONS IN A SYMMETRICAL CHAMBER. P.E. Sharp, J.L. Kubie and R.U. Muller.* SUNY, Brooklyn, NY 11203.

The location-specific firing of hippocampal place cells can easily be brought under the control of experimenter-defined cues. Nevertheless, there is evidence that regions of intense firing (firing fields) are not determined just by immediate sensory input, but also by earlier states of the nervous system (O'Keefe & Speakman, 1987). Here, we report on the roles of visual cues and memory in determining the firing of place cells.

Single cell activity was recorded as rats chased food pellets in a gray cylinder with one or two identical cue cards on the wall; when two cards were used, they were placed 180° apart. If place cells are controlled only by sensory input, the firing pattern in the presence of two identical cards might consist of the field seen with one card, and a second field 180° away. Alternatively, since the rat moves freely in the cylinder, the information is available that pairs of visually identical places are not truly the same, and it is possible that the firing pattern will be different in two such places.

Most cells had just one field after the second card was introduced; the field was the same in the presence of either one or two cards. The field position could be flipped 180° by starting the rat out near one card or the other. Two cells showed paired firing fields 180° apart in the positions expected from sessions with a single card. Neither cell fired in a way consistent with exclusive control by cues. In one case, the second field disappeared with repeated exposures to two cards; in the other the second field persisted when only one card was used. We conclude that place cells are influenced both by the immediate sensory configuration and by internal neural states related to earlier experience in the environment.

51.7

THE EFFECTS OF HEAD ORIENTATION ON THE FIRING OF HIPPOCAMPAL PLACE CELLS. Elizabeth Bostock, Jeffrey Taube, and Robert U. Muller*, SUNY-HSCAB, Physiology Dept., Brooklyn, NY 11203.

Place cells are hippocampal complex-spike cells which fire rapidly only in a localized region of the rat's environment ("firing field"). Previous studies of the effect of direction on place cell firing measured direction from the rat's trajectory. For example, McNaughton, Barnes, & O'Keefe (1983) and Leonard, McNaughton, & Barnes (1987) found that place cell firing depends on the rat's movement direction within the firing field on an 8-arm maze, where both the reward location and the trajectories a rat may take are directionally restricted. We examined whether the firing of place cells is necessarily directional by recording from place cells when neither reward nor the set of possible trajectories were directionally restricted. A 2-diode video detection and recording system was used to track the rat's instantaneous head direction and position within a cylinder at 60 Hz. Hippocampal place cells were recorded while rats chased food pellets randomly thrown onto the floor of a cylinder. None of the 14 place cells recorded to date show strong variation of firing with head orientation. Thus, the firing of hippocampal place cells is not necessarily modulated by the rat's direction; directional modulation of place cell firing may be dependent on the structure of the space accessible to the rat, and the polarization of reward contingencies.

51.8

THE HIPPOCAMPUS AND SPATIAL LEARNING: A REAL-TIME ATTENTIONAL-ASSOCIATIVE MODEL. Nestor A. Schmajuk. Department of Psychology. Northwestern University. Evanston, IL 60201.

We applied the S-P-H model (Schmajuk, Soc. Neur. Abs 13: 1100) to the simulation of several spatial learning paradigms. We assumed that hippocampal lesions (HL) and long-term potentiation (LTP) induction affect computations of different variables in the model. In the normal case, the associability of stimulus CS_i with CS_k is given by: $\alpha_i^k = |\lambda^k - B^k|$, where B^k is the aggregate prediction of CS_k, and λ^k represents the intensity of CS_k. In the HL case, associability is given by $\alpha_i^{US} = |\lambda^{US} - \dot{V}_i^{US}|$, where $\dot{V}_i^k$ is the net associative value between CS_i and CS_k. In the LTP case, CS-CS associations are equal to 1. Computer simulations of normal and HL cases were carried out for: acquisition and reversal in a T-maze, acquisition in a water tank, acquisition in a radial-arm maze, and latent learning (cognitive mapping) in a multiple T-maze. Also computer simulations of normal and LTP cases were carried out for acquisition in a radial-arm maze.

51.9

MICROIONTOPHORETIC ATROPINE ELIMINATES FIRING OF HIPPOCAMPAL THETA CELLS. Mark Stewart and Steven E. Fox. SUNY Health Sci. Ctr., Brooklyn, NY 11203.

The medial septal nuclei are essential for the naturally occurring hippocampal theta rhythm. Evidence that the rhythmic activity of the septum is carried via *cholinergic* afferents to the hippocampus has been: a) the existence of a cholinergic septo-hippocampal projection and b) the sensitivity of one type of theta rhythm (type II) to antimuscarinic agents. The muscarinic action of ACh on pyramidal cells, however, is too slow to carry even a 4 Hz signal.

Eight extracellularly recorded hippocampal theta cells (3 CA1, 5 hilus) in urethanized rats were tested with iontophoretic application of atropine. The spontaneous firing of all theta cells tested could be temporarily *eliminated* by iontophoretic atropine (20-100 nA, 10-60 sec, 50 mg/ml in H_2O). At lower doses or during early recovery, the firing rates of the cells were greatly decreased. During this time, action potentials of phasic theta cells were still phase-locked to the ongoing theta rhythm.

This result is consistent with reports of a fast muscarinic excitation of hippocampal and cingulate interneurons, *in vitro* (Benardo & Prince, Brain Res 249: 315, 1982; McCormick & Prince, J Physiol 375: 169, 1986). It also suggests that: a) the cholinergic cells of the septum are, in fact, rhythmic and b) this connection with interneurons is the mechanism by which theta-frequency activity of the septum is transmitted to the hippocampus.

(Supported by NIH NS17095.)

51.10

FUNCTIONAL CONNECTIVITY BETWEEN PAIRS OF MEDIAL SEPTAL NEURONS. L.V. Colom and B.H. Bland. University of Calgary, Department of Psychology, Calgary, Alberta T2N 1N4.

The medial septum/diagonal band nuclei are critical for the generation of hippocampal theta (θ) rhythm in vivo. The study of the discharge patterns of septal cells and their interactions during hippocampal EEG states will aid in the understanding of the mechanisms involved in the generation of θ activity. In order to examine the relationship between septal cells and the production of hippocampal θ, pairs of cells were recorded in the septum from the same microelectrode, during the spontaneous occurrence of θ and irregular activity (LIA) in the hippocampus, using urethane anaesthetized rats. Out of 25 pairs studied, 7 pairs showed a cross-correlation during both θ and LIA, 11 pairs showed a cross-correlation during θ, 1 pair was cross-correlated during LIA and 6 pairs showed no correlation in either state. Six out of the 7 pairs showing the strongest cross-correlations between both the θ and LIA states were comprised of rhythmic cells. Cell pairs maintaining a strong correlation of their spike trains during both synchronized and desynchronized EEG patterns have a stronger functional connectivity than pairs correlated in only one EEG state. We therefore conclude that pairs of rhythmic cells have a special functional connectivity allowing the recruitment and synchronization necessary to produce a strong rhythmic output to the hippocampus.

51.11

INTRINSIC THETA-FREQUENCY MEMBRANE POTENTIAL OSCILLATIONS IN HIPPOCAMPAL NEURONS IN VITRO INDUCED BY DEPOLARIZING CURRENT. L.S.Leung and C.Y.Yim, Depts. Clin. Neurol. Sci. and Physiology, Univ. of Western Ont., London, Canada.

Intracellular recordings were made in 40 CA3 and CA1 neurons in the in vitro hippocampal slice preparation. Sustained (> 10 s) depolarization of the neurons by current injection (0.1-0.5 nA) through the recording electrode produced an initial burst of action potentials at > 50 Hz, followed by rhythmic membrane potential oscillations (MPO) in the 3-11 Hz frequency range in all recorded CA3 and CA1 cells. The depolarized peak of the MPO was typically accompanied by a single action potential, although the MPO may continue for several cycles without action potential discharge. The MPO were larger and of higher frequency at a more depolarized membrane potential. At resting or hyperpolarized membrane potentials, theta-frequency MPO were less obvious. Tetrodotoxin (TTX, 3 μM) abolished the spiking and MPO in 4 cells; in all cells, membrane resistance decreased and spike threshold increased, although a broad, presumably Ca++ spike could still be evoked. Replacement of Ca++ by Mg++ abolished synaptic transmission and attenuated the MPO. It appears MPO arise when the membrane potential is at or near the spiking threshold, and requires (1) a somatic or dendritic sodium-mediated spike (blocked by TTX) and (2) Ca++, possibly Ca++ mediated K+ currents. (Supported by MRC and PSI)

51.12

DISTRIBUTION OF COHERENCE OF MICRO-EEG IN THE HIPPOCAMPUS OF THE RAT ASLEEP AND AWAKE. T.H. Bullock, M.C. McClune*, G. Buzsaki, Dept. of Neurosciences, Univ. of Calif., San Diego, La Jolla, CA 92093

Recordings of ongoing, compound field potentials were made with 16 implanted semi-microelectrodes (A-P) in a row, 0.2 mm apart, either all in the same layer, stratum radiatum of CA1, or obliquely from str. oriens (A-F) to str. radiatum (I-P, H is just below the pyramids), in three behavioral states. Coherence, (COH) as a useful descriptor, was computed for each pair of electrodes in frequency bands from 1-40 Hz. (1) COH falls slightly with frequency in the 1-40 Hz range, but more consistently with distance between electrodes, most steeply at 20-40 Hz. The array in str. radiatum, parallel to the pyramids, shows decline of COH with distance is steeper than in isocortex (unpubl. data), except the widespread theta (8 Hz) band. Plots of COH vs time show stability of the 60s-mean over hours but in the domain of seconds, significant, nonrandom fluctuations, independent in the several bands. (2) The oblique array shows marked discontinuities across hippocampal laminae. Loci A-G within str. oriens and loci L-P in str. radiatum are each highly coherent among themselves. Locus H, just below the pyramidal layer is moderately and nearly uniformly coherent with A-K and less, yet significantly coherent with L-P. A-G have moderate COH with L-P, and less with I-K, the values depending on the frequency band but the above pattern is similar for all. These statements are true for all three conditions, slow wave sleep, paradoxical sleep (PS), and alert walking (W); PS shows the sharpest localization, whereas W in the theta band shows the least. (3) Delta (3 Hz) activity shows a narrow zone of phase lag of 125 deg below the pyramids (locus I) relative to the uniform phase of str. oriens, (A-H), dipping back to 14 deg lag (J) and then rising again over 0.6 mm to 188 deg lag by locus M, maintained through P deep in str. radiatum. Theta and up to 18 Hz shows only a broad zone at and below the pyramids slowly shifting to 120 deg phase lead of str. radiatum over str. oriens.

51.13

POWER SPECTRAL ANALYSIS OF RHYTHMIC FIELD POTENTIALS IN HIPPOCAMPAL SLICE: A MODEL OF THE EEG? J.H. Schneiderman
Univ. of Toronto., Toronto, Canada M4Y 1J3.

Power spectral analysis (PSA) was applied to spontaneous field potentials recorded from the CA3 distal apical dendritic region of 64 guinea pig hippocampal slices. Spectra distinguishable from those recorded from the bathing medium were obtained in 52 slices. The remaining slices were not viable since they did not respond to stimulation or burst in the presence of 3.4 mM Penicillin (PEN). Power was distributed in a diffuse band up to 15-20 Hz, however, in most of the slices was concentrated under 10 Hz. No consistent peaks were identified. Power was comparable to the 60 Hz peak indicating that the signal was largely buried in background noise. Distinct peaks around 6 Hz appeared in .085 to 0.17 mM PEN. Peak power increased and shifted to the left as PEN was raised to 0.51 mM. Spontaneous bursting was always heralded by non-stationary increases in rhythmic activity. Large peaks under 6 Hz developed in slices which burst spontaneously only after a few stimulations. In contrast, power increased in all frequencies in low Mg++ (0.1 to 0.5 mM) or shifted to the right. Concentrations of Mg++ interfering with synaptic transmission reduced the power both in normal medium and in low PEN. PSA revealed low level rhythmic activity responsive to pharmacologic and ionic manipulations which could provide a model for studying the mechanisms underlying the EEG.

51.14

APV, AN N-METHYL-D-ASPARTATE ANTAGONIST BLOCKS HIPPOCAMPAL THETA RHYTHM. D. J. Stewart, L. S. Leung and K. Desborough*. Depts. of Clin. Neuro. Sci. and Physiology, University of Western Ontario, London, Ontario, CANADA.

Recent work has suggested that excitatory amino acids, such as N-methyl-D-aspartate (NMDA), may be involved in normal hippocampal function. In freely behaving rats, intraventricular injection of the NMDA antagonist, 2-aminophosphonovaleric acid (APV; 10, 20, 40 ug) was associated with a dose-dependent ataxia and suppression of hippocampal theta rhythm. APV appeared to be capable of blocking both atropine-sensitive and atropine-resistant components of the theta rhythm. Theta rhythm following systemic atropine sulfate (i.p., 25 mg/kg) was suppressed by APV (20 ug). Similarly, the atropine-sensitive theta rhythm present under urethane anesthesia was also suppressed by APV (20 or 40 ug). However, the delta-frequency slow waves and hippocampal commissural evoked potentials were not affected by APV. The peak action of APV occurred at about 10 minutes after intraventricular infusion and theta rhythm was affected bilaterally after unilateral infusion of APV. The site of action of APV on the theta rhythm may be hippocampal, subcortical (septal) or some combination.

Supported by NSERC and MRC to LSL.

51.15

THE EFFECTS OF EARLY NEST ENVIRONMENT ON THE MATERNAL BEHAVIOR OF RATS WITH FORNIX TRANSECTIONS. B. Osborne, B.C. Woodside*, J.E. Jans* and K.E. Bjorn*. Department of Psychology, Middlebury College, Middlebury, VT 05753 and Department of Psychology, Concordia University, Montreal, Quebec, Canada H4B 1R6.

Total fornix transection results in marked deficits in pup retrieval, nest building and the pattern of mother-litter contact on Day 2 or 7 postpartum but only small and early (Day 0 or 1) effects on pup integrity (Osborne et al, Neurosci. Abst., 13, 1333). In the first experiment we looked at early postpartum maternal behavior following fornix transection. Fornix transected or sham operated nulliparous females were observed 6 hrs. in light and 6 hrs. in dark on Day 0 postpartum. Fornix transected dams exhibited marked differences in mother-litter contact but also severely reduced pup survival.

Since pup retrieval was severely reduced in comparison to our earlier work and the only changes were in early nest environment (dams in the earlier work were in small nest boxes for 48 hrs. postpartum), in the second experiment we manipulated nest environment. Groups received enclosed nest boxes inside observation cages (aided) or observation cages alone (unaided). Again fornix transection disrupted mother-litter contact but fornix transection decreased pup survival only in the unaided condition.

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SECOND MESSENGERS IV

52.1

PHOSPHATIDYLINOSITOL METABOLISM DURING IN VITRO HYPOXIA. H-M. Huang* and G.Gibson (SPON:D. Levy). Cornell University Medical College at the Burke Rehabilitation Center. White Plains, NY 10605

The phosphatidylinositol (PI) cascade transduces numerous signals across cell membranes. Postdecapitative ischemia is known to activate PI metabolism. An in vitro model of these changes would help elucidate the molecular mechanism of these alterations. K^+-stimulated PI turnover was therefore examined in rat brain slices prelabelled with [^{3}H]-inositol. Depolarization (60 mM KCl) increased inositol phosphates (IP and IP_2) formation in a Ca^{2+} dependent manner. The effects of in vitro histotoxic hypoxia (i.e. 0.5 mM KCN) on the K^+ stimulated PI cascade were determined. At early times (10 sec and 1 min), hypoxia enhanced the cascade as shown by increased IP_2 formation. More prolonged hypoxia (10 min) reduced the cascade as shown by diminished formation of IP and IP_2. Hypoxia did not alter the PI cascade under basal conditions. The early stimulation of the cascade may be due to the hypoxic-induced increase in cytosolic free calcium that we previously demonstrated. Negative feedback regulation by protein kinase C may lead to the subsequent impairment of PI metabolism. An altered PI cascade may lead to neurotransmitter changes and tissue damage that accompany hypoxia-ischemia.

52.2

PROTEIN KINASE C FROM RAT BRAIN: CHARACTERIZATION AND INHIBITOR STUDIES. L.G. Mendelsohn, V.L. Lucaites and J.J. Howbert. Lilly Research Labs, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285

The role of protein kinase C (PKC) in signal transduction in response to receptor stimulation and hydrolysis of phosphatidyl inositol (PI) is well established. Many neurotransmitters cause PI breakdown resulting in activation of PKC and reinforcement of the signal or negative feedback. Given the prevalence of PKC in the brain, the study of PKC inhibitors may allow for better understanding of the role of PKC in intercellular communication within the CNS. Partially purified PKC was prepared from soluble EDTA extracts of rat brain homogenate using DEAE-cellulose chromatography. Silver stained SDS-polyacrylamide gels of the enriched fractions show four major bands, the darkest staining of which is found at the molecular weight of PKC (M_r=80,000). The PKC-rich DEAE fraction was enriched about 90-fold; specific enzyme activity from the homogenate to the final fraction increased from 0.24 pmol ^{32}P incorporated/min/ug protein to 21.5 pmol/min/ug protein. A kinase assay using this fraction was adapted to microtiter plates using 20 mM tris-HCl pH 7.4, 750 ug/ml histone Type III, 5 mM magnesium acetate and 20 uM ATP containing ^{32}P-ATP. Phospholipid and Ca^{2+}-independent kinase activity (background) was measured in the presence of 1 mM EGTA. Phosphatidylserine (PS) and Ca^{2+} dependence of the enzyme was established; basal PKC activity was measured as phosphorylation in the presence of PS and Ca^{2+} minus the background activity. Maximum stimulation with phorbol 12,13-dibutyrate (PDBu) was seen with PS and Ca^{2+} concentrations of 2 ug/ml and 3 uM, respectively. Phorbol stimulation of PKC activity was assessed routinely at low PS, Ca^{2+} and PDBu (7.5 nM) levels, and yielded a maximally stimulated response of approximately 3-fold basal levels. The effects of four kinase inhibitors on PKC were examined at several concentrations. Inhibitors used were staurosporine, a microbial alkaloid, the isoquinolinesulfonamides H7 and W7, and the polypeptide Polymyxin B. All inhibitors showed a dose related effect, with staurosporine being the most potent with an IC_{50} of approximately 10 nM.

52.3

PROPERTIES OF A PROTEIN KINASE C ACTIVITY BOUND TO SYNAPTIC PLASMA MEMBRANE (SPM) AND POSTSYNAPTIC DENSITY (PSD) FRACTIONS FROM CANINE CEREBRAL CORTEX. T. Suzuki* and P. Siekevitz (SPON: J. A. Sechzer) Lab. Cell Biol., Rockefeller Univ., New York, NY 10021

Kinase C activity (phosphorylation dependent on Ca^{2+}/phosphatidylserine and Ca^{2+}/phosphatidylserine/phorbol ester) was detected in SPM with 87k Mr, 60k Mr, 50k Mr and 20k Mr as endogenous substrates and histone III-S as exogenous substrate. In PSD, only 20k Mr protein as substrate was detected, but this protein was also phosphorylated by the PSD Ca^{2+}/calmodulin-dependent kinase II. Both the phosphorylations of histone and of endogenous substrates were enhanced in SPM prepared after treatment of brain homogenate with phorbol 12-mysristate 13-acetate (TPA), but very little enhanced in PSD prepared after such TPA treatment. The phosphorylation of histone and endogenous substrates was inhibited by H-7 but not by calmidazolium in PSD and in SPM prepared with and without TPA treatment. A salient finding was that the SPM kinase C activity (both for endogenous proteins and for histone) enhanced by TPA treatment of brain homogenate was inhibited by calcium (IC_{50}, 3 x10^{-7} M). Others have found that TPA treatment causes a translocation of the kinase from the cytosol to a membrane-bound form. Our results suggest that this modified Kinase C may be under physiological control, dependent on the intracellular Ca^{2+} concentration.

52.4

COMPONENTS OF THE IP_3 SECOND MESSENGER SYSTEM IN RAT BRAIN DIFFER IN DEVELOPMENTAL REGULATION AND REGIONAL LOCALIZATION. A.M. Heacock, E.B. Seguin* and B.W. Agranoff. Neuroscience Lab, U of Michigan, Ann Arbor, MI 48104-1687.

In order to explore the mechanism of regulation of the phosphoinositide-linked second messenger system, we have examined the ontogeny and regional localization of three of its components: inositol 1,4,5-trisphosphate (IP_3) phosphatase, IP_3 kinase and IP_3 receptor. Developmental studies of IP_3 metabolism in rat cortex indicate that IP_3 phosphatase activity is relatively high at birth (50% of adult values) and increases to adult levels by 2 wk postnatal. In contrast, IP_3 kinase activity in cortex is low at birth (8% of adult values) and increases gradually, reaching adult levels by 4 wk postnatal. Cortical IP_3 receptor ontogeny appears to parallel that of the IP_3 kinase. In adult rat brain, IP_3 phosphatase activity in cortex, hippocampus, hypothalamus and pons/medulla was similar, whereas activity in the cerebellum was 8 to 10-fold higher. An enrichment of IP_3 receptor in the cerebellum has also been reported. The distribution of IP_3 kinase is markedly different, with similar activities present in cerebellum, cortex and hippocampus and very low activity in hypothalamus and pons. These results suggest that the principal route of IP_3 metabolism and the ability of IP_3 to release stored Ca^{2+} vary depending on both brain region and stage of development. (Supported by MH 42652 and NS 15413.)

52.5

Activation of Phospholipase D Phosphatidyl Transferase Activity by Protein Kinase C in Neural-derived NG108-15 Cells. M. Liscovitch* (SPON: Eli Hazum). Department of Hormone Research, The Weizmann Institute of Science, Rehovot 76100, Israel.

The activation of cellular phospholipase D by protein kinase C (PKC) was examined in NG108-15 cells by measuring the biosynthesis of the abnormal phospholipid phosphatidylethanol (PEt), produced by phospholipase D in the presence of ethanol by trans-phosphatidylation. PEt production depended on stimulation by 12-O-tetradecanoylphorbol-13-acetate (TPA), in a time- and concentration-dependent manner (EC_{50} = 18 nM). The levels of [^{3}H]PEt in TPA-treated cells were directly related to ethanol concentration in the physiologically attainable range (20-100 mM). The effect of TPA on PEt production could be mimicked by 1,2-dioctanoyl-sn-glycerol in a non-additive manner. The effect of TPA was inhibited by the PKC inhibitor H-7 (0.1 mM) by 70% and was abolished in cells in which PKC was down-regulated by pre-treatment of the cells with TPA. In contrast, TPA did not affect the levels of phosphatidic acid in NG108-15 cells. These results indicate that PEt biosynthesis depends largely on activation of PKC. It is proposed that PKC selectively activates the phosphatidyl transferase activity of phospholipase D, reflecting a signal termination mechanism which may be operative in phospholipase D-mediated signal transduction cascades.

52.6

IDENTIFICATION OF FOUR TYPES OF PROTEIN KINASE C IN RAT HIPPOCAMPUS. Bryan L. Roth#, James Hermiller*, David J. Jacobowitz° and Michael J. Iadarola**, #Department of Psychiatry, Stanford University Medical School, Stanford, CA 94305; °Lab Clinical Sciences, NIMH, Bethesda, MD; *Naval Medical Research Institute and **Lab Neurobiol and Anesthesiol, NIDR, Bethesda, MD.

Protein kinase C (PKC) is a calcium and phospholipid-dependent enzyme which is highly enriched in rat hippocampus. Many hormones and neurotransmitters may activate PKC via production of diacylglycerol from phosphoinositide hydrolysis. Activation of PKC induces a wide variety of biochemical actions including ion-channel opening and closing, long-term potentiation and alterations of PI metabolism. Recently several subtypes of PKC have been identified by cloning and sequencing cDNAs; we sought to determine whether 4 of these (γ, βI, βII, and ε) might be present in hippocampus. Northern blot analysis of total RNA from hippocampus disclosed distinct mRNAs for each PKC subtype and suggested preferential splicing of the β-PKC to the βII type. Antibodies directed against the β-PKC disclosed a heterogenous distribution in rat hippocampus with intense labelling of fibers in the s. oriens adjacent to the pyramidal cells as well as in s. radiata. Analysis of micropunches of rat hippocampus (300 µm each) by Western blots and visualization of PKC by antibodies labelled with [^{125}I]-protein A showed the β-PKC to be preferentially located in the CA1 region.

52.7

PHOSPHATIDYLINOSITOL 4-PHOSPHATE (PIP) AND MG^{2+} REGULATE THE ACTIVITY OF A SYNAPTOSOMAL PROTEIN KINASE. O.B. McDonald* and N. Sahyoun. (SPON: G. Pollard). The Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709.

A synaptosomal protein kinase distinct from protein kinase C avidly phosphorylated a major endogenous M_r 87,000 polypeptide. Of a variety of lipid and lipid-derived potential regulators, PIP, PI and phosphatidylglycerol (PG) inhibited the phosphorylation of the M_r 87,000 substrate as well as ^{32}P-incorporation into two less prominent polypeptides with M_r values of 47,000 and 62,000. PIP was about threefold more potent than PI and PG with a K_i value of 0.3 µM at 1.5 mM $MgCl_2$. Unsaturated fatty acids mimicked the effect of PIP at concentrations above 10 µM. The inhibitory effect of PIP was investigated as a function of the concentrations of protein substrate, ATP and $MgCl_2$; the inhibitory mechanism featured a >fortyfold reduction in the affinity for $MgCl_2$ in the presence of 18 µM PIP. The PIP-sensitive protein kinase was released in synaptosomal hypotonic lysates from which the enzyme was purified by anion-exchange, hydroxylapatite and gel-permeation chromatography. The isolation and characterization of this protein kinase suggest a role for PIP and $MgCl_2$ in synaptosomal signalling.

52.8

PROTEIN KINASE C-MEDIATED PHOSPHORYLATON OF P_O IN PERIPHERAL NERVE FROM NORMAL AND DIABETIC RATS C.L. Rowe-Rendleman*, L.N. Berti-Mattera* and I. Eichberg, Dept. Biochem. Biophys. Sci., Univ. of Houston, Houston, TX 77004.

Previous studies from this laboratory have shown that the phosphorylation of the P_O glycoprotein as well as of myelin basic proteins, is increased in sciatic nerve from chronically diabetic rats. We have investigated whether protein kinase C (PKC) is responsible for this effect. As reported by others (Brunden and Poduslo, J. Neurochem. 49, 1863 (1987)), inclusion of phorbol 12,13-dibutyrate (PDB;100 nM) in incubations of desheathed nerve segments increased the phosphorylation of P_O as much as 500%. Phorbol-12-myristate 13-acetate (PMA) stimulated to a lesser extent and the biologically inactive phorbol ester, 4-a-phorbol, was without effect. Two antagonists of PKC, 1-(5-isoquinolinyl-sulfonyl)-2methylpiperazine (H-7) and staurosporine, depressed control phosphorylation levels and largely prevented PDB-mediated stimulation in a dose-dependent manner. Staurosporine (1 µM) also blocked the rise in P_O phosphorylation over basal levels in nerves from diabetic animals, In these nerves, PDB elicited a 50% greater rise in phosphorylation than in normal nerves. This stimulation was also prevented by H-7 (100 µM) and staurosporine. These results point to a role for PKC in the enhanced phosphorylation of P_O and suggest that additional phosphorylation sites on the protein may be available in diabetic nerve. (Supported by NIH grant DK30577 and NSF Fellowship RCD8758057).

52.9

Bradykinin (Bk)-induced activation of peripheral fibres in neonatal rat tail: receptors and second messenger systems.

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The mechanism by which Bk activates nociceptors may involve specific B1 or B2 receptors coupled to neural second messenger systems (Miller, 1987, TINS 10 226). The present study examines these possibilities in Bk induced activation of peripheral fibres of the neonatal rat.

Spinal ventral root depolarization evoked by activation of fibres in the tail was recorded in an in vitro preparation of the neonatal rat spinal cord/tail. Bk but not D-Arg9-Bk evoked a dose dependent response which was selectively antagonised by D-Arg0-Hyp3-Thi,5,8 D-Phe7-Bk. In low calcium perfusates of the tail 4--phorbol 12,13 dibutyrate (PDBu) also depolarized capsaicin sensitive fibres and the effect of PDBu and Bk was selectively antagonised by the protein kinase C inhibitor staurosporine. Neither nitroprusside, forskolin, dibutyryl cyclic AMP or GMP activated peripheral fibres.

These data suggest that protein kinase-C is involved in the stimulation of peripheral fibres by a phorbol ester or by Bk-B2 receptor activation.

52.10

C-KINASE & THE SLOW VOLTAGE DEPENDENT RESPONSE TO SEROTONIN IN A SEROTONERGIC NEURONE (GSN) OF THE SNAIL.

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In the GSN of Helix aspersa, serotonin (5-HT) modulates a slow voltage-dependent K+ current. This occurs via a second messenger system (Barnes, 1984). We used voltage-clamp to investigate the involvement of C-kinase. Effects of activators of C-kinase, down-regulation and an inhibitor were compared with the 5-HT response. Involvement of C-kinase is suggested by (1). OAG (1-oleoyl-2-acetyl-glycerol) (60nM) effected a similar reversible inward current (15-20nA at Vh=-15mV). (2). PDB (phorbol 12, 13-dibutyrate) also effected an inward current (200nM, 10-14nA at Vh= -15mV) which took 5-10 min. to reach maximum. 200nM PDB evoked a current with an average amplitude 67% (n=20) of the control 5-HT response and further application of 5-HT, with PDB, evoked a small response; 800nM PDB fully mimicked the 5-HT response. An additional second messenger is implicated because (1). Experiments to down-regulate the C-kinase by incubation with PDB for up to 24 hr. have failed to abolish completely the 5-HT response, but it was much reduced. (2). Incubation with the C-kinase inhibitor H-7 (1-(5-isoquinolinyl-sulphonyl)-2-methyl-piperazine) for up to 4 hr. (100uM) did not affect the 5-HT response. H-7 did, however, partially reverse the current evoked by PDB.

52.11

NORADRENERGIC POTENTIATION OF EXCITATORY TRANSMITTER ACTION IN CEREBROCORTICAL NEURONS: EVIDENCE FOR MEDIATION BY ALPHA RECEPTOR LINKED ACTIVATION OF PROTEIN KINASE C.

R. D. Mouradian, F. M. Sessler, and B. D. Waterhouse. Dept. of Physiol. and Biophys., Hahnemann Univ., Phila., PA. 19102-1192

Considerable evidence from intact, anesthetized preparations suggests that norepinephrine (NE) may play a modulatory role within local circuitry of the mammalian neocortex; i.e. iontophoretically applied NE or activation of the coeruleocortical pathway is capable of facilitating neuronal responses to synaptic inputs and putative transmitters. The goal of the present study was to further characterize the effects of NE on somatosensory cortical neuronal responses to putative excitatory transmitters using the in vitro tissue slice preparation. Five-barrel glass micropipets were used to record the extracellular activity of individual rat cortical neurons and to iontophoretically apply drug solutions. Somatosensory unit responses to iontophoretic pulses of acetylcholine (ACh) or glutamate (Glu) (10-60 nA; 5-25 sec. duration) were examined before, during, and after NE (1-35 nA) microiontophoresis. Quantitative analysis of peri-event histograms indicated that both Glu- and ACh-evoked excitatory discharges were routinely (Glu 94%, n = 54; ACh 67%, n = 9) potentiated above control levels during NE administration. In 8 cells, NE revealed robust excitatory discharges in response to otherwise subthreshold iontophoretic doses of Glu. The specific alpha antagonist, phentolamine, routinely blocked NE-induced potentiation of responses to threshold doses of Glu (n = 5). Moreover, activation of protein kinase C by iontophoretic application of phorbol 12,13-diacetate (PDA) (5-15 nA) mimicked the potentiating actions of NE on Glu-evoked excitation. In summary, these results demonstrate that NE can facilitate cortical neuronal responses to threshold and subthreshold levels of excitatory transmitter agents. Moreover, unlike noradrenergic augmentation of GABA-induced inhibition, which results from activation of the B-receptor linked cAMP system, these actions appear to be mediated by an alpha-adrenoceptor mechanism which may be linked to intracellular activation of protein kinase C. Overall, these findings reinforce the notion that noradrenergic modulatory actions on excitatory and inhibitory neuronal responses may involve the activation of separate receptor-linked second messenger systems. (Supported by AFOSR-87-0138, NINCDS 18081 to B.D.W.)

52.12

NORADRENERGIC POTENTIATION OF CEREBELLAR PURKINJE CELL RESPONSES TO GABA: PHARMACOLOGIC SPECIFICITY WITH RESPECT TO ADENOSINE ACTIONS AND GABA RECEPTOR SUBTYPES.

B.D. Waterhouse, F.M. Sessler and R.D. Mouradian. Dept. of Physiol. & Biophysics, Hahnemann Univ., PA, 19102-1192.

Previous in vivo studies from our laboratory have consistently shown that iontophoretically applied norepinephrine (NE) can potentiate GABA-induced depressant responses of cerebellar neurons. Other experiments have further suggested that this NE facilitating action is specific for GABA and results from activation of a beta type adrenoceptor which leads to increased intracellular levels of cAMP. The goal of the present studies was to further characterize this action in terms of GABA receptor subtypes and also determine the extent to which adenosine receptor activation might contribute to effects observed with application of 8-bromo 3',5'-cAMP (BcAMP). Extracellularly responses of both in vivo and in vitro cerebellar Purkinje (P) cells to iontophoretic pulses (5-55nA; 10-20 sec. duration) of GABA or baclofen were examined before, during and after NE, BcAMP or adenosine microiontophoresis. Perievent histograms were generated to assess the effects of NE, BcAMP and adenosine on GABA- and baclofen-induced inhibition of spontaneous firing rate. In contrast to results obtained with BcAMP, adenosine was ineffective (12 of 16 cells) in enhancing P-neuron inhibitory responses to GABA. Moreover, neither iontophoretically applied NE nor BcAMP were effective in augmenting baclofen-induced inhibition (5 of 6 cells), suggesting that GABA-B receptors are not directly involved in the potentiation of GABA by NE. However, baclofen was shown to enhance NE-induced potentiation of GABA inhibition (n = 1), suggesting a possible synergistic interaction between NE and GABA-B receptor stimulation in producing this facilitating effect. In summary, these findings suggest that noradrenergic potentiation of GABA-induced inhibition most likely involves changes which target GABA-A receptor mechanisms and that BcAMP-mediated potentiation of GABA does not directly depend upon interactions with the adenosine receptor system. Overall, these results further specify the receptor subtypes and intracellular mediators of NE's potentiating effect on GABAergic synaptic transmission. (Supported by grants AFOSR-87-0138 and NINCDS 18081 to B.D.W.).

52.13

COMPARISON OF MECHANISM OF N-METHYL-D-ASPARTATE AND PHORBOL ESTER INHIBITION OF CARBACHOL-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN RAT HIPPOCAMPAL SLICES.

C.C. Chow, R.A. Morrisett, J.O.McNamara. Duke/VA Med. Ctr., Durham, NC 27705

NMDA and phorbol esters have been demonstrated to inhibit PI hydrolysis stimulated by the muscarinic agonist, carbachol (Baudry et.al.,Nature, 319:329 (1986), Labarca et. al., Biochem. and Biophys. Res. Commun., 123:703 (1984)). The goal of this study was to determine whether NMDA and phorbol esters act through the same or different mechanisms.

PI hydrolysis was measured by determining the synthesis of radiolabelled IP1, using anion exchange chromatography in rat hippocampal slices. Preincubation in phorbol 12,13-diacetate (PDA), followed by rinsing was sufficient to inhibit PI hydrolysis stimulated by subsequent exposure to carbachol (1mM). The PKC activator, PDA, produced 42% inhibition of carbachol-stimulated PI hydrolysis in a dose (IC50=.6uM) and time dependent manner. Inactive phorbol, 4-phorbol, exhibited no significant inhibitory effects. Preincubation with NMDA(100uM) produced a 76% inhibition. H-7, a PKC inhibitor, reversed the inhibitory effects of PDA dose dependently(IC50=13uM). By contrast, H-7 did not block the NMDA inhibition of carbachol-stimulated PI hydrolysis.

The inhibition by active, but not inactive, phorbol ester confirms the work by Labarca et.al.and suggests that activation of PKC exerts a feedback inhibition of muscarinic receptor-activated phospholipase C activity. The reversal of the phorbol ester, but not NMDA, inhibition of H-7 suggests that NMDA acts through a mechanism independent of PKC.

52.14

N-METHYL-D-ASPARTATE RECEPTOR-COUPLED INHIBITION OF PHOSPHOINOSITIDE HYDROLYSIS IS DEPENDENT UPON CHANNEL ACTIVATION.

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Kindled animals exhibit biochemical evidence of enhanced NMDA receptor function as shown by NMDA inhibition of carbachol-stimulated phosphoinositide hydrolysis; this led us to study the mechanism of the NMDA effect.

Hippocampal slices (400 u) were incubated with ^{3}H-inositol, washed, and treated with drugs; ^{3}H-inositol phosphates were isolated by nonpolar extraction and anion exchange chromatography. Preincubation with NMDA followed by rinsing was sufficient to inhibit PI hydrolysis stimulated by subsequent exposure to carbachol. The inhibitory effect of NMDA was dose-dependent (IC_{50}= 30 uM), maximal by 15 min, persisted unchanged for at least 3 hours, was inhibited by D-APV (100 uM) and the NMDA channel blockers, MK-801 (10 uM) and TCP (1 uM). Omission of Na^{+}, but not Ca^{++}, from the NMDA incubation prevented the inhibitory effect of NMDA.

These results demonstrate that NMDA receptor activation can produce a long-lasting inhibition of carbachol-stimulated PI hydrolysis by activation of the NMDA ion channel, an effect likely dependent upon influx of Na^{+} but not Ca^{++}. Control of muscarinic-receptor coupled PI hydrolysis by NMDA channel activation maybe one molecular means by which brief membrane events are translated into long-lasting modifications of neuronal function.

52.15

INHIBITION OF PROTEIN KINASE C BY MAST CELL DEGRANULATING PEPTIDE. H. Nakabayashi, P. Lazarovici and K.-P. Huang (SPON: W. Tsai) NICHD, NIH, Bethesda, MD 20892.

Mast cell degranulating peptide (MCD) is a polycationic bee venom toxin known to have a potent mast cell degranulating action and convulsion action for the central nervous system. In synaptic membranes, high affinity receptor site for MCD has been identified; however, the function of this receptor and its signaling mechanism is unknown. The effect of MCD on protein kinase C (PKC) isozymes (type I, II, and III) were investigated. Ca^{2+}/phosphatidylserine (PS) and diacylglycerol (DG)-dependent activities of these isozymes were inhibited to different degree by MCD, with an inhibitory response of type I>III>II. Apamin, analogous peptide of MCD, was less potent to inhibit these PKCs. MCD appears to be a specific inhibitor for PKC, it did not inhibit cAMP-dependent kinase, myosin light chain kinase (MLCK), nor casein kinase I and II. In comparison, mastoparan and melittin inhibited PKC as well as MLCK. The activity of PKC measured with protamine sulfate as substrate which is cofactor independent and that of the protease-activated PKC, M kinase, were not affected by MCD. The mechanism of inhibition of PKC by MCD appeared to be complex; the inhibitory response is biphasic and substrate dependent. Increasing concentrations of PS or DG counteracted the inhibition of type I and III kinase by MCD more effectively than that for the type II enzyme. MCD also inhibited the binding of [^{3}H]phorbol 12,13-dibutyrate (PDBu) to the kinase. Ca^{2+}/phospholipid-dependent protein phosphorylation of rat brain proteins in vitro were also inhibited by MCD. These results suggest the possibility that the neurotoxicity of MCD may be mediated by the inhibition of PKC.

52.16

MECHANISMS OF NEUROPEPTIDE INHIBITION OF PROTEIN KINASE C. V.J. Aloyo, M. A. Pullen* and R.F. Walker*. Dept. of Pharmacology, Medical College of PA, Philadelphia, PA 19129.

Several neuropeptides including ACTH, dynorphin and somatostatin are inhibitors of protein kinase C (PKC)-dependent phosphorylation of B-50 (also known as GAP43 and F1) in neuronal membranes. Furthermore, we have previously shown that ACTH-(1-24) is an inhibitor of partially purified PKC. However, the mechanism(s) by which these peptides effect PKC activity is unknown. Thus, we have investigated the kinetics of PKC inhibition by several peptides as well as the prototypical PKC inhibitor, H7. We have found that ACTH-(1-24) and dynorphin A are inhibitors of PKC independent of whether histone or myelin basic protein is used as the phosphate acceptor. However, the potent somatostatin analogue, [D-trp8]-somatostatin, did not inhibit PKC activity even at 100 uM, suggesting that this peptide inhibits neuronal B-50 phosphorylation by an indirect mechanism. Kinetic analysis demonstrated that although ACTH-(1-24) and H7 are equipotent inhibitors of PKC, they act by different mechanisms. We have confirmed that H7 is competitive with respect to ATP and noncompetitive with respect to phosphate acceptor. In contrast, ACTH-(1-24) is noncompetitive with respect to ATP but competitive with respect to phosphate acceptor. Although these results suggest that ACTH may be a phosphate acceptor for PKC, this was not the case under our assay conditions. Further insight into the mechanism of ACTH-induced inhibition of PKC was obtained from order of addition experiments. Preincubation of ACTH-(1-24) with the phospholipid cofactors before addition of histone resulted in significantly greater inhibition of PKC activity when compared to adding the peptide last. These results suggest that ACTH interacts with the phospholipids in order to affect PKC activity. These experiments lend further evidence to the hypothesis that neuropeptides may regulate the signal transducing activity of PKC.

SECOND MESSENGERS V

53.1

GLUTAMATE INDUCES 12-HETE SYNTHESIS THROUGH NMDA RECEPTOR ACTIVATION IN RAT CEREBRAL CORTEX. L. Pellerin* and L.S. Wolfe. Donner Lab. of Exp. Neurochemistry, Montreal Neurological Institute, Montreal, Canada H3A 2B4.

Recent evidence (Piomelli et al., Nature, 328:38, 1987) suggests that 12-hydroxyeicosatetraenoic acid (12-HETE) or its hydroperoxy presursor (12-HPETE) act as a second messanger in Aplysia sensory neurons. Formed by receptor-mediated stimulation, they have been shown to activate a specific K^+ channel in these cells. We believe that in CNS of mammals, lipoxygenase metabolites might play a similar neuromodulatory role. We had previously found that rat cerebral cortex has the capacity to form at least four different hydroxyeicosatetraenoic acids, 5-, 11-, 12- and 15-HETE, with 12-HETE being the major one. However, only 12-HETE was found to be increased by raising intracellular Ca^{2+} concentration with the Ca^{2+} ionophore A23187 (10 M). Different neurotransmitters were then tested for their capacity to induce 12-HETE formation. Glutamate (100 M) and norepinephrine (100 M) induced a two-fold increase in 12-HETE level while other neurotransmitters were ineffective or even had a slightly inhibitory effect. Norepinephrine seemed to act through alpha receptors because isoproterenol, a beta adrenergic agonist, was found to be ineffective. N-methyl-d-aspartate (NMDA) and kainate, two glutamate receptor agonists, were assayed and only NMDA was found to induce 12-HETE synthesis. This points to a specific role for a 12-lipoxygenase metabolite in phenomenon linked to NMDA receptor activation, such as long term potentiation.

Supported by a grant from the MRC Council of Canada. L.Pellerin is a NSERC scholar.

53.2

POSSIBLE ROLES FOR ARACHIDONIC ACID METABOLISM IN THE PHYSIOLOGY AND PATHOPHYSIOLOGY OF CEREBELLAR GRANULE CELLS IN CULTURE.

K.S. Madden, A.E. Schaffner, P. Lysko* and J.L. Barker (SPON: M. Freed). Laboratory of Neurophysiology and *Laboratory of Molecular Biology, NINCDS, NIH, Bethesda, MD 20892.

Cerebellar granule cells from the neonatal rat are commonly raised as primary cultures and under conditions that promote the development of nearly homogeneous (>95%) populations. The advantages of this preparation have now been exploited in standard biochemical studies about the spontaneous and evoked conversion of 1-^{14}C-arachidonic acid (AA) to more polar derivatives. Tests of evoked conversion included exposure to saline that was unmodified or modified by the additions of extra sodium or potassium ions (20 mM) or by glutamate (3-500 µM). High doses of glutamate are neurotoxic and associated with conditions that also increase AA metabolism in the central nervous system, such as cerebral ischemia and epilepsy. As controls for cell death, preparations were also challenged with hypo-osmotic shock or mechanical stress. These last two treatments simulate the effects of positioning the glass microelectrodes needed from electrophysiological recordings from individual granule cells and the cell death that can occur during those studies. The results support a role for AA metabolism in the events of signal transduction in granule cells. This role appears to involve the release of di- and/or monoacylglycerols from cells, probably owing to the turnover of phosphatidylinositol. Since this release is not seen after the integrity of cell membranes is destroyed, it is likely to be a physiological, not a pathophysiological, response.

KSM is an NAS/NRC Research Associate.

53.3

APLYSIA NERVOUS TISSUE CONTAINS PROTEINS THAT INHIBIT OR ACTIVATE PHOSPHOLIPASE A_2 (PLA_2). A. Calignano*, D. Piomelli, B. Wallner* and J.H. Schwartz, HHMI, Columbia Univ. Col. Phys. & Surg., New York, NY 10032 and Biogen, Cambridge, MA 02142.

Release of arachidonic acid from membrane phospholipids is the limiting step in the biosynthesis of eicosanoids, which may be second messengers in Aplysia neurons. We find that PLA_2, an enzyme that participates in arachidonate release, is modulated by proteins in homogenates of Aplysia nervous tissue which were fractionated by size-exclusion HPLC. We detect three heat-stable inhibitory components (M_r app 66,000, 40,000 and 15,000), suggesting the presence of lipocortin-like proteins. Preliminary Western blots with anti-human lipocortin antibodies reveal cross-reacting Aplysia proteins, and Northern blots, possible Aplysia lipocortin transcripts. We also find an M_r 29,000 component that stimulates PLA_2. This component did not contain intrinsic PLA_2; its stimulatory activity is destroyed by boiling and trypsin. Expression of proteins that modulate PLA_2 may be important for long-term regulation of synaptic plasticity in Aplysia.

53.4

SECOND MESSENGER SYSTEMS IN K^+-DEPOLARIZATION OF RAT BRAIN SYNAPTOSOMES. T-N Lin*, M Navidi*, RA MacQuarrie*[1], AY Sun and GY Sun. Sinclair Comp. Med. Res. Farm and Biochem, Dept., Univ. of MO, Columbia, MO 65203, and [1]School of Basic Life Sciences, Univ. of MO-KC, Kansas City, MO 64110.

To elucidate the role of second messenger production through phospholipases A_2 (PLA_2) and C (PLC) in the neurotransmitter release process, intracerebral injection, preincubation and incubation with exogenous substrates were employed for labeling phospholipids in synaptosomes. When synaptosomes were prelabeled with [^{14}C]arachidonic acid (AA) through the acyltransferase system, K^+-depolarization increased the release of labeled AA from phosphatidylcholines (PC) and phosphatidylinositols (PI). Under the same condition, there was an increase in transfer of labeled AA to PI and PC suggesting that the deacylation-reacylation mechanism mediated by PLA_2 and acyltransferases may be involved in the neurotransmitter release process. Attempts to test for K^+-stimulation of polyphosphoinositide breakdown by PLC, either by generating labeled substrate endogenously or adding labeled substrates exogenously, have produced mostly negative results which seem to indicate that the PLA_2 system may prevail over the PLC system. (Supported in part by NSF grant BNS8419063)

53.5

UNSATURATED FATTY ACIDS AND LYSOPHOSPHATIDYLINOSITOL (LYSO PI) ACTIVATE A CATIONIC RAT BRAIN PROTEIN KINASE.
M. Frangakis* and N. Sahyoun. (SPON: B. Cooper). The Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709.

A search for a second messenger role of phospholipase A_2 hydrolytic products led to the isolation by CM-cellulose chromatography of an arachidonic-acid dependent protein kinase. The enzyme phosphorylated several endogenous substrates of which synapsin I was most prominent. The arachidonate effect was observed at concentrations above 6.6 μM with an apparent Km of 89 μM. Oleic acid had a similar effect but was inhibitory at higher concentrations. Lyso PI was also discovered to be an effective activator with peak stimulation at about 20 μM. The effects of unsaturated fatty acids or lyso PI were inhibited by 50 μM trifluoperazine. Interestingly, Ca^{2+}-calmodulin could partially replace the lipid activators, and this property was exploited to purify the protein kinase by calmodulin affinity chromatography. The purification and analysis of the activity of this protein kinase provide a regulatory target for unsaturated fatty acids, and lysophospholipids as well as Ca^{2+}-calmodulin. Elevated Ca^{2+} levels may activate this protein kinase by interaction with calmodulin and by phospholipase A_2 activation.

53.6

HEPOXILIN MAY BE A SECOND MESSENGER IN RAT HIPPOCAMPAL CA1 NEURONS: AN ELECTROPHYSIOLOGICAL STUDY.
N.Gurevich, P.H. Wu*, C.R. Pace-Asciak*, and P.L. Carlen, The Playfair Neurosciences Unit, Toronto Western Hospital, Addiction Research Foundation, Dept. Medicine, Physiology & Pharmacology, The Hospital for Sick Children, University of Toronto, Canada

Hepoxilin (HX) is a hydroxy epoxide derivative of arachidonic acid (AA) and is synthesized from 12-HPETE. It is formed in the rat brain. Hippocampal slices(400μm) from male Wistar rats(150-170g) were used for intracellular electrophysiological recordings by glass electrodes (3M KCl or 3M K-acetate; 60 to 150 Meg Ohm). HX(0.5 to 100μM),AA(5 to 100μM) and other AA metabolites were drop-applied or perfused onto the hippocampal CA1 neurons.

With HX perfusion, a hyperpolarization (< 4mV) developed gradually over a 7 min period. Drop application caused a small(<2mV) hyperpolarization within 10 to 20 sec, lasting up to 40 sec. The afterhyperpolarization (AHP), which is thought to be due to a Ca^{2+}-mediated K^+ conductance, was prolonged up to 50% by perfusion and up to 25% by drop application. EPSPs were not affected. However, a marked enhancement of the IPSP(up to 2-fold) was observed. This increase included both early chloride-dependent and the later K^+-dependent phase of the IPSP. These results suggest that HX may play a second messenger role in the mammalian CNS. Supported by MRC.

53.7

INCREASED MOUSE BRAIN FATTY ACID CYCLOOXYGENASE (CO) ACTIVITY FOLLOWING INCUBATION WITH PROTEIN KINASE (PK)-C.
Thomas W. Lysz and Philip E. Keeting, Dept. of Surgery, New Jersey Med. Sch. Newark, N.J. 07103 and East Orange Veteran's Administration, East Orange, N.J.,07109.

Recent evidence suggests that the brain CO activity is increased following drug induced convulsions (T.W. Lysz et al., Brain Res.408 (1987)6-12. To determine a biochemical mechanism underlying the increased CO activity, brain microsomes prepared from Swiss Webster mice were incubated under different phosphorylating conditions. Initial experiments indicated that microsomes prepared in 0.1M Tris buffer pH7.4 and incubated with 24 uM arachidonic acid (AA), 1 mM glutathione, and 1 mM epinephrine generated between 58 to 65% less PGE2 and PGF2 alpha (measured by RIA) then microsomes incubated in 0.1M phosphate buffer (N=6). The addition of 1 mM ATP,10 mM Mg,10 uM c-AMP and 10 ug c-AMP dependent PK-A to the CO in Tris increased prostaglandin (PG) production only 13%(n=6). However, microsomes incubated with purified rat brain PK-C, diolein, ATP,Ca, and phospatidylserine in Tris, increased total PG synthesis 50 %(N=2). These results suggest that a secondary control point may exist in the AA cascade at the CO level.Supported by a VA Merit Award and NIH grant 529621.

ION CHANNELS: CALCIUM CHANNELS I

54.1

MODULATION OF CALCIUM CURRENTS OF IDENTIFIED DORSAL HORN PROJECTION NEURONS BY GUANINE NUCLEOTIDES.
L.-Y. M. Huang. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

Different types of calcium channels have been identified in acutely isolated, identified rat spinal dorsal horn projection neurons. They are low threshold transient, high threshold inactivating and non-inactivating Ca channels. The effects of intracellularly applied guanosine 5'-O-3-thiotriphosphate (GTP-γS) and guanosine 5'-O-2 thiodiphosphate (GDP-βS) on these Ca channels were studied in the projection neurons using whole cell patch clamp technique. GTP-γS markedly slowed the activation and inactivation of high threshold Ca current. The rate of activation was increased by about 10-fold, and the rate of inactivation was lengthened by about 15- to 20-fold. The effects of GTP-γS were abolished when the cells were treated with pertussis toxin. When GDP-βS was in the pipet solution, the rates of activation and inactivation of the Ca current were similar to those in the control cells. Cyclic AMP and forskolin did not mimic the effects of GTP-γS. These results suggest that guanine nucleotide binding protein modulates directly the activities of Ca channels in identified projection neurons. This work is supported by NS 23061 and NS 01050.

54.2

MECHANISM OF INHIBITION OF CA CURRENTS BY G-PROTEIN ACTIVATION
A.C.Dolphin, S.M. McGuirk* and R.H. Scott* Dept Pharmacology, St. Georges H. M. S., London SW17ORE, UK.

Activation of several receptors (including GABA-B) inhibits Ca currents in cultured rat dorsal root ganglion neurones, and this coupling occurs via a G-protein. The GTP analogue GTPγS also inhibits Ca currents, selectively abolishing the transient component. The remaining current is more slowly activating and sustained, and is distinguished from a control L-type Ca current activated from V_H -30mV by its much slower inactivation. The effect of GTPγS is seen using either Ba or Ca as the charge carrier, and is blocked by pertussis toxin. We have investigated whether a second messenger is involved in the response of Ca and Ba currents to GTPγS. Activation of adenylate cyclase by forskolin (10μM) or inclusion of 50μM cyclic AMP in the patch pipette had no effect on the response to GTP-γ-S. Three inhibitors of protein kinase C: polymixin B (20μM), H7 (50μM) and staurosporine (100nM) were also without effect. Bradykinin (0.1-1μM) stimulated inositol phosphate production in these cultures by 70±14% (mean± SEM,n=8)in 30s, whereas stimulation by the GABA-B agonist (-)-baclofen (100uM) was 22±2% (7). However, baclofen inhibited Ca and Ba currents by 40-70%, whereas bradykinin did not. Arachidonic acid (100μM), applied for up to 5 min., had no effect on Ba currents. These experiments provide no evidence for a second messenger coupling pertussis toxin sensitive G-proteins to Ca channels.

54.3

DIFFERENTIAL BLOCKING ACTIONS OF CHLORPROMAZINE ON SODIUM AND CALCIUM CHANNELS IN CULTURED NEUROBLASTOMA CELLS. Nobukuni Ogata* and Toshio Narahashi (SPON: J. Trubatch). Department of Pharmacology, Northwestern University Medical School, Chicago, IL 60611.

Chlorpromazine (CPZ), a major tranquilizer, affects the excitability of nerve membranes. However, little is known about the mechanism underlying this action. We investigated the effect of CPZ on voltage-gated sodium and calcium channels in cultured mouse neuroblastoma (N1E-115) cells using the whole cell patch clamp technique. CPZ in micromolar concentrations reduced both the sodium and calcium currents. The block was reversible, concentration-dependent, and enhanced by shifting the holding potential toward positive values. The block of sodium channels exhibited a marked use dependence during repetitive stimuli. On the contrary, the block of calcium channels did not appreciably accumulate during repetitive stimuli over a wide range of frequencies used. The lack of apparent use dependence of the calcium channel block was due to a slow onset of the block in the inactivated state and a rapid recovery from this block. This difference between calcium and sodium channels in frequency modulation of the CPZ block might be responsible for certain aspects of the therapeutic as well as the toxic actions of this drug (supported by NIH grant NS14144).

54.4

ROLE OF CALCIUM MODULATORS IN K^+ - STIMULATED NEUROTRANSMITTER RELEASE. V.C. Gandhi and D.J. Jones (Spon: S. Dalterio). Dept. of Anesth. Univ. Texas Health Sc. Cen., San Antonio, TX 78284.

Calcium channel modulators were investigated for their effects on K^+ - stimulated [^{3}H]5HT as well as [^{3}H]NE release in rat spinal cord preparations. Bay K 8644, at 1 uM augmented K^+ - stimulated release of both [^{3}H]5HT and [^{3}H]NE by about 50% without affecting basal release. Dihydropyridine Ca^{++} antagonist nimodipine at 1 uM had no independent effect on K^+ - stimulated release, but blocked the augmentation induced by Bay K 8644.

Quinacrine, a non-dihydropyridine compound produced differential effects on the K^+ - stimulated [^{3}H]NE and [^{3}H]5HT releases. It produced a dose - dependent inhibition (0.1um - 10um) of K^+ - stimulated [^{3}H]NE release with no change in basal release. At 1 uM it also completely antagonized the K^+ - stimulated elevation of [^{3}H]NE release produced by 1 uM Bay K 8644. A large dose-dependent increase in basal [^{3}H]5HT release was produced by quinacrine. This differential effect on the 5HT and NE release pathways may be due to differences in intracellular mediation caused by its other properties.

54.5

INTRACELLULAR AND EXTRACELLULAR MOBILIZATION OF Ca^{2+} IN GUINEA-PIG TRACHEA ARE AFFECTED BY DIHYDROPYRIDINES. **R.R.Ben-Harari*, B.Dalton*, J.Kaplan* and S.Maayani. (SPON: Yahr,M.D.).Department of Anesthesiology, Mt.Sinai Sch. Med.,CUNY, N.Y., N.Y.10029.**

Ca^{2+} blockers have relatively weak effects in the treatment of asthma compared with vascular disorders. This difference may be dependent on the source of Ca^{2+} for contraction. To test this hypothesis, effects of the Ca^{2+} channel activator, Bay K 8644(10uM) and blocker, nifedipine (1uM), were determined on the kinetics of the phasic (intracellular Ca^{2+}) and tonic (extracellular Ca^{2+}) components of the contractile responses to histamine (HA;10uM) and carbachol (Carb;1uM) in the isolated guinea-pig trachea. The experiments were performed in the presence of 1uM indomethacin otherwise basal tension was lost and no phasic response could be generated. Nifedipine eliminated the phasic response. Bay K 8644 increased peak tension but had no affect on the rate of decay of the phasic response. Nifedipine and Bay K 8644 had no affect on peak tension and nifedipine inhibited the rate of generation of the tonic response. Effects of Bay K 8644 and nifedipine were mutually antagonistic. The results suggest that in airway smooth muscle, contractions to HA and Carb mobilize both intracellular and extracellular sources of Ca^{2+}, and that dihydropyridines affect both. (Supported by USPH GM34852)

54.6

BLOCKADE OF DROSOPHILA NEURONAL CALCIUM CURRENT BY TOXINS PURIFIED FROM SPIDER VENOM Hung-Tat Leung*^, Dale Branton, Heidi Phillips*, Lily Jan, and Lou Byerly^. Howard Hughes Medical Institute, UCSF, San Francisco, CA 94143; ^Section of Neurobiology, USC, LA, CA 90089-0371.

The study of ion channels is greatly facilitated by the use of highly specific channel blockers. Dihydropyridines and ω-conotoxin, which are specific blockers for certain vertebrate calcium channels, do not appear to be effective against invertebrate calcium channels. In an effort to identify specific blockers for voltage-activated calcium channels in Drosophila, toxins were purified from Hololena and Plectreurys spider venoms (Bower at al., 1987 and Branton et al., 1987) which block synaptic transmission at the Drosophila larval neuromuscular junction (NMJ). We have now tested these toxins on the Ca current of cultured embryonic Drosophila neurons studied with the whole-cell patch clamp technique. Toxin fractions found to be both active and inactive in blocking the NMJ were tested. A blind study of three fractions from Hololena toxin found that the inactive fraction had no effect while the two active fractions blocked about 50% of the Ca current. A blind study of three fractions from Plectreurys toxin showed that the active fraction blocked 80% or more of the Ca current; the two inactive fractions had no effect in 15 out of 17 trials. These results show that the spider toxins act directly or indirectly to block Drosophila Ca channels.

54.7

AMINOGLYCOSIDE INHIBITION OF OMEGA CONOTOXIN BINDING AND THE ELECTRICALLY INDUCED VAS DEFERENS TWITCH RESPONSE ARE CORRELATED. B.A. Meiners, R. Keith*, R. Stumpo, D. LaMont‡ and A.I. Salama. Department of Pharmacology, ICI Pharmaceuticals Group, ICI Americas, Inc., Wilmington, DE 19897.

The peptide ω-conotoxin GVIA (WCT) inhibits N-type voltage-sensitive calcium channels (VSCC). Furthermore, it has recently been reported that some aminoglycoside antibiotics inhibit the binding of the [125I]WCT (Knaus et al., Naum-Schm. Arch. Pharmacol, 336, 583, 1987. We sought to further profile the effects of these antibiotics by comparing their potency in the electrically stimulated rat vas deferens preparation, an index of neuronal (presynaptic) N-channel function, with their inhibition of [125I]WCT binding. Surprisingly, at low concentrations (100 nM) neomycin caused an enhancement of [125I]WCT binding to levels 30 to 60% over control which appeared to be due to an increase in association rate. The rank order for inhibition of binding and of inhibition of the twitch response was the same for the compounds tested eg WCT > neomycin > gentamycin > kanamycin. The compounds were much more potent against the binding than against the twitch response. For example, the IC_{50} of neomycin in binding was 4 μM and in the twitch response was 300 μM. The observed correlation between the binding and the functional in vitro model supports the hypothesis that aminoglycoside antibiotics can interact with the N-type VSCC in a functionally relevant way.

54.8

DIFFERENTIAL SENSITIVITY OF SYNAPTOSOMAL CALCIUM ENTRY AND ENDOGENOUS DOPAMINE RELEASE TO OMEGA-CONOTOXIN. J.J. Woodward and S.W. Leslie. Division of Pharmacology, College of Pharmacy, University of Texas at Austin, Austin, TX 78712.

The presynaptic neurotoxin omega-conotoxin (w-CgTx) was tested for its ability to inhibit voltage-dependent calcium flux and transmitter release in rat brain synaptosomes. Synaptosomes were prepared from the striata of 2-3 month old Sprague-Dawley rats and were incubated in calcium-free medium with conotoxin (0.001-10 uM). Calcium uptake ($^{45}Ca^{2+}$) and endogenous dopamine release were measured during a three second period with either resting (5 mM KCl) or depolarization (60 mM KCl) medium (100 uM free calcium). Calcium influx was determined using liquid scintillation counting. Dopamine was measured using liquid chromatography with electrochemical detection. Conotoxin (0.001-10 uM) had no effect on calcium uptake or endogenous dopamine release from rat striatal synaptosomes under resting (5 mM KCl) conditions. Net potassium-stimulated calcium influx was partially (20-30%) inhibited by conotoxin at 10 uM. The net release of endogenous dopamine was inhibited by 25% at 0.01 uM and by 60% at 10 uM. These results suggest that a relatively small number of w-CgTx sensitive calcium channels may be linked to the release of synaptosomal dopamine. The majority of synaptosomal calcium influx and dopamine release appears to involve calcium channels insensitive to w-CgTx.

54.9

NIMODIPINE BLOCK OF L-TYPE CALCIUM CHANNELS IN FRESHLY DISPERSED RABBIT DRG NEURONS. H.K. FRY* AND R.T. MC CARTHY*. (SPON: A. Howard) Miles Institute for Preclinical Pharmacology, West Haven, CT 06516, USA

Nimodipine, a 1,4-dihydropyridine, is a potent cerebral vasodilator with beneficial therapeutic effects in aiding recovery from global and focal ischemia. The patch voltage clamp technique has been used to study nimodipine block of voltage-dependent calcium channels in dorsal root ganglion cells(DRG). In whole-cell recordings, a rapidly inactivating T-type channel current can be elicited with weakly depolarizing test pulses from hyperpolarized potentials. This current is almost completely inactivated at potentials positive to -55mV. A second whole-cell current (L-type) remains available at relatively depolarized holding potentials and features little inactivation during test depolarizations (200mS). In cell-attached patch, L-type channels display a slope conductance of 29pS and respond to BAY K 8644 (50nM) with prolonged single channel openings. Nimodipine (20, 200nM) reduces the whole-cell inward current from each of two different holding potentials (V_H=-90 & -55mV) with substantial resting state block ($K_D^H \cong$40nM). In cell-attached patches (V_H=-40mV), nimodipine (50 nM) increased (>3X) the number of null sweeps; hyperpolarization (V_H=-80mV) reversed this block. Direct action of nimodipine on neuronal L-type channels may account for some of its therapeutic effects.

54.10

BLOCKADE OF CALCIUM CHANNELS IN PC12 CELLS

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The persistent depolarization induced by application of high potassium to undifferentiated PC12 cells is accompanied by a biphasic increase in cytosolic calcium as assessed by Fura-2 measurements. The early component of intracellular calcium concentration increase is blocked by -conotoxin whereas the later component is readily abolished by verapamil.

Patch clamp experiments using the whole cell mode, revealed a large (usually 300 pA) calcium current after strong cell depolarizatio from cell resting potential following blockade of potassium currents with intracellular CsCl. Multiple exponential fits of current decay revealed two different time constants. Verapamil (20 uM) was found to selectively abolish the longer lasting current decay.

Our results suggest the existence of at least two different calcium channels in undifferentiated PC12 cells.

54.11

NALOXONE ANTAGONISM OF THE ETHANOL INHIBITION OF CALCIUM CHANNELS IN NG108-15 CELLS. M.D. Herman*, D.A. Twombly*, C.H. Kye*, T. Narahashi. Dept. of Pharmacology, Northwestern Univ. Medical School, Chicago, IL 60611

The effects of ethanol on the nervous system have been studied for many years, yet the precise mechanism of action remains to be seen. Studies have suggested that the cellular activity of ethanol is mediated by the delta-opiate receptor, and that voltage-activated calcium channels are blocked by ethanol. Using the whole cell patch clamp technique, we tested the hypothesis that the delta-opiate receptor mediates the block of calcium channels by ethanol. Ethanol blocked two types of calcium channel currents in neuroblastoma-glioma hybrid cells (NG108-15). Both the transient (type I) component of the calcium channel current and the long-lasting (type II) component were blocked by ethanol. The degrees of block of type I channels at concentrations of 30 mM, 100 mM, and 300 mM were 3%, 19%, and 34%, respectively, and those of type II channels were 13%, 23%, and 32%, respectively. In the presence of 1μM naloxone, ethanol at a concentration of 100 mM had no effect on the type II calcium channels. In contrast to the type II channel effect, naloxone did not antagonize the type I calcium channel block by ethanol.

These results indicate that the block of type II calcium channels by ethanol appears to be mediated by the delta-opiate receptor. Although the inhibition of type I calcium channel currents by ethanol occurs at similar doses to type II calcium channel block, a different mechanism is indicated by the absence of antagonism by naloxone. The delta-opiate receptor mediated block of type II calcium channels may explain the inhibition of transmitter release by ethanol.

54.12

CALCIUM CURRENTS IN DENTATE GYRUS GRANULE CELLS AFTER KINDLING. J.N. Reynolds, I. Mody, M.W. Salter, P.L. Carlen & J.F. MacDonald. Playfair Neurosci. Unit, The Toronto Hosp.& Dept. Physiol., Univ. of Toronto, Toronto, Ont., M5T 2S8

We have investigated voltage-dependent Ca^{2+} currents in granule cells of the dentate gyrus in hippocampal slices obtained from control and commissurally kindled (stage 5) Wistar rats. Neurons were voltage-clamped using the single electrode switching clamp technique (Axoclamp 2A; 4-7 kHz). Electrodes (40-80 MΩ) were filled with 3-M-CsCl or 2-M-CsCl/0.5-M-CsOH/0.25-M-EGTA/10-mM-HEPES. The perfusion media included (in mM): 114 NaCl, 4.25 KCl, 26 $NaHCO_3$, 4 $CaCl_2$, 2 $MgCl_2$, 10 TEA, 5 4-AP, 3 CsCl, 10 d-glucose and 1 μM TTX.

Control granule cells displayed both a transient and a sustained Ca^{2+} current with distinct voltage-dependent properties. In contrast, in kindled granule cells the threshold for the transient Ca^{2+} current was lowered by 10 mV while the sustained Ca^{2+} current was significantly depressed. This depression was not due to an absence of Ca^{2+} channels since intracellular injections of EGTA revealed sustained Ca^{2+} currents in kindled neurons, while having little effect on control cells. Thus, granule cells of the dentate gyrus show marked changes in voltage-dependent Ca^{2+} currents following kindling. These changes may be a consequence of an altered intraneuronal Ca^{2+} homeostasis in kindled granule cells.

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54.13

EFFECT OF IONIZING RADIATION ON CALCIUM CHANNELS IN RAT BRAIN SYNAPTOSOMES. S. B. Kandasamy and W. A. Hunt*. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Exposure to ionizing radiation has been shown to reduce voltage-dependent sodium uptake in rat brain synaptosomes. Since sodium influx can be a stimulus for calcium influx needed for neurotransmitter release, the effect of ionizing radiation on voltage-dependent calcium influx was studied by measuring KCl-stimulated Ca-45 uptake into rat brain synaptosomes. Gamma irradiation (Co-60) (1-100 Gy) reduced calcium uptake after 3 sec in a dose-dependent manner. Non-stimulated calcium uptake was unaffected by radiation exposure. The time course (3, 10, 30 and 60 sec) of calcium uptake by irradiated (3 Gy) and non-irradiated synaptosomes was also measured as well as the effect of variable doses of KCl (15-65 mM). The fastest and highest rate of depolarization-dependent calcium uptake occurred at 3 sec and at 65 mM KCl. Irradiation reduced calcium uptake at all incubation times and KCl concentrations studied. Inositol-1,4,5-trisphosphate (1 nM-1 μM) and prostaglandin F2α (1 nM-1 μM) increased the entry of calcium in non-irradiated but had no effect on irradiated synaptosomes. Since researchers have linked protein kinase C to the opening or closing of ion channels, irradiation might have affected this enzyme, possibly through the lack of mobilization of calcium from intracellular stores.

54.14

EFFECT OF ELECTROCONVULSIVE SHOCK (ECS) ON THE L AND N TYPE CALCIUM CHANNEL IN DIFFERENT RAT BRAIN REGIONS. C.H. Gleiter, C. Cain* and P.J. Marangos. NIAAA/LCS, NIMH/BPB, Bethesda, MD 20892; Human Pharmacology Institute Ciba-Geigy, Tubingen, FRG.

Chronic ECS has been shown to change the number of [3H]nitrendipine binding sites in rat cerebral cortex and hippocampus (Pharmacol. Biochem. Behav. 27:217, 1987[1]). We examined the L-type Ca^{++}-channel using [3H]nimodipine (NI) and the N-type Ca^{++}-channel using [125I]omega-conotoxin GIVA (CO). Rats were sacrificed 30 min after a single ECS (A) or 24 h after 10 once-daily ECS (B). Binding of NI and CO to crude membrane preparations was examined at the respective Kd concentration in the same animals:

	[3H]nimodipine		[125I]omega-conotoxin GIVA	
(A)	CTRL	ECS	CTRL	ECS
Cortex	114 ± 6	99 ± 2*	300 ± 16	249 ± 6#
Cerebellum	33 ± 3	35 ± 2	141 ± 11	153 ± 12
Hippocampus	112 ± 5	116 ± 6	271 ± 5	275 ± 15
(B)	CTRL	ECS	CTRL	ECS
Cortex	98 ± 6	100 ± 3	218 ± 20	220 ± 13
Cerebellum	33 ± 1	33 ± 1	188 ± 20	168 ± 12
Hippocampus	128 ± 7	123 ± 5	289 ± 10	290 ± 15

(fmol/mg protein; mean±SEM; n = 8/group; * $p < 0.05$; # $p = 0.01$)

Conclusions: 1. Binding of the respective ligands to the L and N type Ca^{++}-channel shows a parallel decrease of Bmax (as confirmed by Scatchard analysis of pooled tissue) in cerebral cortex 30 min after a single ECS. 2. Distribution of densities of binding sites is similar for both ligands in the respective brain regions. 3. The earlier report[1] on changes of the L type channel following chronic ECS can not be confirmed.

55.1

VOLTAGE-DEPENDENT Ca^{++} UPTAKE IN CULTURED CEREBELLAR GRANULE CELLS. B.A. Armitage* and P.B. Molinoff (SPON: N.S. Thampi). Dept. of Pharmacology, Univ. of Pennsylvania, Sch. of Med., Philadelphia, PA 19104.

The biochemical properties of voltage-sensitive Ca^{++} channels (VSCC) were studied by measuring $^{45}Ca^{++}$ flux in cerebellar granule cells. Primary cultures were used because the activity of VSCC in neuronal cell lines may differ from that described for neurons *in vivo*. Dissociated cells from the cerebellum of 7 day old rats were plated on poly-D-lysine (PDL) in Basal Eagle's Medium (BME). In control assays the background contributed by PDL-coated plastic dishes was high and variable, whereas that of PDL-coated glass coverslips was negligible. Cells were plated at a density of 10^6 cells per 18mm coverslip. Cells were grown in BME supplemented with 25mM K^+ which is necessary to maintain cell viability. However, chronic depolarization was found to eliminate voltage-dependent Ca^{++} uptake. This activity returned after a 5 hr. incubation at 37°C in BME containing 5mM K^+. Uptake was measured in a balanced salt solution (BSS) containing $^{45}Ca^{++}$ and 5mM or 50mM K^+. Immersing the coverslips in a large volume of cold BSS containing 10mM EDTA for 10 sec. and draining off all excess liquid minimized background. In time course experiments voltage-dependent uptake reached a plateau after 2 min. ($t_{\frac{1}{2}} \approx 30$ sec.). VSCC activity was maximal after 10 days *in vitro*. Maximal voltage-induced uptake was found to be 4 times that of uptake in buffer containing 5mM K^+. Measuring $^{45}Ca^{++}$ uptake in cultured cerebellar granule cells represents a useful model system for studying the biochemistry and pharmacology of VSCC. (Supported by USPHS GM 34781).

55.2

Flow Cytometric Analysis of Calcium Levels in Rat Striatal Synaptosomes. Marina E. Wolf and Gregory Kapatos. Laboratory of Neurochemistry, Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235.

Schieren and MacDermott (Soc. Neurosci. Abstr. 13: 1514, 1987) have previously used flow cytometry and the calcium (Ca^{++}) indicator INDO-1 to monitor receptor mediated changes in cellular Ca^{++} levels. We have used these methods to study the regulation of $[Ca^{++}]_i$ in striatal synaptosomes. While intrasynaptosomal $[Ca^{++}]$ has been previously measured fluorometrically, flow cytometry offers the advantage of enabling detection and separation of synaptosomal subpopulations exhibiting different responses. The ratio of INDO-1 fluorescence at 405 and 485 nm was measured using a Becton-Dickinson FACS 440 and used as an index of intrasynaptosomal $[Ca^{++}]$. Intrasynaptosomal $[Ca^{++}]$ was approximately 120nm under resting conditions and was increased to micromolar levels by the Ca^{++} ionophore ionomycin and by depolarization with 75uM veratridine. Glutamate (10uM) produced a much smaller increase. Nifedipine (10uM), a Ca^{++} channel blocker, produced a paradoxical increase in intrasynaptosomal $[Ca^{++}]$ when administered alone and failed to prevent veratridine-induced increases. Future studies will explore the effects of other Ca^{++} antagonists and neurotransmitters on intrasynaptosomal $[Ca^{++}]$.

55.3

FLOW CYTOMETRIC ANALYSIS OF CALCIUM LEVELS IN PC12 CELLS AND EMBRYONIC MESENCEPHALIC NEURONS. Gregory Kapatos and Marina E. Wolf. Laboratory of Neurochemistry, Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235.

Schieren and MacDermott(Soc.Neurosci.Abstr.13:1514, 1987) have previously combined flow cytometric techniques with the fluorescent calcium (Ca^{++}) indicator, INDO-1, to monitor receptor mediated changes in intracellular Ca^{++} levels ($[Ca^{++}]_i$). The PC12 cell line offers an opportunity to use similar techniques in a homogeneous cell population which maintains multiple Ca^{++} channel subtypes (L and N) and responds to numerous neurotransmitters and growth factors with an increase in $[Ca^{++}]_i$. As expected, PC12 cells responded to ionomycin (10uM) or depolarization with K^+ (56mM) or veratridine (75uM) with a large increase in $[Ca^{++}]_i$ which was dependent on extracellular Ca^{++} ($[Ca^{++}]_o$). K^+ was substantially more effective than veratridine. The response to K^+ was completely blocked by the L-type antagonist nifedipine yet, surprisingly, was potentiated by cadmium (1mM). Incubation with carbacol increased $[Ca^{++}]_i$, but this response was unaltered by nifedipine and independent of $[Ca^{++}]_o$. Similar studies will be reported which examine the development, cell specificity and receptor mediation of changes in $[Ca^{++}]_i$ in embryonic and newborn mesencephalic neurons.

55.4

BLOCK OF ^{45}Ca UPTAKE INTO SYNAPTOSOMES BY METHYLMERCURY: REVERSIBILITY, STATE- AND Na^+-DEPENDENCE. T.J. Shafer* and W.D. Atchison (SPON: J.E. Thornburg). Dept. of Pharm./Tox., Michigan State Univ., E. Lansing, MI 48824.

Characteristics of blocking action of methylmercury (MeHg) on Ca^{2+} influx into isolated nerve terminals were assessed by measuring $^{45}Ca^{2+}$ content of rat forebrain synaptosomes following 1 ("fast phase") or 10s ("total") of depolarization induced by elevated K^+ (77.5 mM), or after 10s of depolarization of synaptosomes that had been predepolarized for 10s in solutions free from Ca^{2+} and MeHg ("slow phase"). Block of the "total" and "fast" phases by MeHg (50 μM) were not completely reversed by increasing $[Ca^{2+}]_e$ from 0.01 to 1.2 mM. However, for $[Ca^{2+}]_e \geq 0.30$ mM, "slow" $^{45}Ca^{2+}$ uptake returned to control levels. When dependence of block of $^{45}Ca^{2+}$ uptake on Na^+_e was tested, block of the "slow" phase of $^{45}Ca^{2+}$ uptake by MeHg (25 μM) was potentiated in the absence of Na^+_e. Furthermore, in the absence of Na^+_e, increasing $[Ca^{2+}]_e$ from 0.15 to 1.2 mM did not reverse block of the "slow" phase by MeHg. When the state dependence of block was tested, similar dose-dependent suppressions of $^{45}Ca^{2+}$ influx by MeHg during depolarization were observed in synaptosomes incubated for 10s in depolarizing or non-depolarizing media prior to measuring 1s of depolarization-induced $^{45}Ca^{2+}$ influx. Finally, MeHg reduced resting $^{45}Ca^{2+}$ uptake at concentrations >125 μM. Results of the present study indicate that 1) the reversibility of block of the "slow" phase of $^{45}Ca^{2+}$ uptake by MeHg is Na^+-dependent; and 2) block of $^{45}Ca^{2+}$ uptake by MeHg is not more pronounced during the inactivated state of the channel. (Supported by NIH grant ES03299.)

55.5

FURA-2 MEASUREMENT OF VOLTAGE-DEPENDENT CHANGES IN INTRACELLULAR CALCIUM CONCENTRATION IN GUINEA-PIG SUBMUCOUS NEURONS. K. Hirai*, Y. Katayama and K. Morita (SPON: E.F. Barrett), Dept. Autonom. Physiol., Med. Res. Inst., Tokyo Med. & Dent. Univ., Tokyo, 101 JAPAN

Voltage-dependent changes in intracellular calcium concentration ([Ca^{++}]i) were investigated by using fura-2 in single voltage-clamped neurons in the guinea-pig submucous plexus. Fura-2 staining of single cells was performed by ionophoretic injection of the dye through the recording microelectrodes.

The resting value of [Ca^{++}]i was about 100 nM. An action potential (AP) increased [Ca^{++}]i by about 80%. The recovery time courses of both the after-hyperpolarization (AHP) and [Ca^{++}]i were single exponentials with time constants of 6 s and 3 s, respectively. In neurons held at -70 mV, [Ca^{++}]i increased with a depolarizing command of 40 mV; the time constant of the increase and the maximum value of [Ca^{++}]i were about 0.5 s and 170% of control. These were almost linearly related to the command amplitude. The recovery time course of [Ca^{++}]i after the depolarization was similar to that after the AP. [Ca^{++}]i was decreased by a hyperpolarization even at the holding potential of -70 mV. This decrease was dependent on the amplitude of hyperpolarizing command. Supported in part by the Nakatani Electronic Measuring Technology Association of Japan.

55.6

FLOW CYTOMETRIC ANALYSIS OF CALCIUM FLUXES IN DISSOCIATED EMBRYONIC RAT HYPOTHALAMIC NEURONS. J. P. Grierson, R. E. Petroski, S. M. O'Connell* and H. M. Geller. Department of Pharmacology, U.M.D.N.J. - Robert Wood Johnson Medical School and The Graduate School, Rutgers University, 675 Hoes Lane, Piscataway, NJ 08854.

The fluorescent, calcium-sensitive dye, INDO-1, has been used in several previous studies to examine stimulus-induced changes in cytoplasmic free Ca^{++} in blood cells. However, calcium mobilization is also known to be important in certain neurons for neurotransmitter transduction and neurotransmitter release. We utilized INDO-1 to probe the expression of functional Ca^{++} channels in dissociated rat neurons obtained from the embryonic day 17 hypothalamus. Neurons were dissociated by enzymatic treatment followed by mechanical dispersion and the cells loaded with 1uM INDO-1 at a concentration of 10^6 cells/ml. Fluorescence was measured on a Coulter EPICS-753 dual laser flow cytometer. The calcium ionophore, ionomycin, induced a substantial increase in the concentration of cytoplasmic free calcium. A significant increase was also induced by the addition of 10-100mM KCl or 50 uM veratridine. The K^+- and veratridine-induced calcium influx was completely inhibited by 3 mM cobalt chloride. Verapamil inhibited the calcium influx and the L-channel antagonist, nifedipine, produced a greater than 50% inhibition of K^+-stimulated Ca^{++} influx. Parallel studies of stimulus-induced membrane depolarization with these cells is presented in one presentation (Grierson et al) and patch-clamp analysis of calcium channels in these cells in another (Petroski et al). Supported by NIH NS25168 and a grant from the UMDNJ Foundation.

55.7

INHIBITION OF THE K^+ STIMULATED RISE IN INTRACELLULAR FREE Ca^{++}, $^{45}Ca^{++}$ INFLUX AND ENDOGENOUS ACETYLCHOLINE RELEASE BY ω-CONOTOXIN GVIA. P.M. Lundy*, J.C. Goulet* M.G. Hamilton*, W. Lee*, R. Frew* and K.A. Stauderman[1] (SPON: J. Maas). Defence Research Establishment Suffield Ralston, Alberta, Canada TOJ 2NO and [1] Merrill Dow Research Institute 2110 E Galbraith Rd. Cincinnati, Ohio 45215, USA.

ω-Conotoxin GVIA (CgTX) is an inhibitor of neuronal N type voltage sensitive Ca^{++} channels (VSCC). We have performed various manipulations to elucidate the effects of CgTX, on K^+ stimulated $^{45}Ca^{++}$ influx, intracellular free Ca^{++} and endogenous acetylcholine (ACh) release using chicken brain synaptosomes and slices respectively. The initial phase of K^+ stimulated $^{45}Ca^{++}$ influx and the rise in intracellular free Ca^{++} measured with Fura-2 was accompanied by release of endogenous ACh as detected by chemiluminescence. ACh release was reduced by 90% in the absence of Ca^{++} ions, or by Cd^{++} (0.1mM). CgTX (1μM) completely blocked the fast phase of $^{45}Ca^{++}$ influx, markedly inhibited the rise in intracellular free Ca^{++} and greatly reduced ACh release. The dihydropyridine VSCC antagonist (-)202791 and agonist (+)202791, by contrast, were ineffective in significantly altering these events. The results are consistent with the suggestion that CgTX inhibits N type VSCC's and that these channels are intimately associated with endogenous ACh release.

55.8

ACTIVITY-DEPENDENT INTRACELLULAR CALCIUM DISTRIBUTION IN GUINEA PIG CEREBELLAR PURKINJE CELLS: AN *IN VITRO* STUDY. M. Sugimori, D.W. Tank, J. Connor, and R. Llinás. Dept. Physiol. & Biophys, NYU Med. Ctr. New York, N.Y. 10016 and Molec. Biophys. Res. Dept. AT&T Bell Labs, Murray Hill, N.J. 07974.

Intracellular Ca concentration ($[Ca]_i$) was measured in adult guinea pig Purkinje cells in cerebellar slices following intracellular fura-2 injections. The ratio of digital micro-fluorometric images at 340 and 380 nm excitation provided a high resolution spatial map of the Ca^{++} distribution in soma, axon and dendrites. Maps were measured at 2 sec intervals during spontaneous .05 Hz oscillatory activity characterized by periods of calcium spiking and silence (Llinas & Sugimori, J. Physiol 1981). Ca^{++} accumulation first peaked in the distal spiny branchlets. Subsequently, $[Ca]_i$ rose in the primary and secondary branches of the main dendritic tree but remained low at the soma. Nearly identical oscillating calcium maps could be repetitively measured for several hours. Following gK blockage by TEA, the dendritic $[Ca]_i$ increased sufficiently to increase slightly the somatic $[Ca]_i$ Bath addition of *Agelenopsis aperta* toxin, known to block calcium spikes in Purkinje cells (Sugimori and Llinas, Soc. Neuroscience 1987), blocked oscillatory activity and produced a spatially uniform very low $[Ca]_i$. These results indicate that calcium entry into Purkinje cells occurs intially at the peripheral dendritic tree, probably in the form of a plateau potential that may serve as a boosting mechanism for the parallel fiber Purkinje cell synapse. When the depolarization becomes high enough, calcium-dependent spikes occur at the main dendrite generating the second phase of calcium entry. Supported by NIH grant NS13742.

55.9

A CA CHANNEL PROBE FOR HUMAN BRAIN: SPECIFIC BINDING SITES FOR ω-CONOTOXIN. M. Litzinger, A. Azimi-Zoonoz* and S. Stensaas*. Depts. of Biology, Neurology and Pathology, University of Utah, Salt Lake City, UT 84112.

ω-Conotoxin GVIA (ω-CgTx), a 27 amino acid positively charged peptide purified from the venom of Conus geographus, a marine snail, is believed to bind to the voltage-sensitive Ca channel (Olivera et al., Science 230, 1338-1343, 1985; Nowcyky et al., Nature 316, 440-443, 1985). Binding with ^{125}I-ω-CgTx was carried out (Cruz and Olivera, J. Biol., 261, 6230-6233, 1986) using crude membranes from two freshly frozen pathology specimens (12-16 hr postmortem) of human forebrain and hippocampus. A ^{125}I-ω specific ω-conotoxin binding site was found in both forebrain and hippocampus. It was displaced by pre-incubation with $2.5x10^{-7}$ M ω-conotoxin GVIA or $1x10^{-4}$ M neomycin. It was not displaced by $2.5x10^{-7}$ M μ-conotoxin (muscle Na-channel specific) or by $5x10^{-6}$ M α-conotoxin GI (nicotinic acetylcholine receptor-specific). Assay conditions employing low ionic strength for binding (Cruz and Olivera, 1986) lead to "false-positive" specific sites when human brain tissue is used; assays carried out under physiologic ionic salt concentrations gave a more reliable estimate of specific binding. High ionic concentrations (> 0.3 M) abolished specific binding in human brain membranes. Further characterization of the human ω-conotoxin binding site is in progress. (Supported by a gift from Miles Laboratories)

55.10

1,4-DIHYDROPYRIDINE-DISPLACING FRACTION FROM BRAIN ACTING ON CALCIUM CHANNELS:ISOLATION AND PARTIAL CHARACTERIZATION A.V. Shrikhande*, D.E. Johnson*, A.D. Howard, R.T. McCarthy* and R.A. Janis* (SPON: R.J. Fanelli). Miles Inc., 400 Morgan Lane, West Haven, CT 06516 USA

The purpose of this study was to determine if a 1,4-dihydropyridine (DHP)-displacing substance that modulates calcium channels could be isolated from mammalian tissue. Bovine brain was subjected to acid extraction and several purification steps. A fraction from brain that eluted from C18 reverse phase columns at 32% acetonitrile inhibited [^{3}H]DHP binding to cardiac membranes. Electrophysiological studies were carried out on rat anterior pituitary cells (GH3) using the whole cell variation of the patch clamp. Addition of the fraction to the inside, but not the outside of the cells produced a block of the sustained, but not the transient calcium channel current (CCC). This inhibition resembled that produced by DHPs. It was time- and voltage-dependent, such that block of slowly-inactivating CCC is maximized by prolonged depolarization. Further purification of this fraction on a sizing column in the presence of 40% acetonitrile yielded a low molecular weight fraction (<1 kDa) and an 18 kDa fraction consisting of myelin basic protein. Both fractions inhibited DHP binding but only the former inhibited CCC. This low molecular weight substance was active when added extracellularly. The results suggest that this fraction contains an endogenous substance that may regulate slowly-inactivating calcium channels.

55.11

KINETIC STUDIES OF [^{3}H]-NITRENDIPINE BINDING IN NORMAL AND CARDIOMYOPATHIC HAMSTER HEARTS. S.E. Howlett & T. Gordon. Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7.

The characteristics of high affinity dihydropyridine binding sites were compared in normal and cardiomyopathic (CM) hamster hearts to probe for possible defects in the Ca channel which could lead to Ca overload and, in turn, muscle necrosis. Kinetic studies of the temperature dependence of [^{3}H]-nitrendipine ([^{3}H]-NTP) binding to ventricular homogenates from 60-day-old normal and CM hamsters showed that, in normals, the rate of dissociation (0.049±0.006 min $^{-1}$ @ 25°C) was highly temperature-dependent (Q_{10} = 4.40±0.69) and that neither the rate nor the temperature dependence were influenced by disease. The rate of association (1.12±0.11 min $^{-1}$ nM^{-1} @ 25°C) was weakly temperature dependent (Q_{10} =1.25±0.04) and similarly unaffected by disease. The rates of [^{3}H]-NTP dissociation were increased and decreased by the Ca antagonists verapamil and diltiazem, respectively with little effect on the association rates. Allosteric interactions were identical in normal and CM hearts and, together with the normal temperature sensitivity, show that there is no abnormality at the related binding sites for nitrendipine, verapamil and diltiazem in the Ca channel of the CM hamster heart. Supported by the AHF and the AHFMR.

55.12

APPEARANCE OF SLOW CALCIUM CHANNEL ACTIVITY IN THE MYOTUBES OF MUTANT MICE WITH MUSCULAR DYSGENESIS (*mdg*). R.Bournaud[1,*], T.Shimahara[1,*], L.Garcia[2,*] and F.Rieger[2]. (SPON: Y.Brunner). Lab. of Neurobiologie Cellulaire et Moléculaire, CNRS, 91198 Gif/Yvette cedex France. Groupe de Biologie et Pathologie Neuromusculaires INSERM U153, 17 rue du Fer à Moulin 75005 Paris France.

Skeletal muscles from *mdg/mdg* fetuses are characterized by an absence of excitation-contraction (E-C) coupling and a significant reduction in the number of binding sites for dihydropyridine (D.H.P.). In vitro the high threshold slow Ca current is totally absent and contractile activities could be restored when these myotubes are co-cultured with spinal cord cells from normal mice. We have studied : 1) the modifications of physiological phenotype of *mdg/mdg* myotubes cultured with or without spinal cord cells. 2) the role if any, of the functional synaptic transmission in the appearance of normal physiological activities. We establish that both slow calcium current and E-C coupling were restored only when mutant myotubes are co-cultured with spinal cord cells from normal mice. Such a restoration of physiological phenotype did not require functional synaptic transmission since it was also obtained when neuro-muscular transmission was chronically blocked with α-bungarotoxin. The restored slow Ca conductance was identical to that observed in normal myotubes. The spinal cord cells from *mdg/mdg* mice, however, failed to induce normal muscle activity in the mutant myotubes. Partial restoration of the slow calcium current was also observed in aged *mdg/mdg* myotubes cultured without spinal cord cells. Though, in our experimental condition we did not observe an E-C coupling establishment in aged *mdg/mdg* myotubes. We conclude that: 1) *mdg/mdg* myotubes are capable of expressing slow calcium channel activity. 2) spinal cord cells stimulates the synthesis of D.H.P. receptors which are supposed to control both slow calcium current and E-C coupling. 3) functional neuromuscular transmission is not involved in the reversion of the *mdg* phenotype.

55.13

CALCIUM CURENTS EXHIBIT ALTERED SENSITIVITY TO BAY K8644 IN PC12 CELLS DIFFERENTIATED WITH NGF AND V-*SRC*. D. Lewis, D. Rausch, L. Eiden, and J. Barker (SPON: J. Lechleiter). Neurophysiology, NINCDS, NIH and Cell Biology, NIMH, Bethesda, MD 20892.

Ca currents were recorded with the patch clamp technique in PC12 cells under normal growth conditions and following differentiation induced by NGF or v-*src* oncogene. Following infection with a recombinant retrovirus carring the gene for v-*src*, PC12 cells extended neuron-like processes similar to that seen following NGF (D. Rausch & L. Eiden, these abstracts; Alemá et al., *Nature* 316:557, 1985). Peak Ca current in control PC12 cells increased in the presence of (-)Bay K8644 by 50.2 ± 7.8% (n=18), but was unaffected in v-*src* and NGF differentiated cells.

It has been reported that secretion in NGF-treated PC12 cells is insensitive to Bay K8644 (Kongsamut,S. and Miller, R. *PNAS*., 83:2243, 1986) implying that NGF-induced differentiation results in secretion mediated via dihydropyridine insensitive Ca channels (Miller, R. *Science*, 235:46, 1986). Our results indicate that PC12 cells differentiated with NGF or v-*src* do indeed develop Ca currents which have altered sensitivity to Bay K8644. Our macroscopic current recordings did not indicate the appearance of dihydropyridine insensitive Ca currents similar to the T and N types (Nowycky, M. et al., *Nature*, 316:440, 1985). The Ca currents recorded here were of the high-threshold, slowly inactivating or L type. It is possible that dihydropyridine insensitive channel types are expressed which have macroscopic properties different from the N and T types. Single channel analysis must confirm the channel types and their dihydropyridine sensitivities with neuronal differentiation.

55.14

EFFECTS OF NERVE GROWTH FACTOR ON CALCIUM CURRENTS IN PC12 CELLS. J.Streit*, H.D.Lux (SPON:H.R.Lüscher). Department of Neurophysiology, MPI for Psychiatry, D-8033 Planegg-Martinsried, GFR.

In the rat pheochromocytoma cell line PC12 cell sprouting is activated by nerve growth factor (NGF). We investigated, whether NGF treatment alters calcium currents in these cells in a way which could be related to neurite outgrowth. Whole cell calcium currents were recorded in the somata of PC12 cells using the patch clamp method. Peak current amplitudes (I_*), times to peak (ttp) and the fraction of inactivated current after 200ms pulse duration [F_i=1-(I_{200}/I_*)] were sampled for various groups of cells with different NGF treatment protocols. All three parameters were increased in cells treated with NGF compared to untreated cells [I_*: 256 ±12pA (n=91) versus 69±6pA (n=35), ttp: 19.9±1.0ms (n=109) versus 11.9±0.8ms (n=39), F_i: 0.39±0.01 (n=104) versus 0.27±0.01 (n=39), all ±SEM]. The increase in ttp was stronger correlated to neurite bearing cells than to cells treated with NGF, indicating that it was due to the changed electrotonic properties of differentiated cells and not to a change of the activation kinetics of the calcium channels. The increase in I_* became prominent only after 5 days of NGF treatment, clearly after the sprouting of the cells had occurred. However, it persisted when the cells lost their neurites due to a subculturing procedure, thus showing that it was not a consequence of neurite outgrowth. The NGF-induced increase in F_i was most prominent in the first three days of NGF treatment, that is during neurite outgrowth. It was strongly correlated to the NGF treatment, much less to the existence of neurites and not to I_*. These findings were confirmed when NGF was applied directly to previously untreated PC12 cells during whole cell recordings. In periods up to 60 minutes no effect of NGF on ttp or I_* was observed, whereas F_i was increased after 10 to 20 minutes of NGF application. This effect was partially reversible after washout of NGF. Due to the lack of temporal correlation of the NGF effects on I_* and F_i we conclude, that these effects can not be explained by an NGF-induced synthesis of an altered calcium channel, which is inactivated to a greater extent. The results are, however, in good agreement with a hypothesis based on calcium current distribution measurements (Streit,J. and Lux,H.D., Pflügers Arch.,411(sup.):R143, 1988), where a NGF induced clustering of calcium channels leading to more current inactivation is proposed.

55.15

Monoclonal Antibodies to the Alpha1 Subunit of the Muscle Voltage-gated Calcium Channel Identify Related Proteins in Human Small Cell Carcinoma and Neuroblastoma Cell Lines. Mary E. Morton* and Stanley C. Froehner. Department of Biochemistry, Dartmouth Medical School, Hanover, N.H. 03756.

Lambert-Eaton Syndrome (LES) is a neuromuscular disorder characterized by defective transmitter release and thought to involve an autoimmune response to presynaptic calcium channels. LES is frequently associated with small cell carcinoma of the lung (SCCL). We have investigated the possibility that SCCL cells express calcium channels similar to those found on the nerve terminal. A monoclonal antibody, mAb 1A, that recognizes the alpha$_1$ subunit of the voltage-gated calcium channel from skeletal muscle identified a related component on SCCL cells. FACS analysis demonstrated the presence of mAb 1A-reactive molecules on the surface of cultured human SCCL cells. An alpha$_1$-like polypeptide immunoaffinity purified from these cells exhibits an M_r similar to that of alpha$_1$ from skeletal muscle and is recognized by two distinct anti-alpha$_1$ mAbs. In addition, similar experiments showed that an alpha$_1$-like protein was present on the surface of IMR-32 cells, a human neuroblastoma line. These results indicate that small cell carcinoma and a cell line derived from peripheral neurons share surface proteins related to calcium channels. The data support a model which proposes that cross-reactivity of anti-tumor cell antibodies with presynaptic calcium channels plays a role in the development of Lambert-Eaton Syndrome.

55.16

CHARACTERIZATION OF Ca^{2+} TRANSPORT SYSTEMS IN RAT BRAIN MICROSOMES. J. Shah*[1] and H.C. Pant[2]. [1]Biophys. Inst., Boston Univ. Sch. of Med., Boston, MA 02118 and [2] Lab. of Neurochem., NINCDS, NIH, Bethesda, MD 20892.

We have characterized the different Ca^{2+} transport systems existing in the microsomes isolated from rat brain. A Mg^{2+}-ATP-dependent Ca^{2+} uptake was observed in K^+ loaded microsomes which was inhibited by sodium ortho-vanadate. A maximal 34% of total sequestered Ca^{2+} was released by an inositol 1,4,5-trisphosphate (IP_3)-gated channel and this release was insensitive to various Ca^{2+} channel blockers e.g. dihydropyridines (nitrenedipine, nifidipine), ω-conotoxin, Co^{2+}, ruthenium red, verapamil or dantroline. However, this IP_3-induced Ca^{2+} release was blocked by tetraethyl ammonium chloride (TEA) and 9-TEA (potassium channel blockers). The addition of IP_3, also results in a K^+ influx, suggesting that IP_3-induced Ca^{2+} release requires an opposite flow of K^+ ions. However, coexistence of another kind of Ca^{2+} transport system was found in these microsomes which was activated by Cd^{2+}. Cd^{2+} produced a rapid, large release of Ca^{2+} in a concentration-dependent manner. Maximal release (80% of the total A23187 releasable Ca^{2+}) was noticed at 250 uM Cd^{2+}. The Cd^{2+} stimulated Ca^{2+} release was inhibited by cysteine. Similar effects were shown by $AgNO_3$, although Ca^{2+} release was less pronounced compared to Cd^{2+} stimulated release. Different Ca^{2+} channels may play different physiological roles.

55.17

RECONSTITUTION OF A CALCIUM CHANNEL FROM OF A HIGHLY PURIFIED PREPARATION OF MOUSE BRAIN SYNAPTOSOMES. R. Etcheberrigaray* and E. Rojas* (SPON: J. Rinzel) LMCN, NINCDS and LCB & G, NIDDK, NIH, Bethesda, MD 20892.

It is generally accepted that, regardless of the origin of the Ca^{2+} required for exocytotic release of neurotransmitters, intracellular Ca^{2+} stores play a fundamental role in secretion. Membrane channels provide the most efficient mechanism to furnish the Ca^{2+} required for release. However, the role of the endoplasmic reticulum (ER) Ca^{2+}-channels in nerve cells has not yet been elucidated. A highly purified synaptosome preparation was used to obtain intra-synaptosomal membranes. Application of the freeze and thaw method to the synaptosomes generated a membrane preparation rich in ER fragments. Ca^{2+}-channels were reconstitued into bilayers made with phosphatidylinositol (Avanti Polar Lipids). Membranes were formed at the tip of patch clamp pipets by the "double dip" method. A low [Ca^{2+}] solution (mM: 100 CsPipes, 1 CaHepes, pH = 6.4) was used in the chamber containing the membranes fragments (cis side) and a high [Ca^{2+}] solution in the pipet (mM: 100 CsPipes, 50 CaHepes, pH = 7.6). Under these conditions, we were able to incorporate Ca^{2+}-channels with the following properties: 1) Strong voltage sensitivity. Fractional open-time increased from 32.4 (measured at 20 mV) to 84.5% (at 60 mV). 2) Single Ca^{2+}-channel currents varied linearly with pipet potential to give a slope conductance of 20 pS. 3). The channel was activated by ATP (500 uM) and caffeine (10 mM) and was blocked by ruthenium red (10 uM). Nifedipine (10 uM) was without effect (drugs were added to the cis side). These pharmacological properties support the idea that the Ca^{2+}-channel originated from internal organelles such as the ER and emphasize its possible role in Ca^{2+} mobilization from internal stores.

56.1

DISTRIBUTION OF T,L AND D CHANNELS IN HIPPOCAMPAL GRANULE CELLS: SIMULATION OF VOLTAGE CLAMP DATA AND CALCIUM SPIKES.

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Although hippocampal granule cells were previously supposed to possess weak calcium conductances, recent singlé-electrode voltage clamp studies (Blaxter et at, in press) have revealed the presence of three types of calcium channels (T,L and D) similar to the well known T, L and N channels found in other preparations (see Fox et al J Physiol 394:173,1987a). In this study, we have attempted to simulate the clamp voltage versus peak current relation (I-V curve) as well as the shape of the clamp current over time, using detailed descriptions for these channels derived from whole-cell patch-clamp data (Fox et al J Physiol 394:149,1987b). Steady-state gate opening curves were fitted to the data of Fox et al (1987b) for each of the calcium channels (each with m^2h kinetics). Special attention was paid to the relative positions of the activation and inactivation curves on the voltage axis and the ranges of the inactivation time constants. These channels were incorporated into a model of the electrical properties of granule cells (Yuen and Durand Soc Neuro Abs 13:1353 with gNa=gK=0) and their I-V curves and clamp currents were simulated as a function of the distribution, type and density of the channels.

The experimentally obtained I-V curve was essentially reproduced by a model with T and L at the soma and D in the proximal dendrites at properly adjusted conductance values. However, the appearance of the I-V curve is sensitive to a number of other factors such as the rate of intracellular calcium ion removal and the spatial location of the D channels on the dendrites. Whereas **the simulated clamp currents for T and L resembles the experimental data, the inactivating portion of the D-current (observing from soma) gives a different appearance from the data.** Introducing calcium dependence to the inactivation gate for D improves the fit but at the expense of a fairly large (6.5 mS/sq.cm.) channel density and a shifted I-V curve to positive voltages. This discrepency between the simulated and the observed D-current may therefore be due to the experimental difficulty of obtaining adequate space-clamp. This simplified model of the distribution of T, L and D (somatic T and L, dendritic D) is also capable of generating calcium spikes similar to those obtained experimentally under TTX and TEA treatments. Simulations to date confirms (see Blaxter et al) that **a dendritic D-current is probably involved in the calcium spike** as suggested by: (1) the failure to generate a proper rising phase for the calcium spike in the absence of dendritic D channels at various values of somatic T and L conductances (2) preferential localization of the D-channel to the dendritic tree as suggested by experimental studies and (3) failure to obtain calcium spike with somatic T, L and dendritic T.

56.2

CALCIUM-DEPENDENT INACTIVATION OF CALCIUM CURRENTS IN HIPPOCAMPAL NEURONS: EFFECTS OF TETRAETHYLAMMONIUM AND NIMODIPINE. L.W. Campbell*, S.Y. Hao*, and P.W. Landfield (Spon: J. Robinson) Dept. of Phys./Pharm., Bowman Gray Sch. of Med., Winston-Salem, NC 27103.

Recent studies have indicated that Ca conductance (G_{Ca}) in rat hippocampal neurons exhibits a form of apparent Ca-dependent feedback inactivation (Pitler, Landfield, *Brain Res.* 410: 1987) similar to that described for invertebrate neurons (Eckert, Tillotson, *J. Physiol.*, 1981). However, hippocampal neurons were also shown recently to exhibit a brief Ca-and voltage-dependent K current (I_C) (Storm, *J. Neurophysiol.*, 1987), that could confound measures of G_{Ca} inactivation. This I_C is blocked by TEA, and the present studies therefore compared Ca-dependent inactivation of G_{Ca} before and after TEA application.

Ca currents were measured in cesium-loaded, TTX-treated rat hippocampal slice CA1 cells, in which the slow K-mediated afterhyperpolarization was blocked. TEA was applied by pressure ejection, and TEA efficacy was assessed by a several-fold lengthening of the Ca spike in current-clamp. Single-electrode voltage-clamp methods were used to study Ca currents in response to depolarizing commands below Ca spike threshold. TEA administration did not alter the time course or percentage inactivation during 2- or 5- Hz trains of command steps, indicating that I_C is not a confounding factor in the apparent inactivation of Ca current. However, the application of nimodipine, a DHP Ca-channel antagonist, reduced baseline current amplitude and slowed the rate of inactivation. These studies further indicate that a Ca-sensitive negative feedback mechanism inactivates Ca currents and limits the influx of Ca into mammalian brain neurons. (Supported by AG04542).

56.3

IDENTIFICATION OF TWO CA-CURRENTS IN NEURONS FROM THE RAT LATERAL GENICULATE NUCLEUS. A. HERNANDEZ-CRUZ* and H.-Ch. PAPE* (SPON: ENA), Howard Hughes Institute at Stony Brook and Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Ca-conductances have been proposed to crucially determine firing properties of thalamic neurons (Jahnsen & Llinás, J. Physiol., 349: 205, 1984). Here we characterize Ca-currents in acutely dissociated multipolar neurons of the lateral geniculate nucleus (LGN). Cells were obtained after papain incubation (14 u/ml) of LGN slices from rats at postnatal days 13 and 14. Bath // pipette solutions contained respectively (in mM): 5 $CaCl_2$, 15 TEA, 3 4-AP, 10 K-HEPES, 24 glucose, 5 KCl and 236 sucrose // 50 CsCl or CsAc, 50 CsF, 10 TEA, 10 Cs-HEPES, 5 Cs-EGTA or 10 Cs-BAPTA, 2 Na-ATP, 10 phosphocreatine and 50 u/ml creatine phosphokinase. Whole-cell recordings showed two components of the Ca-current similar to the "T" and "L" type currents found in other central neurons. 1) The low-threshold transient (T) component: a) Is best elicited from holding potentials <-80 mV. b) Peaks in 35 to 6 ms and decays with a time constant ranging from 30 to 22 ms in the activation range -50 to -25 mV. c) Its steady-state inactivation occurs in the range -80 to -30 mV with half-inactivation near -60 mV. d) Is relatively insensitive to 50 μM $Cd^{++}$and blocked by 50 μM Ni^{++}. e) Is less permeant to Ba^{++} than to Ca^{++}. 2) The high-threshold sustained (L) component: a) Is selectively activated from less negative holding potentials. b) Shows no decay over pulses 100 ms or longer. c) Its activation ranges between -22 and 3 mV. d) Is relatively insensitive to $Ni^{++}$and very sensitive to block by Cd^{++}. e) Is less permeant to Ca^{++} than to Ba^{++}. To assess the role of these two currents in normal firing behavior, relay cells from the same animals were recorded in the in vitro LGN slice preparation (Ca^{++} concentration 1.5-2.5 mM). Cells in the dorsal LGN showed characteristic thalamic responses, including low-threshold Ca-spikes (LTS). LTS were de-inactivated with hyperpolarization <-80 mV, readily blocked by Ni^{++}(0.2-2 mM), whereas block by Cd^{++}(1-1.5 mM) was slower and incomplete. We conclude that a "T" type current underlies LTS in thalamic neurons, being this current dominant in the near-threshold voltage range.

56.4

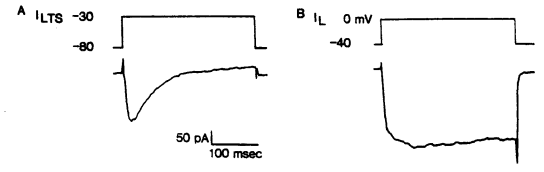
CALCIUM CHANNELS IN HYPOTHALAMIC NEURONS IN DISSOCIATED CELL CULTURE R. E. Petroski and H. M. Geller. Dept. of Pharmacology, UMDNJ - Robert Wood Johnson Medical School and The Graduate School, Rutgers University, Piscataway, NJ 08854.

We previously demonstrated that cultured embryonic hypothalamic express functional sodium and potassium channels as measured by whole cell patch clamp recordings. We now report that at least two kinds of calcium channels can be recorded from these neurons. Hypothalami are dissected from E17 rat brains and plated onto a confluent monolayer of cortical astrocytes. Whole cell recordings were made beginning 1 day in vitro. Input resistances range from 1000-5000 MΩ on these neurons which are 10-25 uM in diameter. Calcium currents are recorded with an electrode containing Cs+/TEA to block potassium currents. Choline was used in the bath to eliminate sodium currents and barium was utilized as the charge carrier. By 1 d.i.v., most neurons display an inactivating inward current carried by Ca^{++}/Ba^{++}. The time course of this current resembles that of the T-channel described in many neuronal preparations. At this time, little if any sustained inward current can be detected. As the neurons develop in vitro, a non-inactivating inward current develops. This is usually seen as a "notch" current with a variable lag period before activation. Additionally, prolonged "tail" currents are seen. The inadequate space clamp control suggests that the channels which carry this current are located in the processes of the neuron. This is temporally correlated with the appearance of long processes in the neurons. Parallel experiments with flow cytometric methods have also detected voltage-dependent calcium fluxes in freshly dissociated embryonic hypothalamic neurons (see Grierson et al, this meeting).

56.5

CHARACTERIZATION OF THE IONIC CURRENT UNDERLYING THE LOW-THRESHOLD CALCIUM SPIKE IN ENZYMATICALLY ISOLATED THALAMIC NEURONS. **Setsuo Suzuki* and Michael A. Rogawski, Medical Neurology Branch, NINCDS, NIH, Bethesda, MD 20892.**

Current clamp recordings in thalamic slices have demonstrated low-threshold (LT) Ca^{2+} spikes that are believed to mediate the shift from tonic to burst firing that occurs with hyperpolarization to -80 mV (Jahnsen & Llinas, *J. Physiol.* 349:205-247). We recorded similar LT spikes in neurons acutely isolated from the adult guinea pig dorsal lateral geniculate nucleus (LGNd), and, using whole cell voltage clamp recording techniques (140 mM *N*-methyl-D-glucamine internal solution), we characterized a novel Ca^{2+}current in these cells that may underlie the LT spike. Step depolarization to -30 mV and above from a holding potential of -80 mV activated an inward Ca^{2+} current (I_{LTS}) that rose to a peak within 25-35 msec and inactivated during the subsequent 100-200 msec; the current was reduced by Ba^{2+}. I_{LTS} was completely inactivated at -40 mV; depolarization from this potential elicited a slowly activating, minimally inactivating Ca^{2+}current (I_L) that was reduced by nimodipine. The characteristics of I_{LTS} are generally comparable to those of the N-type Ca^{2+} current of sensory neurons.



56.6

TWO TYPES OF SINGLE CALCIUM CHANNEL CURRENTS IN NG108-15 CELLS. H. Kojima* and T. Narahashi (SPON: A.B. Butler). Department of Pharmacology, Northwestern University Medical School, Chicago, IL 60611.

Single calcium channel currents were recorded from neuroblastoma-glioma hybrid cells (NG108-15) using the cell-attached patch clamp technique. The cells used were 5-6 days old in culture. The pipette solution contained 110 mM $BaCl_2$ and 10 mM HEPES (pH 7.5). The external solution contained 140 mM K-aspartate, 10 mM EGTA and 1 mM $MgCl_2$ (pH 7.3). Under this condition the membrane potential was maintained close to 0 mV. All experiments were performed at room temperature (22°C).

Two types of calcium channel currents were observed during step depolarizing pulses in the same membrane patch. One type of currents was generated during a depolarizing step to -20 mV from a holding potential of -80 mV, and had relatively short (1-3 msec) durations. This type of currents was observed frequently. The other type was generated during a depolarizing step to +20 mV from a holding potential of -20 mV, and had relatively long (2-8 msec) durations. This type of current was observed much less frequently than the former type. The short and long single calcium channel currents correspond to type I (T-type) and type II (L-type) whole cell calcium channel currents, respectively (supported by NIH grant NS14144).

56.7

CHARACTERIZATION OF VOLTAGE-DEPENDENT BARIUM CURRENT IN DORSAL ROOT GANGLIA x NEUROBLASTOMA HYBRID CELLS. L.M. Boland and R. Dingledine, Neurobiology Curr., Univ. North Carolina, Chapel Hill, NC, 27599.

The whole cell patch clamp technique was used to study voltage-dependent ionic currents in F-11 cells (see Dingledine and Boland, this meeting). F-11 cells expressed outward K and inward Na and Ca currents. The properties of the whole cell Ba current through voltage-dependent Ca channels were studied using 30 mM external Ba and an internal Cs solution that contained a nucleotide regenerating system to minimize washout. In most cells, the Ba current had a transient and a sustained component. The transient current was activated at -30 mV (peak at -10 mV) and exhibited voltage-dependent inactivation (half inactiv. at -30 mV). The sustained current was activated at -10 mV (peak at +20 mV) and could be isolated from the transient by holding the cell at -30 mV. Cadmium (20 μM) blocked 35$\pm$5% of the transient current and 95$\pm$3% of the sustained current (n=5-6). At a holding potential of -80 mV, 1 μM nimodipine blocked 18$\pm$5% of the transient current and 47$\pm$11% of the sustained current (n=5-6). Perfusion of F-11 cells with the opioid agonist DPDPE (30 nM) blocked 17$\pm$11% of the transient current and 16$\pm$6% of the sustained current (n=5-6). The percent block was not reduced further by increasing the concentration of DPDPE to up to 5 μM or by using other opioids (DAGO, Dynorphin A, U50,488). Thus, similar to DRG neurons from which this cell line is derived, a small fraction of the Ba current is blocked by opioids. (Supported by NS23804)

56.8

Possible existence of two types of Ca^{++} channels in bovine chromaffin cells. **R.Y.K. Pun and J. Picone* Dept. of Physiol. and Biophys., Coll. of Med., Univ. of Cincinnati, Cincinnati, OH 45267.**

There is ample evidence implicating the presence of multiple Ca^{++} channel subtypes. To determine whether release of catecholamines in chromaffin cells is mediated by a specific Ca^{++} channel, we studied the Ca^{++} currents in these cells using whole-cell "tight-seal" voltage clamp technique. Bath solutions contained (in mM): 5 Ca^{++}, 2 μM TTX, 100 TEA; and 110 Cs, 25 TEA, 2 ATP, 5 creatinine phosphate, 1.1 EGTA in the pipette solution. All drugs were applied from a perfusion pipette placed near the cell. At a holding potential (V_h) of -90 mV, depolarizing steps to between -30 and -10 mV evoked a sustained inward current. Depolarizing steps to between -10 and 0 mV evoked a large inward current which peaked within a few msec of the step and decayed slowly. This current was blocked by the addition of Mn^{++} (2 mM) or removal of Ca^{++} from the perfusion medium. In some cells, at a V_h of -90 mV, both nitrendipine (1 μM) and nifedipine (1-10 μM) enhanced the sustained inward current and slightly attenuated the slowly decaying current. When the V_h of the same cell was changed to -30 mV, the inward current evoked during depolarizing steps was greatly attenuated by the dihydropyridines. Peak current-voltage plots further showed that in the presence of Cd^{++} (5-20 μM), currents evoked positive to 0 mV from a V_h of -90 mV were blocked revealing an outward current. An inward current could still be recorded at negative potentials. Our results indicate that in chromaffin cells there may exist two types of Ca^{++} channels.

56.9

SPATIAL HETEROGENEITY OF NEURONAL CALCIUM CURRENTS. Man-Son-Hing, H.J. and Haydon, P.G. Department of Zoology, Iowa State University, Ames, IA 50011.

While neuronal somata are known to contain multiple types of calcium currents, it is unclear whether all types of calcium currents are distributed throughout the neuronal arbor, or whether they exist only in localized regions. To address this question, neurons were isolated from adult specimens of *Helisoma*, and plated in cell culture where the macroscopic calcium currents of the soma and growth cone were examined.

Acutely-isolated somata and somata which had extended neurites were found to contain three types of voltage-sensitive calcium currents. Using electrophysiological and pharmacological manipulations, the macroscopic current was separated into its three components: a low-voltage-activated decaying current, a high-voltage-activated decaying current, and a high-voltage-activated sustained current.

Cell bodies adjoined to their original axons were plated in culture. The first growth cone to arise from the axon was physically isolated and the local calcium current examined. Only a single type of calcium current was present in growth cone, a high-voltage-activated sustained current.

The high-voltage-activated sustained currents of the soma and the growth cone were both reduced by 10μM Cd^{++} and/10μM racemic Bay K8644. These data demonstrate that multiple types of calcium currents can be localized in the soma while the growth cone contains one specific type of current.

This work was supported by an NIH grant NS24233.

56.10

Ca CURRENT OF A PARASYMPATHETIC NEURON. R.B. Clark*, A. Tse and W.R. Giles (SPON: T.M. Dwyer). Dept. of Physiology, Faculty of Medicine, University of Calgary, Calgary, Canada.

Parasympathetic neurons from the interatrial septum of bullfrog (Rana catesbeiana) heart were isolated with an enzymatic dispersion procedure (Tse et al, Soc. Neurosci. Abst. 13,535) and maintained in short-term (1-6 d) tissue culture. Ca currents were recorded with whole-cell patch clamp methods. Na and K-dependent currents in these cells were blocked by replacing external Na and K by either n-methyl-d-glucamine or tetraethylammonium (TEA), and by an internal pipette saline containing Cs-aspartate, TEA Cl and 5 mM EGTA.

Ca current activated near -40 mV, and reached peak values at about +10 mV. The peak current density in 2.5 mM external Ca, was 34 $\pm$ 3 μA/μF (S.D.; n=10); the capacitance of the same sample of cells was 22.9 $\pm$ 9.1 pF. The Ca current appeared to be predominantly "L-type", and it inactivated by 19.1 $\pm$ 5% (n=12) during 200-300 ms depolarizations to about +10 mV. The amplitude of the Ca current was reduced by up to 30% by depolarizing prepulses of 0.5-0.9 sec in duration, but only for prepulse potentials more positive than about -10 mV, i.e. in the range where significant Ca current was activated. "T-type" currents could not be detected, even after holding at hyperpolarized potentials (-80 to -100 mV) for 1-3 min. The peak of the I-V relation was shifted negative by about 10 mV when Ca was replaced by a equimolar amount of Ba, but the peak current magnitudes were similar. After Ca replacement, the time course of inactivation of Ba current was essentially unchanged. These data suggest that inactivation of the Ca current in these neurons results from a small component of "N-type" Ca channels.

56.11

TRANSIENT CALCIUM CURRENT OF SOLITARY BIPOLAR CELLS FROM THE MOUSE. L. H. Pinto, A. Kaneko and M. Tachibana. Northwestern University, Evanston, IL 60201, and National Institute for Physiological Sciences, Okazaki 444, Japan.

We obtained solitary bipolar cells by enzymatic (papain) dissociation of the adult mouse (C57BL/6J) retina and measured the membrane currents of these cells by whole-cell patch clamp. Hyperpolarization from the holding voltage, V_h, of -40 mV evoked a slowly-activated, Cs-sensitive, inward current (probably an h-current), and depolarization evoked a TEA-sensitive, outward current (probably a combination of $I_{K(V)}$ and $I_{K(Ca)}$). Depolarization from more negative V_h (*e.g.*, -80 mV) evoked a transient inward current that had maximal amplitude between -40 and -20 mV. This current was identified as a Ca current: its amplitude was increased with elevated $[Ca^{2+}]_o$ and was decreased with reduced $[Ca^{2+}]_o$, and it was blocked by Co^{2+} (4 mM), but not by TTX (5 μM). The Ca current was insensitive to Cd^{2+} (50 μM) and ω-conotoxin (1 μM). The Ca conductance was activated by voltage steps to potentials >-60 mV and inactivated fully at potentials >-20 mV. This voltage range includes the presumed physiological range; thus, this current may participate in synaptic transmission. The transient character of the current may also help to shape transient responses of ganglion cells. (Supported by NEI R01EY01221, NSF INT8613447 and Japan Society for the Promotion of Science)

56.12

CALCIUM CURRENTS IN COCHLEAR HAIR CELLS OF THE DOMESTIC CHICK. P.A. Fuchs and M.G. Evans*, Dept. Physiology, C240 Univ. Colorado Med. School., Denver, CO 80262.

We have examined calcium currents (I_{Ca}) in hair cells isolated from apical and basal regions of the chick's cochlea. Solitary tall hair cells were voltage-clamped using whole-cell tight-seal recording methods. Large, non-inactivating, outward K currents (these are Ca-activated K currents (I_{Kc}) in basal cells) dominate the net current-voltage relationship. Therefore, inward currents were studied under a variety of experimental conditions designed to eliminate the larger outward currents. Inward currents were measured in some cells in the presence of 10-20 mM tetra-ethyl ammonium (TEA) or in solutions in which Ba substituted for Ca. In both of these conditions I_{Kc} was eliminated. Alternatively, substitution of internal K by Cs eliminated outward currents. This last procedure proved to be necessary for cells from the cochlear apex with large voltage-activated K currents that were insensitive to external TEA and barium. When outward currents were blocked the residual inward current required external Ca but also could be carried by Ba. I_{Ca} activated positive to -50 mV and reached maximum amplitudes of 75 pA (in 5.6 mM Ca) at 0 mV. When care was taken to block all outward current, no inactivation of I_{Ca} was evident for at least 200 msec. The activation time constant (derived from the fit of a squared exponential) decreased with depolarization and was approximately 0.4 msec at -10 mV (23 °C) in all cells examined. There appeared to be no significant differences in magnitude or kinetics of I_{Ca} in apical versus basal hair cells. Supported by grants NS21454 and NS01007 from NIH/NINCDS.

56.13

VERY SLOW, VOLTAGE-DEPENDENT INACTIVATION OF CA CURRENT IN INSULIN-SECRETING HIT CELLS. L.S. Satin* and D.L. Cook* (SPON: S. Fatherazi). Depts. of Physiol/Biophys and Medicine, Univ. of Wash. and VA Medical Ctr, Seattle, WA 98108.

The whole-cell patch clamp technique was used to voltage clamp single cultured HIT cells, a pancreatic β-cell line which secretes insulin in response to glucose. Na current was blocked by TTX (.5μM), outward currents by internal Cs (140mM), 4-AP (10mM), EGTA (1mM) and ATP (2mM) and external TEA (10mM). Depolarization-activated Ca currents inactivated in two phases: about 50% in <100 msec; the remaining in >10 seconds. The fast phase appears to depend on Ca influx since it was prolonged by replacing Ca with Ba and was proportional to Ca current but not depolarization. In contrast, the slow phase was voltage-dependent since long depolarizations inactivated the current in the absence of Ca influx (e.g. below the ICa threshold and above its reversal as well as in Ba). Recovery of inactivated Ca current was biphasic, consisting of rapid (<2 secs) and much slower (tens of seconds) components. Fast and slow inactivation may correspond to low and high-threshold Ca currents respectively (Satin and Cook, Pflugers Archiv, in press) since: 1) the rapid phase predominated at relatively low depolarizations and 2) 100 msec prepulses inactivated the rapid phase to reveal a slowly inactivating high threshold component. These properties may be useful for understanding the electrical activity observed in pancreatic β-cells in response to elevated glucose.

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56.14

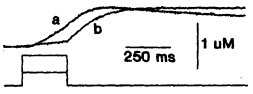
CALCIUM CURRENT IN PARASYMPATHETICALLY ANEURAL CHICK HEARTS. Tony L. Creazzo* (SPON: D. Stoney). Dept. of Anatomy, Medical College of Georgia, Augusta, GA 30912.

Chick hearts were made parasympathetically aneural by bilateral ablation of the nodose placodes and the neural crest which seeds pharyngeal arches 1-3 at H.H. stages 8 or 9 (Kirby M.L., J. Neurosci., in press). In conjunction with an absence of intracardiac ganglion cells the hearts from lesioned embryos suffer from failure of conal truncal septation (persistent truncus arteriosis; PTA). Therefore, hearts were used for these experiments only if diagnosed as having PTA. The atria and ventricles from 3 sham operated and 3 lesioned embryos were separated and enzymatically dissociated to yield single cells. A total of 33 cells from sham and experimental atria and ventricles were examined using the whole cell variation of the patch clamp. K+ currents were blocked by replacing K+ with Cs+. The extracellular solution contained 1.8 mM Ca++ and 3 μM TTX to block Na+ current. Inward Ca++ current (ICa) was elicited with 400 msec depolarizing pulses from Ehold = -80 mV to potentials ranging from -60 to 60 mV in 10 mV increments. Peak ICa was about 30% greater in experimental ventricles when compared to the sham at depolarizing potentials from -40 to 0 mV while not significantly different at positive potentials. At 0 mV the peak current density was 3.2 +0.7 pF/pA (SEM, n=7) and 2.1+0.4 pF/pA (n=8). Peak ICa in experimental atria was not significantly different from sham. Currently, experiments are underway to determine whether the increased ICa is due to the absence of innervation, the PTA or both.

56.15

TUBULAR INWARD CALCIUM CURRENT CAN DIRECTLY CONTRIBUTE TO MYOPLASMIC CALCIUM TRANSIENTS. E. Stefani, M. Amador* & J. García*. Baylor College of Medicine, Dept. Physiology and Molecular Biophysics, One Baylor Plaza, Houston, TX, 77030.

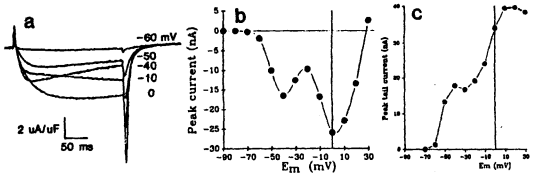
To detect any contribution of inward Ca currents to the myoplasmic Ca transients, it becomes necessary to reduce Ca release and removal. Experiments were performed in cut single skeletal fibers isolated from the semitendinosus muscle of Rana pipiens by using the indicator dye Antipyrylazo III. The fiber was held at -90 mV with the vaseline gap voltage clamp technique; temperature= 16 °C. The intracellular solution contained 1.0 mM-EGTA. Ca transients progressively decreased and became slower after repetitive stimulation (0.02 to 0.03 Hz) with 250 ms pulses to 0 mV. As Ca transients became smaller, it was more evident their relation with the amplitude and the time integral of the inward Ca current. Ca transient amplitude followed the electromotive force for Ca; during a large pulse to +100 mV they were greatly reduced and became large after repolarization during the inward tail current. In agreement, with the addition of 2 μM-Bay K 8644, Ca transient increased parallel to the inward current. The figure shows the Ca transients obtained with Bay K to pulses delivered to a: 0 mV and b: +100 mV. Supported by NIH.



56.16

TWO TYPES OF CALCIUM CURRENTS IN MAMMALIAN SKELETAL MUSCLE. J. García*, R. Gamboa-Aldeco*+ and E. Stefani (Spon: E. R. Decker) Baylor College of Medicine, Dept. Physiology and Molecular Biophysics, One Baylor Plaza, Houston, TX, 77030.

Voltage clamp experiments were made in cut single fibers from extensor digitorum longus muscle from rats and rabbits using the vaseline gap technique. Holding potential was -90 mV. External solution contained 2 mM-Ca. Two populations of calcium channels were found according to their kinetics and voltage dependence (fig. a). A low threshold current was detected at -70 mV in 21 out of 31 fibers from rats, reached its maximum amplitude (-1.9 uA/uF) at about -30 mV, and the time constant of deactivation at -70 mV was 80 ms. A higher threshold current was detected at -20 mV, its maximum (-6 uA/uF) was at 0 mV (fig. b), and the time constant of deactivation at -70 mV was 10 ms. This current was DHP-sensitive: it was blocked by 10 uM-nifedipine and potentiated by (-)-Bay K 8644 (1 uM). Peak tail amplitudes showed two plateaus, at -40 mV and 10 mV, corresponding to each current (fig. c). In 4 out of 5 fibers from rabbits both currents with similar properties were found. Supported by NIH.
+U. Juarez Autonoma de Tabasco Fellowship.



ION CHANNELS: MODULATION AND REGULATION I

57.1

EARLY APPEARANCE OF NEUROMODULATORY MECHANISMS IN THE EMBRYONIC CHICK CHOROID. D. Zelazny* & D.B. Gray. Dept. Physiol. & Neurobiol., Univ. Connecticut, 75 N. Eagleville Rd., Storrs, CT 06268.

Somatostatin (SS) and morphine have recently been shown to completely inhibit K+-evoked release of ^{3}H-ACh from isolated nerve terminals of the ciliary ganglion (CG) in the choroid layer of the chick eye (Gray, D. & G. Pilar, Soc. Neurosci. Abstr., 13: 790, 1987). To determine if this capacity for neuromodulation is present in immature synapses, ^{3}H-ACh release from CG terminals in St 40 (E 14) choroid was measured in normal and 55mM K+ Tyrodes. In these preparations, K+-evoked release per choroid was 5-10 fold lower than after hatching. Only 30% of ^{3}H-choline uptake (in 1μM choline) was sensitive to 0.5μM hemicholinium (a specific antagonist of Na+-dependent, high-affinity choline uptake) at St 40 as opposed to 85% at hatching. In spite of these differences, K+-evoked release of ^{3}H-ACh from CG terminals in the choroid at St 40 is abolished by both 100nM SS and 10μM morphine. As in the hatchling chick this inhibition of ACh release is sensitive to a 1-hr preincubation in pertussis toxin (200 units/ml). These studies suggest that even in immature synapses between CG neurons and vascular smooth muscle in the choroid, the membrane apparatus mediating neuromodulation is already in place.

Supported by NSF grant#BNS 8410581 and NIH grant #NS 10338.

57.2

THE TRANSIENT K+ OUTWARD CURRENT DEVELOPS LATER THAN THE DELAYED K+ CURRENT IN THE RAT PINEAL CELL. Luis G. Aguayo and Forrest F. Weight. Section of Electrophysiology, Laboratory of Physiologic and Pharmacologic Studies, NIAAA, Rockville, Md 20852.

The outward currents of pineal cells acutely dissociated from Sprague-Dawley rats aged 1 and 2 weeks were studied with the whole-cell variation of the patch-clamp technique and compared with those of adult rats. All adult cells displayed both a transient and a delayed voltage-activated K^+ outward current. The delayed current, which was activated from a holding potential of -50 mV, had an activation threshold of -20 mV. This current was activated in all the cells from 1-week-old rats and the amplitude was about 70% of the amplitude in adult cells. In young and adult cells no evidence for a Ca^{2+}-activated K^+ current was found.

The transient current was activated from a holding potential of -100 mV with commands potentials positive to -60 mV. With more positive holding potentials the transient current displayed voltage-dependent inactivation with 50% inactivation at -76 mV. The transient current was observed in only 40% of the cells dissociated from 1-week-old rats. The peak amplitude of the transient current measured in cells from rats up to 2 weeks of age was no more than 25% of the amplitude in adult rats. Comparison of the time course and voltage dependence of the delayed and transient currents in young and adult cells showed no significant differences. The appearance of the transient current was associated with a marked acceleration of the rate of decay of the voltage response produced by a depolarizing constant current pulse.

The results suggest that the K^+ channels carrying the transient current in pineal cells are expressed later than those carrying the delayed current.

57.3

LONG-TERM REGULATION OF POTASSIUM CHANNELS IN PC12 PHEOCHROMOCYTOMA CELLS BY NERVE GROWTH FACTOR. B. Rudy and J.D. Pollock. Dept. of Physiol & Biophys., NYU Med. Ctr., New York, NY 10016 and Division of Biology, Caltech, Pasadena, CA 91125

PC12 cells are a clonal cell line isolated from a rat pheochromocytoma (L.A. Greene and A.S. Tischler, PNAS, 73: 2424, 1976). In the presence of nerve growth factor (NGF) PC12 cells cease dividing and differentiate from chromaffin-like cells to sympathetic-like neurons. As PC12 cells differentiate the amount and types of sodium and calcium channels expressed changes (B. Rudy, J. Neurosci.2: 1405, and S. Kongsamut, PNAS 83: 2243, 1986). We have explored if the amount and types of potassium channels expressed in PC12 cells is altered after long term NGF treatment.

Ten day NGF treatment of PC12 cells increased the net outward current fourfold without changing the current density. However, a significant change in potassium current kinetics is observed after ten day treatment with NGF. Undifferentiated PC12 cells show a transient outward current that is elicited by commands to 0 mV or greater from a holding potential of -90 mV. The total current decays by 31.3 $\pm$ 1.02% n=30 at the end of a 400 ms step to 30 mv. The transient component is sensitive to 200 μM TEA. We have identified a 21 pS channel whose ensemble average resembles the macroscopic current observed in undifferentiated cells. The transient component is no longer apparent in PC12 cells treated with NGF for ten days and appears to be replaced or obfuscated by a more slowly inactivating current. In these cells the total potassium current decays by 12.9 $\pm$ 1.8% n=16 at the end of a 400 ms step to 30 mV. We have observed a 12.5 pS channel whose ensemble average resembles the macroscopic current in NGF differentiated cells.

57.4

HUMAN AND BOVINE bFGF BUT NOT cAMP INDUCE SODIUM CHANNELS IN PC12 PHEOCHROMOCYTOMA CELLS. J.D. Pollock, M. Krempin*, and B. Rudy (Caltech, Pasadena, Ca. and NYU School of Medicine, NY, NY)

Nerve growth factor (NGF) has been previously reported to induce neurite outgrowth and sodium-dependent action potentials in PC12 cells (L.A. Greene and A.S. Tischler. PNAS, 73: 2424, 1976 and B. Rudy, et al., J. Neurosci., 2:1405, 1982). The effect of NGF on neurite outgrowth can be mimicked with human and bovine basic fibroblast growth factor (bFGF) (R. Rydel and L.A. Greene, J. Neurosci., 7: 3639, 1987). Other substances such as EGF and cAMP lead to some of the early events elicited by NGF but not to later differentiating events (D.G.B. Leonard, et al., Mol. and Cell. Biol. 7: 3156, 1987). Since induction of sodium channels is a key feature of the late neuronal-like differentiation of PC12 cells by NGF we asked the question whether bFGF or cAMP also induces sodium channels in PC12 cells.

Ten day treatment of PC12 cells with either 50 ng/ml human bFGF or 10 ng/ml bovine bFGF caused neurite outgrowth and a significant increase in sodium current and sodium current density. The sodium current in bFGF cells starts to activate at -50mV and peaks at about -10 mV. The midpoint for steady-state inactivation of the sodium current is around -70 mV and can be completely blocked with 1 uM TTX. Single channel recording of sodium channel currents suggest a single channel conductance of 10-11 pS. On the other hand, 300 uM treatment with CPT-cAMP for ten days failed to induce sodium currents.

These results suggest that sodium channel is a final common pathway of late gene expression induced by bFGF and NGF but not cAMP. Moreover, any possible elevation of cAMP by NGF or bFGF is not by itself sufficient for the induction of sodium channels in PC12 cells.

57.5

REGULATION OF IONIC CURRENTS IN PC12 CELLS BY NERVE GROWTH FACTOR AND DEXAMETHASONE. S. S. Garber, T. H. Hoshi*, and R.W. Aldrich, Dept. of Neurobiology, Stanford Univ., Stanford, CA 94305-5401

Differentiation of pheochromocytoma (PC12) cells can be influenced by growth factors and hormones. On application of Nerve Growth Factor(NGF), PC12 cells extend neurites and become morphologically similar to sympathetic ganglion cells (SGCs), whereas incubation of PC12 cells with a steroid, dexamethasone (DEX), causes an increase in tyrosine hydroxylase activity suggesting that the cells become chromaffin cell-like. SGC's are known to express voltage-dependent Na^+ currents and more then one type of Ca^{++} current. Chromaffin cells express voltage-dependent Na^+ current and only "L-type" Ca^{++} current. We measured voltage-dependent Na^+ currents (23%) and transient "T-type" Ca^{++} currents (20%) were expressed by only a fraction of PC12 cells grown for up to 21 days in the absence of either NGF or DEX. In the presence of 200 ng/ml NGF, the percent of cells with Na^+ currents increased within 7 days to 94%, as has been previously documented. The fraction of cells with "T-type" Ca^{++} current increased to 46% after 7 days of exposure to NGF. Cells incubated with 1μM DEX become large and irregular in shape over 2-3 weeks. Cells grown up to 21 days in DEX, showed an increase over control cells in the expression of Na^+ current (75% over 23%) and a small but significant increase in "T-type" Ca^{++} current (40% over 20%). After 21 days, this difference declines because of a gradual increase in the fraction of control cells expressing Na^+ and "T-type" Ca^{++} current. Incubation of cells in both NGF and DEX prevented the rapid increase in the appearance of the currents observed with NGF alone. All cells (control, NGF and DEX) retained "L-type" Ca^{++} current and no changes in K^+ currents were observed. These results suggest that the temporal expression of ionic currents are differentially regulated by NGF and DEX. Supported by NS23294, NS07158, and a NIH postdoctoral fellowship to T.H.

57.6

HORMONAL CONTROL OF MEMBRANE CURRENTS IN SOMATOTROPHS. Stephen M. Sims* and Jacob Kraicer*. Dept of Physiology, Univ. of Western Ontario, London, Ontario, Canada. N6A 5C1 (SPON: P. Grigg)

Control of growth hormone secretion was studied with patch-clamp recording of freshly dispersed, normal rat somatotrophs. In whole-cell voltage-clamp mode, depolarization elicited a number of currents, including a transient inward Na^+ current, a slowly inactivating Ca^{++} current, and outward K^+ current. Inward Ca^{++} channel current was studied in isolation with Cs^+ in the recording pipette and 10 mM Ba^{++} in the bath. The inward current, which was fully blocked with 1 mM Cd^{++}, inactivated slowly during 100 ms test depolarizations and was partially blocked by nifedipine. Outward currents were studied with K^+ in the patch electrode, and evidence was consistent with K^+ being the charge carrier. The reversal potential for tail currents was close to the predicted K^+ equilibrium potential, and shifted 56 mV/10 fold change of $[K^+]_{out}$. Furthermore, TEA blocked the outward current. Cd^{++} reduced the K^+ current, suggesting the presence of Ca^{++}-activated K^+ current. In these respects normal rat somatotrophs resemble clonal growth hormone secreting cell lines,(eg. GH_5/GH_4). Growth Hormone Releasing Factor (GRF) (2-76 nM) reversibly suppressed outward K^+ current, an effect which could underlie the excitatory, depolarizing action of GRF, and trigger growth hormone release. (Supported by MRC Canada)

57.7

CIS-UNSATURATED FATTY ACIDS ATTENUATE VOLTAGE-DEPENDENT SODIUM CURRENT IN N1E-115 NEUROBLASTOMA CELLS. D.J. Linden* and A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, IL 60208.

Cis-unsaturated fatty acids such as oleic acid have been shown to modulate synaptic efficacy (J. Neurosci. 7:3783) and to activate purified protein kinase C (PKC) in vitro (J. Biol. Chem. 261:15424). We wished to determine the ionic basis of this modulation and its potential link to PKC in a simplified system and thus turned to the whole-cell voltage clamp preparation, using the N1E-115 neuroblastoma cell differentiated in medium containing 4% DMSO.

Oleic acid bath-applied at 5 uM produced no alteration of the resting potential (-45 mV) and only a slight decrease of the baseline input resistence (135 megohm). Analysis of families of currents evoked under voltage clamp by depolarizing steps from a holding potential of -85 mV during application of 5uM oleate showed a 40% reduction in the peak inward current with no appreciable shift in the I-V relation, and no detectable alteration of outward current. Na current was then isolated by application of external Mg, internal F and TEA and replacement of internal K with N-methylglucamine (Ikeda et al., J. Neurophys. 55:527). This isolated current was completely and reversibly abolished by TTX or removal of external Na. The cis-unsaturated fatty acids oleate, linolenate and linoleate were seen to attenuate isolated Na current with approximate ED_{50}'s of 5, 5, and 20 uM respectively. Elaidate (a trans-unsaturated isomer of oleate) and stearate (a saturated fatty acid), which do not activate PKC, had no effect on the isolated Na current when applied at concentrations of 1-50 uM. Since cis-unsaturated fatty acids are known to fluidize membranes as well as to activate PKC, we sought to dissociate these functions by applying compounds that fluidize membranes but do not activate PKC: methyloleate and lysophosphatidylcholine. Neither compound affected Na current when applied at concentrations of 1-50 uM, consistent with the view that the reduction of Na current by cis-unsaturated fatty acids is mediated by activation of PKC. (Supported by MH25281-13 and AFOSR87-0042 to A.R. and an Air Force Laboratory Graduate Fellowship to D.J.L.)

57.8

ALTERED MEMBRANE FATTY ACID COMPOSITION MODIFIES NA-CHANNEL INACTIVATION. C.C. Park* and Z. Ahmed. Dept. of Physiology, State Univ. of New York, Buffalo, NY 14214.

Dissociated embryonic neurons (E16) from rat diencephalon were grown in a defined medium. Membrane fatty acid composition was modified by supplementing culture media with either the n-3 or n-6 class of polyunsaturated fatty acid (PUFA). Lipid analysis showed that the ratio of PUFA to saturated fatty acid (SFA) was increased about 2 fold. This increase was due to a class-specific incorporation of PUFA.

Electrophysiological studies were performed on 5-10 days old cultures using the whole-cell voltage-clamp technique. Neurons grown in PUFA supplemented media showed an age-dependent increase in input capacitance and a decrease in input resistance. Control cultures showed no significant change. The peak-Na current-voltage relationships in the supplemented and control cultures were not significantly different. The inactivation time course of Na-currents can be described by a sum of two exponentials with a slow (tl) and a fast (t2) time constant. In PUFA supplemented cultures, tl was reduced 40% from that of the control while t2 was not significantly affected.

These effects are not due to the metabolites of the n-6 class of PUFA since they occurred with both classes. Rather they are probably due to the increased ratio of PUFA/SFA in the membrane. Supported by NSF Grant BNS 84-17003.

57.9

REGULATION OF MEMBRANE-MEDIATED CHRONIC MUSCLE DEGENERATION IN DYSTROPHIC HAMSTERS (DH) BY ATP-$MgCl_2$. Syamal K. Bhattacharya and Denise M. Riedel*. Surgical Research Laboratories, University of Tennessee, Memphis, TN 38163.

Membrane-mediated excessive intracellular Ca accumulation (EICA) appears to be a fundamental pathogenetic event associated with chronic muscle degeneration in humans and hamsters with hereditary muscular dystrophy (Muscle & Nerve, 10:168, 1987). Dystrophic muscle is deficient in ATP which stabilizes the membrane fluidity and fuels the intracellular Ca-pump in the regulation of excessive myoplasmic Ca^{2+}. Hypothesizing that ATP and $MgCl_2$ possess membrane stabilizing and Ca-antagonistic properties, we studied whether subcutaneous (sc) ATP-$MgCl_2$ could regulate EICA and improve the associated dystrophic lesions in the heart, diaphragm, and rectus femoris of CHF-146 strain DH. Equimolar ATP-$MgCl_2$ (100 micromoles/100 g BW/day) was administered sc to 8 DH and 8 CHF-148 strain normal hamsters (NH) for 87 days, beginning at the age of 20 days. Six DH and 5 NH served as vehicle controls. Muscle and plasma [Ca], [Mg], and [P_i] as well as plasma CK were determined. Histologic evaluation was also performed on the tissues. The ATP-$MgCl_2$ treated DH showed significant reduction in EICA, plasma CK activity and overall improvement in the muscle morphology and phosphate sequestration. We conclude that ATP-$MgCl_2$ lowers EICA and improves membrane-mediated dystrophic lesions in DH and thus, may have therapeutic applications in the regulation of dystrophic pathobiology in humans. (Supported by NIH grant #AR-38540)

57.10

EFFECT OF ALTERATIONS IN EXTRACELLULAR MAGNESIUM CONCENTRATION ON EXCITABILITY AND LTP IN THE RAT HIPPOCAMPAL SLICE. K.E. VanLandingham, J.M. Stringer, and E.W. Lothman. Dept. of Neurology, University of Virginia Medical Center, Charlottesville, VA 22908.

Extracellular magnesium ($[Mg^{++}]_o$) is known to regulate NMDA receptors and synaptic transmission in the central nervous system. In the hippocampal slice, removing $[Mg^{++}]_o$ causes epileptiform activity and high $[Mg^{++}]_o$ suppresses stimulus induced responses. However, intermediate variations in $[Mg^{++}]_o$ have not been studied systematically. We examined the effects of 0, 0.5, 1.0, 1.5(control), 2.5, and 3.5mM $[Mg^{++}]_o$ on input-output (I/O) curves, long-term potentiation (LTP), and epileptiform activity in the CA1 region of rat hippocampal slices. Extracellular calcium and potassium were kept at 1.5 and 3.1mM, respectively. $[Mg^{++}]_o$ below 1.5 mM always caused a left shift in the I/O curve and an increase in the maximal population spike (PS) amplitude. Raising $[Mg^{++}]_o$ above 1.5mM always caused a right shift of the I/O curve and a decrease in the maximal PS amplitude. LTP was completely blocked in 3.5mM $[Mg^{++}]_o$, suppressed in 2.5mM, but constant in $[Mg^{++}]_o \leq 1.5$mM. Spontaneous or stimulus-locked epileptiform bursts were present in $[Mg^{++}]_o$ lower than 1.5mM, but never in 1.5mM or higher. The percentage of slices showing spontaneous epileptiform discharges was: 79% (0mM), 40% (0.5mM), and 9% (1.0mM) and the percentage of slices showing only stimulus-locked bursts was: 7% (0mM), 50% (0.5mM), and 45% (1.0mM).

57.11

FAST AXONAL TRANSPORT SPEED AUGMENTATION BY Ca^{++} CHANNEL AGONISTS. M.B. Atkinson*, C.M. Ferrario and A.C. Breuer (SPON: D. Averill). Depts. of Neurology and Brain and Vascular Research, Cleveland Clinic Foundation, Cleveland, OH 44195.

The interruption of fast axonal transport (FAT) by Ca^{++} depletion (Ca^{++} free buffer or Ca^{++} channel antagonists) has been documented by several investigators (Ochs, S., *et al*, Nature, 270:748-750, 1977; Lavoie, P.A., *et al*, J. Neurochem, 32:1745-1751, 1979). We report here on the results of increased Ca^{++} flux into the axons to further modify FAT rates.

Axonal preparations from male Sprague-Dawley rats were treated with either 1 μM BAY k 8644 (a Ca^{++} channel agonist) or depolarized by 50 mM K^+ (to open Ca^{++} channels). Axonal transport was then video-recorded under DIC optics for later analysis by computer methods. BAY k treatment resulted in an anterograde (ANTERO) FAT speed increase of 9.9 ± 1.9% ($p < 0.05$) and a retrograde (RETRO) FAT increase of 9.5 ± 1.1% ($p < 0.001$). K^+ depolarization increased the ANTERO and RETRO FAT speed by 14.9 ± 1.9% ($p < 0.001$) and 10.6 ± 1.6% ($p < 0.001$) respectively. Pre-treatment of the axonal preparations with nitrendipine (a calcium channel antagonist) with subsequent exposure to either BAY k or K^+ depolarization completely eliminated the observed FAT speed increases (not significantly different from controls in either direction). These results substantiate a critical role for calcium in the modulation of fast axonal transport speeds.

57.12

DIFFERENT EFFECTS OF CAPSAICIN ON SUBTYPES OF DORSAL ROOT GANGLION NEURONS IN VITRO. M.J. McLean and S.E. Barron. Departments of Neurology and Pharmacology, Vanderbilt Univ. Med. Ctr., Nashville, TN 37212.

Subtypes of adult mouse dorsal root ganglion neurons (DRGn) in monolayer dissociated cell culture were recognized by comparison of action potential (AP) waveforms with those of DRGn in intact ganglia whose type was determined by conduction velocity and stimulus specificity (Lawson and Harper, J. Physiol., 1985; Rose et al., Neurosci. Lett., 1986). Neurons (SDn) which fired one or a few short-duration (2 msec), tetrodotoxin (TTX)-sensitive APs during depolarizing steps resembled mechanoceptors in situ. Neurons in cell culture (LDn) which sustained repetitive firing of long-duration (2-5 msec), TTX-resistant APs resembled nociceptors in situ. Action potentials of both SDn and LDn were sodium-dependent. Pressure ejection of capsaicin (CAP, 1 uM) onto quiescent LDn, but not SDn, caused depolarization and AP firing. Firing of depolarization-elicited APs by SDn was reversibly abolished by CAP applied in Ca^{++}-containing, but not Ca^{++}-free, solution. Depolarization of LDn was less when CAP was applied in Ca^{++}-free solution, but was not abolished. These findings suggest different mechanisms of action of CAP on functionally distinct DRGn.

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57.13

EFFECTS OF LIDOCAINE AND SAXITOXIN ON THE TETRODOTOXIN-RESISTANT CURRENT OF MAMMALIAN DORSAL ROOT GANGLION NEURONS. S.E.Barron, P.B.Bennett* and M.J.McLean. Depts of Neurol. and Pharm., Vanderbilt Univ. Sch. Med., Nashville,TN 37212.

Freshly dispersed dorsal root ganglion neurons (DRGn) have tetrodotoxin (TTX)-resistant and sensitive sodium currents (I_{Na})in varying proportion (McLean et al.,1987). Neurons with a large proportion of TTX-resistant I_{Na} (I_{Na}-R) resemble nociceptor-like DRGn in cell culture which fire TTX-resistant action potentials. Effects on the I_{Na}-R may help determine cellular mechanisms of action of analgesic drugs such as lidocaine (LIDO). To characterize the I_{Na}-R further, whole cell currents were recorded from dispersed adult mouse DRGn. TTX(3uM), saxitoxin(STX,1uM) and LIDO(37uM) were added to the extracellular solution (in mM: NaCl 65;CsCl 65;$MgCl_2$ 2;$CaCl_2$ 1;dextrose 10;TEA-Cl 10;HEPES 10; and pH 7.35) at 15 to 18°C. Patch pipettes were filled with solution containing (in mM:CsF 110;NaF 10;CsCl 30;$MgCl_2$ 2;EGTA 2; HEPES 10; and pH 7.35). The I_{Na}-R was reduced tonically by STX and both tonically and in use-dependent manner by LIDO. Thus, LIDO produces use dependent block of a unique I_{Na}-R of DRGn,similar to its blockade of nerve TTX-sensitive I_{Na}. Supp: Mallinckrodt Fellowship and NIH Grant #NS01194.

57.14

INHIBITION OF THE Na^+-Ca^{++} EXCHANGE SYSTEM BY AMILORIDE ANALOGUES STIMULATES DOPAMINE RELEASE FROM TUBEROINFUNDIBULAR HYPOTHALAMIC NEURONS. L.Annunziato, L.M.T. Canzoniero*, S.Amoroso*, G.F.Di Renzo*, E.J.Cragoe*and M.Taglialatela* Inst. Pharmacology,Univ.Naples and Chieti,Italy,and Merck Sharp & Dohme,Res.Lab.,West Point,PA 19486,USA.

In the last years a great deal of attention has been pa to the membrane Na^+-Ca^{++} exchange system as an important regulator of Ca^{++} transport across the neuronal membrane. In the present study we investigated the effect of amiloride and of some of its derivatives, which are provided with different inhibitory potency in inhibiting the Na^+-Ca^{++} antiporter, on ^{3}H-dopamine (^{3}H-DA) release from superfused tuberoinfundibular hypothalamic (TIDA) neurons. Amiloride (0.3-1mM) and its 2',4'-dimethylbenzamyl derivative (2',4'-DMB)(30-300uM),which inhibited Na^+-dependent Ca^{++} uptake in synaptosomes obtained from TIDA neurons, dose-dependently stimulated ^{3}H-DA release from these neurons. Furthermore, 2',4'-DMB (50uM) was able to prevent the stimulatory effect on ^{3}H-DA release elicited by the cardioactive glycoside ouabain (0.5mM),which is known to promote neurotrasmitter release through the stimulation of Ca^{++} entrance into the neurons mediated by the Na^+-Ca^{++} antiporter. On the other hand, 5-N-methyl-N-(guanidinocarbonylmethyl)-amiloride, an amiloride derivative which lacks of inhibitory properties on the Na^+-Ca^{++} exchange activity, but displays high selectivity in inhibiting the Na^+-H^+ antiporter, failed to modify basal release of ^{3}H-DA from TIDA neurons. Collectively, these results for the first time demonstrate that the inhibition of the Na^+-Ca^{++} exchange system can modulate neurotrasmitter release from central synapses.

57.15

ANTIBODY TO THE 45 kD GAP-JUNCTIONAL PROTEIN BLOCKS DYE COUPLING BETWEEN CARDIAC MYOCYTES. R. Lal, S. B. Yancey*, J.-P. Revel*, Division of Biology, Caltech,Pasadena, CA 91125

Gap junctions mediate rapid intercellular transfer of electrical signals and synchronous activity of the excitable tissues. In heart, the major constituent of the gap junction is believed to be a 45 kD protein. Here we present evidence that site-specific antibodies raised to a synthetic peptide corresponding to the first 20 amino acids of the 45 kD heart gap junction protein blocks intercellular communication between pairs of neonatal rat myocardial cells in culture. All experiments were conducted at room temperature, between 3-6 days of culture. In isolated pairs, one cell was iontophoretically injected with 5% Lucifer Yellow CH (LY) or 0.2% Texas Red (TR) in 150 mM LiCl. Dye transfer to the other cell of the pairs indicated normal coupling between myocardial cells in culture. In one cell of a dye-coupled pair, antiserum diluted 1:4 in 150 mM LiCl buffered to pH 7.6 with 10 mM HEPES was iontophoretically injected by a large (~100 nA) hyperpolarizing pulse at 1 Hz for 5 sec. After 3-5 mins.,the other cell of the pair was injected with the second dye. In 27 of 30 cell pairs which showed an intercellular transfer of the first dye (LY or TR) the injection of antiserum showed unequivocal blockage of transfer of the second dye (TR or LY). The remaining instances were questionable for various reasons. In 22 of another 25 dye-coupled pairs an identical injection of pre-immune serum had unequivocally no effect on transfer of the second dye. Thus our results are consistent with the notion that the heart gap junctions mediating cell-cell communication are formed by 45 kD proteins. Further our finding that the intracellular injection of the antibody disrupts dye-coupling indicates that the amino terminus of the protein resides in the cytoplasm and that this portion of the molecule may play role in regulating channel functions.Supported by NIH grant LH37109.

57.16

MODULATION OF APLYSIA CELL MEMBRANE RESISTANCE BY A SINUSOIDAL ELECTRIC FIELD. A.R. Sheppard, M.R. Wacker,* S.M. Bawin and W.R. Adey. Research Service, J.L. Pettis VA Medical Center, and Departments of Physiology, Neurosurgery and Surgery, Loma Linda University, Loma Linda, CA 92350.

Membrane resistance of silent cells of the Aplysia abdominal ganglion was studied by hyperpolarizing current clamps during a 4 h period which included two 0.5 h exposures to a 10-Hz, 1 V/m rms electric field (produced by constant current between Ag-AgCl electrodes in artificial seawater perfusate, ~22 C). Resting membrane potential and single electrode clamp data were obtained regularly by a minicomputer interfaced to a digital 'scope and microelectrode amplifier via custom hardware and software. Delayed, gradual reductions of 5 to 30% in membrane resistance occurred with a mean latency of 20 min after start of exposure. Often, the first exposure had little or no effect, but larger changes occurred during the second exposure. Cell resting membrane potential also changed, but the magnitude and direction of changes were not tightly linked to resistance changes. These results suggest a modulation of one or more membrane channels as a result of changes in slowly responding intracellular biochemical pathways. (Sponsors: Dep't of Energy (AI01-85CE76260) and General Motors Corporation.)

57.17

PATCH CRAMMING: A NEW METHOD FOR DETECTING INTRACELLULAR MESSENGERS IN INTACT CELLS. Richard H. Kramer Graduate Dept. of Biochemistry, Brandeis University, Waltham, MA 02254.

Intracellular messengers are ubiquitous regulators of cellular processes. However, little is known about the intracellular concentrations, dynamics, and spatial distributions of most of these molecules. To address these questions I have been developing a method for detecting intracellular messengers in intact cells. The "patch cram" method utilizes ion channels sensitive to a messenger as probes for detecting that molecule inside a cell. The method is conceptually simple: A conventional patch pipette is used to obtain a patch of membrane housing the "detector" ion channel, and the pipette is then "crammed" into the cytoplasm of a recipient cell. The activity of the detector ion channel will then reflect the intracellular concentrations of the messengers to which it is sensitive. Ion channels directly sensitive to Ca, cAMP, cGMP, and IP_3 have been described; hence all of these should be detectable with the patch cramming technique.

I have obtained detector ion channels either by 1) patch clamping a cell known to contain the detector ion channels, and then excising the patch (inside-out patch configuration), or 2) reconstituting the ion channel in an artificial lipid bilayer formed at the tip of the patch pipette ("tip-dipping"). Patches containing the detector channels have succesfully been crammed into neurons from Aplysia and Helix. Even though these patches were forceably thrust through the membrane of the recipient cell, in more than 75% of cases the patch continued to exhibit a gigaohm seal, and channel gating activity continued unabated for up to 20 min. In addition, the recipient cell often quickly recovered from the impalement, exhibiting normal resting potential, action potentials, and complex spontaneous behavior, such as bursting pacemaker activity.

In initial experiments, I have used a Ca-activated K channel from Helix neurons to detect intracellular Ca. After cramming the patch into a recipient Helix neuron, the channel open probability decreases as calcium diffuses away from the patch. If the patch is depolarized, channel open probability increases, and then quickly returns to baseline after the depolarization. In contrast, depolarizing the cell, either with action potentials, or under voltage-clamp, results in a increase in channel opening which long outlasts the depolarization. I suggest that this change in gating activity is due to increase in intracellular calcium induced by opening voltage-dependent calcium channels in the recipient cell. Ongoing experiments are aimed at confirming this suggestion. Additional experiments are aimed at detecting cyclic GMP in Helix neurons and in other cells, utilizing cyclic GMP detector ion channels from vertebrate rods.

Thanks to Irwin B. Levitan and NIH grant NS17910 for supporting this research.

57.18

DISCERNING MULTIPLE CONDUCTANCES USING A NOVEL METHOD TO ANALYZE VOLTAGE RECORDS. S.D. Hocherman,* R. Werman* and Y. Yarom* (SPON: A.J. Susswein), Department of Neurobiology, Hebrew University, Jerusalem 91 904, Israel.

We have developed a procedure which enables us to calculate the time course of a conductance change as well as its reversal potential from voltage recordings by calculating the neuronal input resistance at different times during a voltage response. We used this procedure to study the kinetics of the long afterhyperpolarization (*AHP*) that follows a train of action potentials in the vagal motoneuron in guinea pig brainstem slices. The *AHP*'s were superimposed on long hyperpolarizing pulses of varying intensities. The voltage-current relation of each sampled point (digitized at 500 *Hz*) was plotted to give the instantaneous input resistance of the cell, while the intersection of each plot with the control voltage-current relation gives the reversal potential. This procedure reveals that the vagal *AHP* results from activation of at least two conductances that differ in their time course, amplitude and reversal potential. As these conductances overlap, we used a differential blocker, norepinephrine (*NE*), in order to resolve their kinetics. Application of 50-200 μM *NE* does not change the Ca^{2+} action potential but blocks the main component of the *AHP*, leaving a brief (decays with τ=200 msec) Ca^{2+}-dependent AHP. This *AHP* apparently involves at least two ions (from reversal potential measurements). The main component of the *AHP*, which is blocked by *NE*, is produced by a calcium-dependent K^+ conductance that rises monotonically, to peak in about 1 sec, and then decays slowly ($\tau \sim 1.5$ sec). The maximum amplitude of this conductance increases linearly with the number (1-10) of action potentials. The unusual, relatively simple tool introduced here is powerful in the detailed analysis possible with its use.

PEPTIDES: PHYSIOLOGICAL EFFECTS I

58.1

SOMATOSTATIN INCREASES THE HEIGHT OF ACTION POTENTIALS IN HIPPOCAMPAL PYRAMIDAL NEURONS. T.W.J. Watson and Q.J. Pittman. Neuroscience Research Group. University of Calgary, Calgary, Alberta, Canada T2N 4N1.

In addition to previously reported actions on various neural and endocrine cells, we have found that somatostatin (SS14) causes an increase in height of action potentials (AP) in CA_1 pyramidal neurons. Intracellular voltage records were obtained by conventional methods using transverse rat hippocampal slices that were submerged and perfused with oxygenated artificial CSF at 30-32 C. Drugs were applied through the perfusate. SS14 ($2x10^{-6}$M) consistently resulted in a 3-10 mV increase in the amplitude of evoked or spontaneously occuring AP and this action was associated with an increased rate of rise of the depolarizing phase of the AP. This was not related to a change in membrane potential and was not associated with any change in width of the AP. The effect persists in 0.5mM $CdCl_2$ thus it appears to be a direct action not involving a calcium conductance. The increase in AP height is seen with 2mM $BaCl_2$ in the extracellular fluid which suggests it is independent of the previously described action of SS14 on the m-current (Moore et al Science 239: 278-80). This action is not seen with somatostatin 1-12. The increase in amplitude of the AP is blocked by 10^{-6}M TTX and may represent an effect on the fast sodium current.

58.2

GALANIN DECREASES MEMBRANE EXCITABILITY OF MUDPUPPY PARASYMPATHETIC POSTGANGLIONIC NEURONS. R.L. Parsons and L.M. Konopka*. Department of Anatomy and Neurobiology, College of Medicine, University of Vermont, Burlington, VT 05405.

Galanin inhibited action potential firing in mudpuppy parasympathetic neurons which exhibited spontaneous activity. This effect remained after the membrane potential was returned electrotonically to pregalanin values. Quiescent parasympathetic postganglionic neurons produced a brief train of spikes during 400 msec depolarizing current pulses. Following application of galanin, the number of spikes in the train was decreased. This effect also occurred in cells in which the galanin-induced hyperpolarization was negated. Galanin shifted the value of the threshold for spike generation and decreased the MRR, amplitude and MRF of sodium spikes. The spike HAP was also significantly prolonged by galanin. Galanin decreased the amplitude and duration of TTX-insensitive spikes initiated in cells maintained in a solution containing 9mM calcium, 20mM TEA and 1.5uM TTX. These results demonstrated that galanin decreases excitability of individual neurons in the mudpuppy cardiac ganglion. The decrease in excitability is due to an action of this peptide on a number of membrane conductance components and occurs independently of the galanin-induced hyperpolarization. Supported by PHS RO1 23978 and NSF BNS 8605611.

58.3

CHARACTERISTICS OF THE GALANIN-INDUCED HYPERPOLARIZATION OF MUDPUPPY PARASYMPATHETIC POSTGANGLIONIC NEURONS. L.M. Konopka* and R.L. Parsons (SPON: I. Young). Department of Anatomy & Neurobiology, College of Medicine, University of Vermont, Burlington, Vermont 05405.

Many parasympathetic postganglionic neurons in the mudpuppy cardiac ganglion are hyperpolarized by local application of the 29 amino acid neuropeptide, galanin (Neel et al., Neuroscience Abstracts 13:739, 1987. The present study was undertaken to establish the properties of the galanin-induced hyperpolarization. Application of galanin produced a hyperpolarization which developed within a few seconds and persisted for tens of seconds after the galanin application was terminated. The galanin-induced hyperpolarization was present after pretreatment with either 1uM atropine, 50uM curare, or 1.5uM TTX. Desensitization of the galanin-induced hyperpolarization occurred in only a small percentage of cells. In nine cells maintained in 2.5mM extracellular potassium, the reversal potential for the galanin-induced hyperpolarization was -105.4 $\pm$ 2.7mV. In four cells the reversal was shifted by 38.7 $\pm$ 4.9mV with a fourfold elevation of the extracellular potassium concentration. The galanin-induced hyperpolarization exhibited rectification when the membrane potential was made more positive than E_K. The results of these studies suggest that the galanin-induced hyperpolarization results from the activation of a potassium conductance. Supported by PHS RO1 23978 and NSF BNS 8605611.

58.4

SOURCE OF GALANIN-LIKE PEPTIDE IN THE MUDPUPPY CARDIAC GANGLION. T.W. McKeon and R.L. Parsons. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Galanin has been demonstrated to have inhibitory effects on parasympathetic postganglionic neurons in the mudpuppy cardiac ganglion. The present study was undertaken to determine the source of galanin-like peptide in this preparation. Using immunohistochemical techniques, galanin-immunoreactive fibers were seen to form complexes over many mudpuppy parasympathetic postganglionic neurons in the cardiac ganglion. These fibers remain two weeks following bilateral vagotomy and appear to be processes of postganglionic parasympathetic neurons and SIF-like cells. In many instances, processes from galanin-immunoreactive SIF-like cells were the origin of the pericellular immunoreactive complexes around the postganglionic neurons. We hypothesize that the SIF-like cells in the mudpuppy cardiac ganglion may function as inhibitory interneurons and that in many cases a galanin-like peptide may mediate this inhibition. Supported by PHS RO1 23978 and NSF BNS 8605611.

58.5

NPY STIMULATION OF LHRH RELEASE FROM RAT MEDIAN EMINENCE: EFFECTS OF ESTROGEN AND CALCIUM. F.D. Sabatino and J.K. McDonald. Dept. of Anatomy and Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

NPY stimulates LHRH release from median eminence (ME) fragments in vitro from ovariectomized (OVX) rats treated with estrogen (E) (Sabatino & McDonald, Neurosci. Abstr. 13: 1310, 1987). We examined the effects of physiological doses of E on NPY stimulation of LHRH release and the role of extracellular Ca^{2+}. Sprague-Dawley rats were OVX and 2-4 weeks later implanted with silastic tubes containing vehicle or E (1E, 2E and 4E), which produced plasma E levels of 17, 30 or 73 pg/ml. MEs were incubated in medium before and after application of NPY (10^{-5} & 10^{-6} M). In other experiments, MEs from rats treated with the 2E dose were incubated in medium containing 2.5, 1.0 or 0.25 mM Ca^{2+} or in Ca^{2+} free medium containing 2.0 mM EGTA and in some cases 2.5 mM Co^{2+}.

NPY caused a dose-related stimulation of LHRH release which was enhanced by increasing plasma levels of E. No effects were observed in vehicle-treated OVX rats. Treatment with 2E and 4E increased ME concentration of LHRH, while NPY levels were significantly greater only with the 4E dose. KCl (56 mM) stimulation of LHRH release was reduced in a dose-dependent fashion by decreasing Ca^{2+} levels and absent in Ca^{2+} free medium. Reductions of Ca^{2+} or omission of Ca^{2+} $\pm$ inclusion of Co^{2+} had no effect on NPY (10^{-5} M) stimulation of LHRH release. The results show E-dependent modulation of NPY stimulation of LHRH release from the ME and suggest that extracellular Ca^{2+} may not be required for this effect. Supported by NIH HD19731, HD00727 and March of Dimes 5-524.

58.6

INHIBITION OF SMOOTH MUSCLE CELL CYCLIC AMP ACCUMULATION AND DISRUPTION OF LIPOSOME STRUCTURE BY NEUROPEPTIDE Y (NPY). S.H. Buck, B. Baron, J.L. Krstenansky* and L.R. McLean*. Merrell Dow Research Institute, Cincinnati, OH

The 36-amino acid peptide, NPY, and its receptors are found in the CNS and in the periphery. Recently, NPY was reported to inhibit forskolin- or agonist-induced adenylate cyclase activity in CNS and peripheral tissues with an EC_{50} of 10-100 nM. In the CNS, a second phase (nonreceptor mediated?) of inhibition was also observed at NPY concentrations above 5 µM. We have found similar complex inhibition by NPY of cyclic AMP accumulation stimulated by 10 µM isoproterenol (ISO) in cultured rat aortic smooth muscle cells. As NPY concentration is increased, there is an initial, small inhibition (30%, EC_{50} = 3 nM), a disappearance of inhibition (approximately 100 nM), and a subsequent second phase of intense inhibition (> 70%) commencing at NPY concentrations greater than 1 µM. Deletion of Ca^{2+} from the incubation medium abolished the disappearance phase resulting in continuous, dose-related inhibition by NPY at 1-1000 nM (EC_{50} = 24 nM). In dimyristoylphosphatidylcholine liposomes, addition of 12 µM NPY induced a rapid, nearly complete loss of turbidity monitored by absorbance at 400 nm. Thus, some of the inhibitory effects of NPY on adenylate cyclase may involve membrane perturbation due to the amphipathic helical conformation proposed for the unfolded peptide. It is not yet clear if similar structural requirements underlie the receptor-mediated physiological effects of the peptide.

58.7

BRADYKININ INDUCES AN INWARD CATION CURRENT AND AN INCREASED MEMBRANE CONDUCTANCE IN CULTURED RAT SENSORY NEURONS. D.S. McGehee* and G.S. Oxford. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599.

The excitatory effects of the nonapeptide bradykinin (BK) were examined on sensory neurons in primary culture. Whole cell patch clamp techniques were used to measure BK responses from rat DRG neurons 1-20 days in vitro. BK (10-1000nM) was applied directly to cells via U-tube perfusion. Concentration-dependent depolarizations and conductance increases were seen in BK responsive cells. Action potneitla frequency increased and many cells (20-30um diam.) fired in bursts. These effects lasted 1-3 min in the continued presence of agonist, and repeated applications gave attenuated responses. Responses to BK were attenuated or absent in 0mM external Ca^{++} or with internal EGTA. Under voltage clamp conditions, BK causes an inward current with a time course similar to the depolarizations. This inward current was potentiated by the inclusion of 100uM GTP in the patch electrode. Identical BK effects could be recorded from cells in young (1-2 day) cultures without obvious synaptic connections, indicating a postsynaptic effect. Gammon et al. (in press) have reported BK-induced increases in phosphatidylinositol metabolites in DRG cutlures with time courses which closely match those seen electrophysiologically. We are undertaking experiments to dtermine which portions of the PI second messenger cascade are involved in the excitatory response to BK. Supported by NIH grant NS23804.

58.8

BRADYKININ AGONIST AND ANTAGONIST RESPONSES IN SENSORY NEURONS AND SMOOTH MUSCLE ASSAYS. D.M. Rock, M.J. McLean, J.J. Geer*, L.W. Cooke* and C.P. Taylor. Parke-Davis Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105 and Dept. Neurol., Vanderbilt Univ., Nashville, TN 37212.

Bradykinin (BK) is a nonapeptide that causes pain and inflammation. BK depolarizes unmyelinated dorsal root ganglion neurons (DRGNs) releasing neurokinins peripherally[1]. We studied BK responses with intracellular recordings from cultured mouse DRGNs and with contractile responses of several in vitro preparations: isolated rabbit iris muscles[1] (contractions from activation of BK receptors on sensory neurons), rabbit jugular vein segments[2] and guinea pig ileum segments[2] (smooth muscle responses).

We appljed BK and peptide BK antagonists[3] [$Thi^{5,8}$,$DPhe^{7}$]-BK or $DArg^{0}$-[Hyp^{3},$Thi^{5,8}$,$DPhe^{7}$]-BK to cultured DRGNs from blunt micropipettes. The peptide antagonists did not depolarize DRGNs and appeared to block BK responses. The peptides were competitive antagonists of smooth muscle BK responses. Seven peptide derivatives of BK had similar relative potencies as agonists of neuronal (rabbit iris) and contractile responses.

These data are consistent with the existence of a single BK receptor type in sensory neurons and certain smooth muscle preparations.

1. Ueda et al.,(1984) J. Pharm. Exp. Ther. 230:469-473.
2. Regoli et al.,(1980) Pharm. Rev. 32:1-46.
3. Schachter et al.,(1987) Brit. J. Pharm. 92:851-855.

58.9

IN VIVO ELECTROCHEMICAL STUDIES OF THE EFFECTS OF CHOLECYSTOKININ ON EVOKED OVERFLOW OF DOPAMINE (DA) IN THE CAUDATE NUCLEUS AND NUCLEUS ACCUMBENS OF THE ANESTHETIZED RAT. G.A. Gerhardt[1,2], M.S. Brodie[4], A.P. Gratton[2], T.W. Vickroy[4], G.M. Rose[2,3] and B.J. Hoffer[2] Depts. of Psychiatry[1] and Pharmacology[2], Univ. of Colorado & VAMC[3], Denver, CO 80262 and Neuroscience Research[3], Pharmaceutical Discovery, Abbott Laboratories, Abbott Park, IL.

Peptides related to cholecystokinin (CCK) have been reported to have both facilitory and inhibitory actions on transmitter release in DA pathways in the central nervous system. In order to further investigate this phenomenon, in vivo electrochemical studies of DA release were conducted in the striatum and nucleus accumbens of the urethane anesthetized rat. High-speed chronoamperometric measurements (5-25 Hz; IVEC-5 Instrument, Medical Systems Inc.) were performed using Nafion-coated recording electrodes fastened to double-barrel micropipettes containing solutions of potassium and cholecystokinin octapeptide (CCK-8). Potassium-induced overflow of DA was studied both before and after CCK-8 ejection. Locally applied CCK-8 consistently augmented potassium-evoked overflow in both brain areas. In addition, parenterally administered CCK-8 also augmented potassium-evoked overflow of DA. Thus, our data support that CCK-8 possesses a facilitory action on the presynaptic elements of the nigrostriatal and mesolimbic DA systems. (Supported by USPHS grants AG06434 and NS09199, NSERCC, and the VAMRC.)

58.10

EFFECTS OF CERULEIN ON THE IN VIVO RELEASE OF NEUROTRANSMITTERS FROM RAT STRIATUM. M.Shimoyama* and S. Kito. 3rd Dep. of Int. Med. Hiroshima Univ. Sch. of Med., 1-2-3, Kasumi, Minami-Ku, Hiroshima, 734, Japan.

In 1980, Hökfelt et al. showed that cholecystokinin(CCK)-immunoreactivity was co-localized with dopamine (DA) in some neurons in the rat ventral tegmental area which projected to various limbic structures. This finding has led to the suggestion that CCK-peptides may modulate the DA function in the brain. The present study examined the effects of cerulein, a decapeptide analog of CCK-8, on the in vivo release of DA and acetylcholine (Ach) from the rat striatum through intracerebral dialysis. A thin dialysis tube was inserted transversally through the rat caudate nuclei and the tube was perfused with the Ringer solution. The perfusion arrangement allowed the rats to move freely. The perfusates were assayed for DA, 3,4-dihydroxyphenylserine(DOPAC), homovanillic acid(HVA) and Ach by HPLC with electrochemical detection. Intraperitoneal and intrastriatal administrations of cerulein were without effect on the spontaneous release of both DA and Ach in striatal perfusates. Elevated K^+-induced DA release was inhibited by cerulein(100 µg/kg, i.p.). The inhibitory effect of cerulein on the high potassium-evoked DA release disappeared in bilateral vagotomized rats. This results suggested that the inhibitory effect of systemically administered cerulein on the rat striatal DA system was mediated by vagal CCK receptors.

58.11

INHIBITION OF POTASSIUM-EVOKED RELEASE OF CHOLECYSTOKININ (CCK) FROM RAT CAUDATO-PUTAMEN INCUBATED IN VITRO BY PHENCYCLIDINE (PCP) AND RELATED COMPOUNDS. L.R. Allard*, P.C. Contreras and M.C. Beinfeld* (SPON: S.E. Dryer). Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104 and G.D. Searle/Monsanto Co., Chesterfield, MO 63198.

We have suggested that CCK release from rat caudato-putamen (cp) is under inhibitory control by another substance which is released from the cp along with CCK by a Ca^{+2}-dependent mechanism. Since we have found that glutamate inhibits CCK release, we examined whether PCP (which is known to interact with NMDA receptors) influences CCK release. PCP at 10^{-5}M produces a significant inhibition of CCK release, greater than that produced by 10^{-5}M glutamate. However, PCP and glutamate appear to be acting differently because while the inhibitory action of glutamate could be blocked by APB (amino-phosphono-butyric acid), the inhibitory action of PCP was increased by APB. Several other PCP-like drugs were also found to inhibit CCK release. These results suggest that it is possible that PCP may inhibit CCK release in vivo and that inhibition of CCK may be involved in PCP-induced psychosis.
(Supported in part by NIH grant NS 18667 (to MCB)).

58.12

CHOLECYSTOKININ (CCK) DEPOLARIZES RAT SUPRAOPTIC NUCLEUS (SON) NEUROSECRETORY NEURONS. C.R. Jarvis, C.W. Bourque and L.P. Renaud. McGill Univ. Centre for Research in Neuroscience, Montreal, Canada H3G 1A4.

The rat hypothalamic SON demonstrates CCK-like immunoreactive cells and fibers as well as high density CCK binding. Intracellular recordings obtained from 26 phasic and 17 non-phasic SON neurons in superfused explants revealed dose-dependent, reversible and TTX resistant membrane depolarizations (2-11 mV) during brief infusions of 0.05 - 2.0 µM CCK-8 (both sulfated and non-sulfated), CCK-4 and caerulein. A reversible inward current accompanied by a small increase in chord conductance were seen under voltage clamp. Proglumide (1 mM) reversibly blocked CCK but not glutamate-evoked depolarizations. These data suggest that activation of a postsynaptic CCK receptor can excite SON neurosecretory neurons.

Supported by FRSQ, FCAR and the MRC.

58.13

CALCITONIN GENE-RELATED PEPTIDE ENHANCES CALCIUM CURRENT OF THE RAT DORSAL ROOT GANGLION NEURONS AND SPINAL EXCITATORY SYNAPTIC TRANSMISSION. P. D. Ryu, G. Gerber*, K. Murase and M. Randic, Dept. of Vet. Physiology and Pharmacology, Iowa State University, Ames, IA, 50011.

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide formed in neural tissue by alternative splicing of mRNA of the calcitonin gene. The presence of CGRP-like immunoreactivity in the rat dorsal root ganglion (DRG) neurons and spinal dorsal horn has been demonstrated. Our experiments were designed to examine the actions of CGRP on the Ca^{2+}-dependent action potentials and the voltage-dependent Ca^{2+} current in rat DRG neurons in vitro. In addition we tested the effect of CGRP on excitatory synaptic transmisison in the rat spinal dorsal horn.

Experiments were performed on the isolated preparations of lower lumbar (L4-L6) DRGs attached to a length of dorsal roots and horizontal spinal cord slices with attached dorsal roots and ganglia prepared from 14-28-day-old rats. Single-electrode voltage-clamp recordings were made from DRG neurons and intracellular current-clamp recordings from dorsal horn neurons. Synthetic rat CGRP was applied into the bath in known concentration.

CGRP (10^{-8} to 2 x 10^{-6}M for 1-3 min) evoked a slow, reversible dose-dependent hyperpolarization accompanied by an increase in neuronal input resistance in DRG cells. In addition, some large DRG neurons were depolarized by CGRP. CGRP produced a reversible increase in the amplitude and the duration of the Ca^{2+} spike of DRG neurons and directly increased the voltage-dependent Ca^{2+} current by enhancing both the transient and the sustained components of the current.

CGRP caused a pronounced and long-lasting increase in the amplitude of the fast e.p.s.p.s. recorded in dorsal horn neurons in response to high intensity electrical sitmulation of a lumbar dorsal root. In addition, CGRP increased the frequency and amplitude of presumptive spontaneous e.p.s.p.s.

The increase in the Ca^{2+} current is likely to be responsible for the increase in the Ca^{2+} spike and facilitation of excitatory synaptic transmission. These results are consistent with a neuromodulator role for CGRP in the rat spinal dorsal horn. Supported by NSF and USDA.

58.14

CHARACTERIZATION OF DIFFERENT FACTORS WITH FMRF-AMIDE-LIKE ACTIVITIES FROM NEUROHAEMAL ORGANS OF THE LOCUST. M. Schiebe*, R. Watts*, I. Orchard, A. Lange and H.L. Atwood. Dept. Physiology and Zoology, Univ. of Toronto, Ont. M5S 1A8

The extensor tibiae muscle of the locust Schistocerca gregaria is modulated by FMRF-NH2-like peptides. Immunohistological data suggested that a FMRF-NH2-like factor is concentrated in neurohaemal organs (NHO). Extracts of these organs were processed using reversed phase HPLC, and were split up on an acetonitrile gradient. The fractions were assayed by examination of their effects on muscle kinetics using muscle fiber bundles innervated by the slow motor axon in both Schistocerca and Locusta. Five different fractions with FMRF-NH2-like properties were identified. However, at the retention times of FLRF-NH2, YGGFMRF-NH2 and YGGFLRF-NH2, the known FMRF-NH2 agonists, no bioactive material was found. The peptides TNRNFLRF-NH2 and SDRNFLRF-NH2 from Homarus (Trimmer, B.A. et al., J. Comp. Neurol. 266:16, 1987) which are as potent as YGGFLRF-NH2/YGGFMRF-NH2, were eluted at the retention time of one bioactive NHO fraction. Part of the biological activity of NHO extracts may be provided by these peptides.

58.15

EFFECTS OF TACHYKININS AND OPIOID PEPTIDES ON NEUROTRANSMISSION IN THE AVIAN CILIARY GANGLION. L. Mossman, C.-H. Feng*, O.A. Ramirez* and V.A. Chiappinelli. Dept. of Pharmacol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

In addition to acetylcholine, some presynaptic terminals in the avian ciliary ganglion contain substance P-like and/or leucine-enkephalin-like immunoreactivity (Erichsen et al., J. Neurosci. 2:994, 1982). We have described the physiological effects of substance P on choroid neurons and presynaptic terminals within intact chick ciliary ganglia in an in vitro chamber (Dryer, S.E. and Chiappinelli, V.A., J. Neurosci. 5:2654, 1985 and Brain Res. 336:190, 1985). We now report the pharmacological profile of the tachykinin receptors present on choroid neurons. These receptors mediate a slow, depolarizing response which mimics a slow EPSP activated by brief 5-20 Hz preganglionic stimulation. A putative tachykinin antagonist, (D-Arg^{1},D-Pro^{2},D-Trp^{7,9}, Leu^{11})-substance P, acts either as a pure antagonist or as a mixed agonist/antagonist at these receptors.

Several opioid peptides, including leucine-enkephalin, DADL (Tyr-D-Ala-Gly-Phe-D-Leu) and DAGO (Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol) reduce nicotinic transmission through the choroid neurons, an effect which may be due to presynaptically located delta- and mu-opioid receptors. The effects of the opioid peptides are blocked by naloxone.
(Supported by NIH Grant EY06564 to V.A.C.)

PEPTIDES: PHYSIOLOGICAL EFFECTS II

59.1

BRAIN NATRIURETIC PEPTIDE AS A SYMPATHETIC PREGANGLIONIC NEUROTRANSMITTER. M.M. Moga, N.J. Dun, P. Needleman and C.B. Saper, Depts. of Pharm. & Physiol. Sci. and Neurol., Univ. of Chicago, Chicago, IL 60637, Dept. of Pharm., Loyola Univ., Maywood, IL 60153 and Dept. of Pharmacology, Wash. Univ. Sch. Med., St. Louis, MO 63110.

Brain natriuretic peptide (BNP) is a 25 amino acid peptide recently isolated from porcine brain that shares substantial sequence homology with atrial natriuretic peptide (ANP), as well as eliciting similar hypotensive and diuretic-natriuretic responses (Sudoh et al., Nature 332:78, 1988). Several studies suggest that ANP can influence the synthesis and release of norepinephrine by sympathetic ganglion cells. However, we have found no sympathetic preganglionic neurons that are ANP-like immunoreactive (ir). We now report that many neurons in the sympathetic preganglionic cell groups in the spinal cord, including the intermediolateral and intermediomedial columns and the dorsal commissural nucleus, are BNPir. BNPir fibers leaving these cell groups enter the ventral roots, and can be traced into sympathetic ganglia.

We further examined the responses of neurons in sympathetic ganglia to microapplication of BNP. In the inferior mesenteric ganglion of the guinea pig in vitro, BNP produced a depolarization and increase in membrane conductance, suggesting the opening of a cation channel. These results suggest that BNP may be an important modulator of sympathetic postganglionic neurons.

59.2

DOPAMINERGIC MEDIATION OF THE DIURETIC AND NATRIURETIC ACTION OF CENTRALLY ADMINISTERED RAT ATRIAL NATRIURETIC FACTOR (99-126). M. Torres*, Y. Barbella* and A. Israel. U.C.V. and U.C., Venezuela.

Atrial natriuretic factor (ANF) has actions on the brain. Central administration of ANF inhibits salt appetite, water intake, vasopressin and prolactin secretion and increases urinary volume and sodium excretion. The mechanism of central-rANF-induced diuresis and natriuresis (D&N) is unknown. The interaction of ANF with brain dopaminergic systems has been demonstrated (EJP 131:171). We examined therefore, the possible involvement of brain dopaminergic systems in the D&N produced by intracerebroventricular (IVT) administration or rANF. Male S-D rats (230-280 g) were implanted with a cannula in the left lateral cerebroventricle and were divided in the following groups: C= control; H= receiving a s.c. dose of haloperidol (2.5 and 1.25 mg/kg) or V= vehicle at 18 and 2 h before the experiment; 6OHDA= receiving a IVT dose of 250 ug/5ul of 6-OH-dopamine or V'= vehicle 72 and 48 hr before the experiment. Half of the rats receiving each treatment were IVT injected with saline (S) or rANF (5ug/5ul) (A) followed by 20 ml/kg p.o. water. Urine was collected at 1,3 and 6 h and samples were assayed for Na+ and K+ content. rANF-IVT to conscious hydrated rats resulted in an increase in urinary volume and sodium excretion at 1,3 and 6 h period collection. This effect was prevented by 6OHDA pretreatment and by the unselective dopamine antagonist haloperidol. Uri.Vol (ml/100g) at 3h= C-S: 1.5 $\pm$ .3; C-A: 2.8 $\pm$ .2*; 6OHDA-S: 1.8 $\pm$ .2; 6OHDA-A: 1.5 $\pm$ .1; H-S: 1.5 $\pm$.12; H-A: 1.8 $\pm$ .1. Na+ (uEq/100g) at 3h= C-S:67 $\pm$ 20; C-A: 203 $\pm$ 15*; 6OHDA-S: 63 $\pm$ 20; 6OHDA-A: 81 $\pm$ 16; H-S: 101 $\pm$ 13; H-A: 103 $\pm$ 14; *$p < 0.01$. Our findings strongly suggest that ANF exerts its centrally mediated effects on sodium and water metabolism, at least in part, via a dopaminergic mechanism.(Supported by grants CDCH F.07.20.878, UCV).

59.3

STIMULATORY EFFECTS OF ATRIAL NATRIURETIC PEPTIDE (ANP) AND ANGIOTENSIN II (AII) ON LUTEINIZING HORMONE (LH) RELEASE IN FEMALE RATS. M.K. Steele, Dept. of Physiology, University of California, San Francisco, CA 94143

Intracerebroventricular (ICV) ANP antagonizes the increase in blood pressure, fluid ingestion and vasopressin release due to ICV-AII. In addition, ICV-ANP inhibits the release of LH in male rats (Samson, et al, 1988), an animal model in which ICV-AII also inhibits LH secretion (Steele, Murakami and Ganong, unpubl.). The present experiment investigated the effects of ANP or ANP plus AII on LH release in ovariectomized rats treated with estradiol and progesterone, a model in which ICV-AII stimulates LH secretion (Steele, et al, 1985).

Conscious rats were bled from a jugular cannula prior to and following ICV injection of vehicle, AII (50 ng), ANP (1-28, 200 ng), or ANP plus AII. Blood LH levels and water intake were measured. Vehicle injection did not modify LH values or water intake. AII or ANP increased LH levels 100% at 10 min and 50% at 20 min postinjection. Coinjection of ANP plus AII resulted in increases in LH values of 200% at 10 min and 100% at 20 min. AII-induced water intake was attenuated by coadministration of ANP. These data show that low doses of ANP affect LH release in the same way as AII. The effects of ANP plus AII on LH are additive, whereas the effects on water intake are antagonistic.

Supported by HD18020 and HL29714.

59.4

INTERACTION OF METHYSERGIDE AND THYROTROPIN-RELEASING HORMONE ON SPINAL REFLEXES IN NEONATAL RATS. S.B. Deshpande and J.E. Warnick (SPON: S. Das Gupta). Dept. of Pharmacol. & Exp. Ther., U. of MD Sch. of Med., Baltimore, MD 21201.

Thyrotropin-releasing hormone (TRH) is known to enhance the excitability of motoneurons and potentiate the monosynaptic reflex (MSR) while methysergide (MeSG) was found to depress the MSR. This study was begun to examine the ability of TRH to reverse the depression of the MSR by MeSG and to elucidate the specificity of this action in the neonatal rat spinal cord. Spinal cords from male Wistar rats (8-9 days old) were hemisected, placed in an experimental chamber and superfused with oxygenated physiological solution. Stimulation of an L_{3-5} dorsal root evoked a MSR in the corresponding ventral root and a dorsal root reflex (DRR) in an adjacent dorsal root. MeSG produced a dose-dependent depression of the MSR with 50% depression at 7-9 nM, without affecting the DRR. TRH, which selectively increases the magnitude of the MSR without affecting the DRR, reversed the depression of the MSR by MeSG (30 nM) in a dose-dependent manner with 50% reversal at 40-50 nM TRH. The K-channel blocker 3,4-diaminopyridine (10 μM) reversed MeSG-induced depression of the MSR, but it evoked segmental polysynaptic discharges and also potentiated the DRR. The results suggest that TRH, unlike 3,4-diaminopyridine, selectively acts on α-motoneurons to reverse MeSG-induced depression of the MSR. (Supported by USPHS grant NS-21312.)

59.5

INTRACISTERNAL INJECTION OF TRH PRECURSOR TRH-GLY PRODUCED STIMULATION OF GASTRIC ACID SECRETION IN RATS.

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It has been established that TRH in the mammalian brain is formed by post-translational processing of a larger precursor molecule. Reports indicate that the prohormone contains five copies of the sequence pGlu-His-Pro-Gly, termed TRH-Gly. Intracisternal injection of TRH produces a marked stimulation of gastric acid secretion in rodents (Nature 1980; 287: 149-151). The effects of TRH-Gly on gastric acid secretion was investigated in urethane-anesthetized rats with gastric fistula. Intracisternal injection of TRH-Gly (1, 10 and 100 μg) produced a dose dependent stimulation of gastric acid secretion (μmol-/2h: 12 ± 5; 37 ± 12 and 162 ± 43; n = 4-15). In contrast, iv injection of 100 μg TRH-GLY produced no stimulation of gastric acid secretion (umol/2h: 2 ± 1; n = 5).Cerebrospinal fluid collection (20 μl) every 15 min and replacement by artificial CSF was performed to assess the rate of disappearance of TRH-Gly and the appearance of TRH (n = 9) using sensitive radioimmunoassay with no cross reactivity between TRH and TRH-Gly. Intracisternal injection of TRH-Gly (10 μg), induced a marked increase ($2x10^4$) in TRH-Gly and a 30 fold elevation in CSF levels of TRH 15 min post injection. These results demonstrate that intracisternal TRH-Gly like stimulates TRH gastric acid secretion, although doses required are 100 fold higher 2) TRH-Gly is rapidly converted to TRH in the CSF, with peak levels occurring at the first period (15 min). TRH-GLY bio-logical activity may result from its conversion into TRH, or direct activation of TRH receptors or its own specific receptor.

59.6

ω-CONOTOXIN "SHAKER" PEPTIDE CAUSES INCREASED GLUCOSE USE IN VESTIBULAR NUCLEI AND CEREBELLUM OF RATS AND MICE.

S.R. Nelson, G. Liang*, F.E. Samson, T.L. Pazdernik*, R.S. Cross* and B.M. Olivera*. Depts. of Anatomy, Pharmacology and the R.L. Smith Research Center Laboratories, Univ. of Kansas Sch. of Med., Kansas City, KS 66103, and Dept. of Biology, Univ. Utah, Salt Lake City, UT 84112.

The intraventricular injection of "shaker" peptide, ω-conotoxin GVIA, from venom of fish eating cone snails (Conus geographus), causes prolonged "shaking," characterized by a posture of spread limbs and rapid shaking of head, body and limbs. Local cerebral glucose use was determined during the shaking period in peptide-treated rodents (2 nmole into the lateral ventricle) by the [^{14}C]-2-deoxyglucose method. High glucose use occurred in vestibular nuclei and paraflocculus, flocculus, ventral vermis and cortex of the cerebellum. The findings suggest that "shaker" peptide activates vestibular nuclei in the floor of the 4th ventricle, and secondarily, the cerebellum. Disruption of normal interactions between vestibular nuclei and cerebellum may account for the characteristic posture and "shaking" induced by the peptide. The peptide is known to block voltage-activated Ca^{2+} channels; thus, blockade of ω-conotoxin sensitive Ca^{2+} channels in rat CNS may interfere with the normal inhibition/excitation balance in the vestibular system. Supported in part by DAMD17-86-G-6038 and NIH GM 22737.

59.7

EFFECT OF ANGIOTENSIN II (AII) AND AII RECEPTOR BLOCKERS ON PROLACTIN (PRL) SECRETION.

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Previous work has shown that a high dose of intracerebroventricular (ICV) AII suppresses levels of PRL in ovariectomized (OVX) rats (Steele et al., 1981). The present study investigated the sensitivity of this effect on PRL secretion by using varying doses of AII in OVX female and intact male rats. Additionally, AII receptor blockers, Saralasin (SAR) or Sarthran (SART) were given to OVX rats.

Blood samples were drawn from a jugular cannula from conscious rats prior to and following ICV injections of vehicle, AII (50 ng, 500 ng or 5000 ng), SAR (25 μg), or SART (10 μg). Water intake and plasma levels of PRL were measured. In males, AII significantly suppressed PRL levels up to 60 min. In OVX rats AII (50 ng) significantly suppressed PRL at 15 min, with levels returning to preinjection values at 60 min. Neither SAR nor SART were found to alter PRL levels compared to injection of vehicle in OVX rats.

These data show that low doses of AII can suppress basal PRL secretion in both male and OVX female rats. However, the lack of response when endogenous AII is blocked suggests that AII is not acting tonically to suppress PRL.

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59.8

REGULATION OF STRIATAL TYROSINE HYDROXYLASE BY CRF.

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We investigated the effect on synaptosomal dopamine (DA) synthesis of corticotropin-releasing factor (CRF),a putative brain neuromodulator of stress-induced responses. DA synthesis was assayed in a synaptosomal preparation from rat or mouse striatum by measuring the release of $^{14}CO_2$ from L-[1-^{14}C] tyrosine. In rat striatum, CRF caused a 34% increase in DA synthesis with half-maximal and maximal stimulations at 40 and 300 nM, respectively. In mouse striatum CRF increased DA synthesis by 48%. The CRF effect was accompanied by a stable change in the activity of tyrosine hydroxylase (TH) assayed in extracts of lysed synaptosomes. The exposure of synaptosomes to CRF resulted in 30% and 64% increase of TH activity in rat and mouse striatum,respectively. The stimulatory effect of CRF on synaptosomal DA synthesis was significantly counteracted by the specific CRF receptor antagonist α-hel CRF 9-41 (1 μM). The effect of CRF was dependent on the concentration of Ca++ in the incubation medium and was antagonized by polymyxin B,an inhibitor of protein kinase C,with an IC50 value of about 30 μM. These results indicate that CRF can regulate striatal TH activity via presynaptic receptors which operate through a Ca++-dependent mechanism.

59.9

VIP EXCITATION OF LOCUS COERULEUS NEURONS: EVIDENCE FOR A G-PROTEIN-MEDIATED INWARD CURRENT.

Y.-Y. Wang and G.K. Aghajanian, Depts. Of Pharmacol. and Psychiat., Yale Univ. Sch. Med., New Haven, CT 06510.

VIP (vasoactive intestinal polypeptide) receptors and immunoreactive nerve terminals have been localized in the locus coeruleus (LC). The purpose of this study was to characterize the electrophysiological actions of VIP in the LC and the possible mediation of these actions by guanine nucleotide regulatory proteins (G proteins).

Brain slices containing the LC (male albino rats) were perfused with standard artificial CSF. Intracellular recordings and single-electrode voltage-clamping were performed using an Axoclamp-2; electrodes were filled with 2M KCl or 2M KCl with GTP*r*S (10 mM). Puffs of VIP (125-500 μM) were applied by pressure ejection.

VIP produced a reversible increase in the firing rate of LC neurons. Voltage-clamping at -60 mV revealed that VIP induced an inward current which slowly recovered within about 5-10 min. The inward current persisted in the presence of Ca channel blockers (e.g., 2 mM Co or Mn), suggesting a direct action of VIP at postsynaptic sites. Partial replacement of Na with choline (about 80%) reduced the VIP-induced inward current by about 60%. Current-voltage plots of VIP did not show any reversal at E_K. When electrodes containing GTP*r*S, a hydrolysis-resistant analog of GTP, were used, the VIP-induced inward current became irreversible (observed up to 2 hrs), implying that VIP receptors are coupled to G proteins. In contrast, the inward current generated by N-methyl-D-aspartic acid (NMDA, 50 μM) was not prolonged by GTP*r*S. After multiple applications, responses to VIP tended to become smaller, indicating the possibility of receptor desensitization.

In summary, our results indicate that VIP directly excites LC neurons by inducing a Na-dependent inward current; as this effect becomes irreversible in the presence of intracellular GTP*r*S, mediation through a G protein is suggested. Since VIP is known to activate adenylate cyclase in brain, we are investigating the possible involvement, if any, of cAMP in these actions.

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59.10

ALTERED REGULATION OF GLYCOGEN METABOLISM BY VASOACTIVE INTESTINAL PEPTIDE (VIP) AND K^+ IN THE NEOCORTEX OF THE SPONTANEOUSLY EPILEPTIC MOUSE MUTANT QUAKING.

P.J. Magistretti and P.R. Hof, Département de Pharmacologie, CMU, 1211 Geneva 4, Switzerland.

K^+ at concentrations reached in the extracellular space during normal neuronal activity (i.e. 5-12 mM) promotes a concentration- and calcium-dependent hydrolysis of ^{3}H-glycogen newly synthesized by mouse cerebral cortical slices. The quaking mutant (qk) is characterized by a severe myelin deficiency in the CNS due to an impaired differentiation of oligodendrocytes. We have observed a supersensitive response to the glycogenolytic effect of low concentrations of K^+ in cerebral cortical slices prepared from homozygous (qk/qk) mutants. This supersensitive response to K^+ was only observed in qk/qk older than 8 weeks, with differences between qk/qk and controls of 28 % at 10 weeks, 50 % at 16 weeks and 17 % at 30 weeks for 8 mM K^+. VIP induces glycogen hydrolysis in neocortical slices with an EC50 of 26 nM. We have recently observed a new effect of VIP on cortical glycogen levels. VIP at non-glycogenolytic concentrations, i.e. pM, strongly inhibits the K^+-evoked ^{3}H-glycogen hydrolysis in normal mice. Thus, VIP 10 pM inhibits by 75 % the glycogenolysis elicited by 8 mM K^+. This novel action of VIP is not observed in qk/qk mutants, suggesting the presence of an impaired function of the VIP intracortical neuronal system in the cerebral cortex of the qk mutant. Supported by FNRS Grant No 3.357-0.86.

59.11

VASOPRESSIN AND OXYTOCIN CONTENT IN HYPOTHALAMIC NUCLEI: RELATION TO FOOD DEPRIVATION AND SUBSEQUENT REFEEDING. M. Jhanwar-Uniyal, M. L. Chapleur*, A. J. Burlet* and S. F. Leibowitz. The Rockefeller Univ. New York, NY 10021 and Laboratoire de Biologie Cellulaire, Faculte de Medecine, INSERM U 308, 54000 Nancy, France.

The peptide hormones, vasopressin (AVP) and oxytocin (OT), appear to play a role in the control of food intake. Recent evidence suggests that norepinephrine (NE), in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei, is involved in controlling the release of these hormones, as well as in controlling eating behavior. An increase in NE turnover in the PVN was demonstrated in 48 hrs food deprived (FD) rats. The present study examined the effect of FD (48 hrs), or food intake (FI, 6 hrs) following 42 hrs FD, on the content of AVP and OT in 7 discrete hypothalamic sites and the neurohypophysis.

Eight hypothalamic areas, namely, PVN (M; magnocellular), PVN (P; parvocellular), dorsomedial nucleus (DMN), median eminence (ME), perifornical lateral hypothalamus (PLH), SON, suprachiasmatic nucleus (SCN), and ventromedial nucleus (VMN), were micropunched and assayed for AVP and OT content via RIA. The results demonstrate that: 1) after 48 hrs. FD, AVP and OT levels in the hypothalamic nuclei remain stable, except in the VMN where AVP is reliably increased ($p<0.05$); 2) after 6 hrs of refeeding, AVP levels are significantly reduced in the PVN-P ($p<0.05$), ME ($p<0.05$), SON ($p<0.01$), and VMN ($p<0.05$) and unaffected in other hypothalamic areas and neurohypophysis; and 3) after 48 hrs FD, a small decline in OT levels in the PVN and ME ($p<0.05$) occurs. No change is seen after refeeding. These studies demonstrate that hypothalamic AVP, and to a lesser extent OT, may vary in relation to feeding behavior.

59.12

ARGININE-VASOPRESSIN; A RELEASING FACTOR FOR POMC PEPTIDES IN THE BRAIN. V.M. Wiegant*, C.G.J. Sweep*, I. Barna* and D. De Wied*. Rudolf Magnus Institute of Pharmacology, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands.

Arginine-vasopressin (AVP) is well established as a hypothalamic neurohormone involved in the regulation of the activity of pro-opiomelanocortin (POMC) cells in the anterior pituitary gland. We have obtained evidence that AVP may also function as a releasing factor for brain POMC activity. Intracerebroventricular (icv) treatment of rats with AVP (pg-ng) resulted in a rapid, dose dependent increase in the concentration of β-endorphin immunoreactivity (βEIR) in cerebrospinal fluid (CSF). This effect could not be obtained with several fragments of AVP, nor with structurally related peptides such as oxytocin and N^{α}-acetyl-AVP. In addition, pretreatment (icv) with a specific vasopressin pressor antagonist completely prevented the effect of AVP. This suggests the involvement of vasopressin receptors in the brain, possibly of the V1 type. A possible site of action for this effect of AVP may be located in the mediobasal hypothalamus, as AVP (10^{-7} - 10^{-9} M) stimulated the in vitro release of βEIR from slices prepared from this brain region. Indeed, it has been reported that the arcuate nucleus, which contains cell bodies of POMC neurons, is rich in putative vasopressin receptors as evidenced by high binding of the vasopressin pressor antagonist (Van Leeuwen, F.W. et al., Neurosci. Lett., 80: 121, 1987).

59.13

MAINTENANCE OF LONG-TERM POTENTIATION IN THE LATERAL SEPTUM REQUIRES VASOPRESSIN.
P. van den Hooff,* I.J.A. Urban* and D. De Wied.* Rudolf Magnus Institute for Pharmacology, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands.(SPON: EBBS)

Excitatory postsynaptic potentials (EPSPs) and field-potentials (FPs), evoked by stimulation of the fimbrial (Fi) afferents, were recorded in rat brain slices of the lateral septum (LS) in order to examine long-term potentiation or LTP of synaptic efficacy following brief high frequency stimulation of the Fi fibers. We observed that the amplitude of the EPSPs and the amplitude of the negative wave (N-wave) in the FPs of Wistar (WI) rats were long lasting increased following tetanic stimulation of the Fi fibers. Slices prepared from vasopressin (VP) deficient Brattleboro rats consistently failed to maintain a tetanus induced increase in the N-wave amplitude. This could be restored by VP supplementation to these rats and also by VP or picrotoxin administration to slices from these rats. In addition, the V1 vasopressor antagonist, $d(CH_2)_5Tyr(Me)AVP$, prevented the maintenance of a tetanus induced increase in the N-wave amplitude in control Long-Evans (LE) and WI slices.

It is concluded that VP is important for maintenance of LTP in excitatory transmission in the LS. It is postulated that the effect of VP on memory could originate from increased synaptic efficacy similar to the LTP phenomenon.

59.14

A PROBABLE MECHANISM FOR VASOPRESSIN ACTION IN THE HIPPOCAMPUS. D. Albeck* and T. Smock (SPON: S. Calisher). Behavioral Neuroscience Program, Dept. of Psychology, Univ. Colorado, Boulder, CO 80309.

Arginine vasopressin (AVP) is contained in fibers that project to the hippocampus and is released from neural terminals upon stimulation. Receptors for the peptide exist in the hippocampus and the effect of the AVP on various hippocampal cell types can be blocked by a selective pressor (V_1) antagonist.

Nevertheless, confusion as to the actual mechanism of AVP action impedes evaluation of the peptide as a central neurotransmitter candidate. At first, single unit recording in the slice preparation suggested a direct excitatory action on CA1 neurons (Mühlethaler et al., 1982, Nature 296:749), but subsequent studies implicated direct, excitatory action on inhibitory interneurons and secondary inhibition of pyramidal cells (Mühlethaler et al., 1984, Brain Res. 308:97). Intracellular recording from pyramidal cells revealed an excitatory action, but constriction of slice microvessels was also observed, suggesting that the excitation may have been a byproduct of direct vascular activation (Smock et al., 1987, Exp. Brain Res., 66:401).

Here we report results from field potential recordings from the slice that reconcile these seemingly disparate findings. Male Sprague-Dawley rats (150-250 g) were used to prepare hippocampal slices with standard techniques. Monopolar DC pulses of 75 µsec duration (submaximal voltage) were applied to stratum radiatum and composite postsynaptic potentials and population spikes were recorded from the pyramidal cell body region of CA1. AVP, bath applied at 1 µM, had negligible action on the rising phase of the synaptic potential and a slight inhibitory effect on the falling phase. However, the population spike due to pyramidal cell discharge was profoundly inhibited, falling from an average of 1.67 mv (± .35) to .41 mv (± .22) in thirty minutes postapplication (n = 11). The inhibition produced by AVP was completely blocked in the presence of a specific V_1 pressor antagonist for the peptide (also 1µM, n = 9).

These data imply inhibition of pyramidal cell somata with little effect on presynaptic excitability or dendritic mechanisms. The results are consistent with a model that posits direct AVP action on inhibitory interneurons and slice microvessels and reveal pyramidal cell inhibition and excitation to be secondary consequences of the two direct effects.

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59.15

VASOPRESSIN MEDIATION OF MICROVASCULAR SPASM IN THE RAT BRAIN. R. Cach*, C. Durboraw*, T. Smock and D. Albeck. School of Medicine and Behavioral Neuroscience Program, University of Colorado, Boulder, Colorado 80309.

The coagulation reaction produces a substance that elicits regional constriction of penetrating microvessels in the rat hippocampal slice (Cach et al., 1987, Brain Res. 414:1). The spasmogen, probably related to an eicosanoid of the thromboxane series, employs neural mechanisms to bring about the vasoconstriction (Cach et al., 1987, Brain Res. 421:370). Here we show that this neural mechanism probably involves a subpopulation of vasomotor neurons that secrete the polypeptide, arginine vasopressin (AVP).

In the first series of experiments, spasmogenic serum and candidate spasmogens were applied to the slice and the evoked perforant path-to-granule cell field synaptic potential and population spike were recorded. In contrast to control serum (n=7), dilute spasmogenic serum obtained by incubating coagulated blood inhibited the evoked neural responses in each case (n=8). Thus, the spasmogen, which employs neural mechanisms for its action, does not do so by generally enhancing the neural excitability of the slice. A subpopulation of vasomotor neurons must be involved.

Alone among several candidate vasomotor transmitters tested, AVP consistently duplicated the constricting response to spasmogenic serum (Smock et al., 1987, Exp. Brain Res. 66:401). We therefore used a specific AVP antagonist to block the action of spasmogenic serum. The vascular response evoked by spasmogenic serum (62 ±4 microns of blood movement, n=5) was completely absent in the presence of 1µM antagonist (2±0.5 microns, n=6).

Taken together, these data implicate AVP in the etiology of the acute phase of cerebral microvascular spasm and present the intriguing possibility that structural AVP antagonists might prove to be valuable probes in future studies of this condition.

Supported by grants from the Epilepsy Foundation of America, the Colorado Heart Association and NSF grant BNS8520622.

59.16

DIFFERENTIAL AFFECTS OF DOPAMINE AND NOREPINEPHRINE ON GONADOTROPIN-RELEASING HORMONE NEURONS HARVESTED FROM ADULT MALE RATS[1]. P. A. Melrose. Dept. Vet. Anatomy, LSU School Vet. Med., Baton Rouge, LA 70803.

The gonadotropin-releasing hormone (GnRH) neurons of 40 adult male Sprague-Dawley rats were harvested to determine if dopamine (DA) or norepinephrine (NE)produced acute, in vitro, affects on the neurosecretion of GnRH or on cellular GnRH concentrations. Cell fractions were recovered as previously described (Melrose et al., Endo.121 1987). Neurons from each rat were then plated in an individual culture dish; the cells were incubated for 5 days. On day 6, cultures were treated with vehicle, 1000 ng DA, 2500ng DA, 500ng NE, or 1000ng NE (8/group). Media samples for GnRH RIA were collected at 2 min intervals for 30 min during the pretreatment period(P) and for 60 min during the treatment P. The cellular GnRH concentrations were determined 24 hrs after the initiation of treatment.

Neuropeptide release from cultures, during both the pre- and post-treatment P, was pulsatile. There was no treatment affect ($P>0.05$) on mean media GnRH concentrations or on the overall mean post-treatment frequency and amplitude of GnRH pulses. Significant treatment affects which were observed are summarized in Table 1. The 2500ng DA treatment lengthened the interval to, and increased the amplitude of, the first post-treatment GnRH pulse. In contrast, NE treatments reduced cellular GnRH concentrations at 24 hours of treatment. Results from this experiment suggest dopamine and norepinephrine may produce differential short-term affects on GnRH neurons of adult male rats. Further, these results suggest that unprimed GnRH neurons rapidly become refractory to the direct affects of dopamine.

TABLE 1: AFFECTS OF CATECHOLAMINES ON GNRH NEURONS[2]

group	cellular GnRH pg/culture)	interval to 1st post-treat. pulse (min.)	amplitude 1st post-treatment pulse (as % pre-treatment)
control	12905 ± 72.8	7.0 ± 2.0	100.4 ± 5.1
1000ng DA	11700 ± 57.4	10.0 ± 0.0	91.5 ± 4.0
2500ng DA	12681 ± 59.4	23.6 ± 6.2*	162.2 ± 9.0*
500ng DA	6825 ± 18.7*	11.6 ± 3.8	110.0 ± 7.5
1000ng NE	6431 ± 34.8*	14.0 ± 3.6	103.6 ± 8.8

[1]Supported by N1H grants NS19315 & PHS-2 SO7RR05403 [2]Mean ± SE *P<0.05

60.1

CONTRASTING EFFECTS OF ENALAPRILAT AND PHOSPHORAMIDON ON BOMBESIN INDUCED HYPOTHERMIA IN THE RAT. R. T. Layer and R. O. Warwick, Jr. Dept. of Pharmacology and Toxicology, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104.

Bombesin (BN) is a potent hypothermic neuropeptide with slow onset and long duration of action when given intracerebroventricularly (i.c.v.) to cold exposed rats. Little is known of the in vivo inactivation of BN, although BN-degrading enzymes are present in rat brain synapses. We studied the effects of enalaprilat (ENP), a peptidyl dipeptidase inhibitor, and phosphoramidon (PAN), a endopeptidase 24.11 inhibitor, on BN hypothermia. All agents were administered into the right lateral ventricle of rats at 6° C ambient temperature. BN (0.01 ug) hypothermia (33.5° C ± 1; n = 6) was potentiated ($p < 0.05$) 10% by simultaneous administration of PAN (50 ug i.c.v.), and was abolished ($p < 0.05$) by simultaneous administration of ENP (100 ug i.c.v.). The results suggest that BN administered i.c.v. is inactivated by endopeptidase 24.11 and activated by peptidyl dipeptidase. Additionally, these data support in vitro evidence that these peptidase inhibitors differentially modulate BN-induced inhibition of serotonin release.

60.2

INDOMETHACIN-INDUCED ANTIPYRESIS IN THE RAT. D.M. Fyda, K.E. Cooper and W.L. Veale. Department of Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1.

These experiments were undertaken to determine whether indomethacin given into the ventral septal area (VSA) could suppress a prostaglandin E_1 (PGE_1) hyperthermia and, if so, to assess whether the antipyresis was evoked by means of vasopressin receptors. Thirty-two male, Long Evans rats had cannulae stereotaxically implanted which were aimed at the lateral cerebral ventricle (LCV) and bilaterally at the VSA. The rats were fitted with indwelling radio-transmitters which allowed for the remote monitoring of core temperature. During control infusions of artificial cerebrospinal fluid into the VSA, PGE_1 (30 ng/µl) into the LCV evoked a 1.0°C increase in core temperature; whereas during indomethacin infusions (15 µg/µl/hr) the PGE_1-induced hyperthermia was reduced to 0.3°C. When a V_2-receptor blocker was injected into the VSA the temperature response to PGE_1 was not altered. Conversely, when a V_1-receptor blocker was administered into the VSA the hyperthermic effect of PGE_1 was abolished; whereas the antipyretic effect of indomethacin was maintained. Neither indomethacin nor the two receptor blockers affected core temperature when administered individually. The results suggest that although indomethacin is an effective antipyretic against PGE_1-induced fever, its mode of action does not involve V_2-receptors and may not involve V_1-receptors.

60.3

MICRODIALYSIS EVALUATION OF BRAIN ANTIPYRETIC CONCENTRATIONS OF AVP FOLLOWING ICV INJECTION. N.W. Kasting and M.F. Wilkinson*, Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada.

Microdialysis is an adaptation of the push-pull technique that keeps the perfusate separated from the ECF by a dialysis membrane to allow exchange of only small molecules. Microdialysis tubing (MWCO 9000) was fastened between two stainless steel cannula so that a length of 1 mm of membrane was exposed. The cannula was stereotaxically implanted into the Ventral Septal Area (VSA) of the anesthetized rat brain along with an icv cannula. In vitro experiments utilizing labelled AVP determined that the exchange rate between external fluid and perfusate was 0.04%. During in vivo experiments, rats were injected with 10 ng of AVP icv and the perfusate collected at 5 min intervals and assayed for AVP by RIA. Peak levels were typically obtained in the perfusate 10 to 15 min after icv injections. The peak levels also indicated that levels of AVP in the VSA reached values of 10.7 ± 0.5 nM after 10 ng icv injections. The threshold response for antipyretic effects is 1 ng icv, which would indicate that the threshold AVP concentration in the ECF of the VSA would be 1.07 nM. According to recent receptor binding analysis of AVP receptors from the VSA, the K_D for AVP is 1.06 nM. Thus, we conclude that icv injections of AVP reach the receptors in the VSA at physiologically relevant levels. Supported by MRC of Canada.

60.4

CENTRAL NERVOUS SYSTEM EFFECTS OF LIPOPHILIC ACE-INHIBITORS IN RODENTS: BEHAVIORAL AND BIOCHEMICAL EVIDENCE. H.J.Gerhards*, F.J.Hock, U.Leven*, J.Stechl* and G.Wiemer*. Hoechst AG., 6230 Frankfurt/M.-80, FRG.

Inhibitors of Dipeptidyl-peptidase A (ACE) have been described to possess distinct psychotropic effects in the clinic. Since these compounds are not easily crossing the blood brain barrier, we tested some more lipophilic ACE-Inhibitors and their prodrugs for CNS effects in behavioral and biochemical models in mice and rats.

From a series of derivatives of the ACE-inhibitor Hoe 498 (Ramipril) the compounds S84 8882 and S86 4065A were selected for a comparison of their antagonism against scopolamine amnesia in a passive avoidance model and their inhibition of brain ACE (in vitro and ex vivo in vitro autoradiography using 125J-MK3251A as ligand).

The potency of these compounds to displace 125J-MK351A from its binding sites in brain (in vitro and ex vivo in vitro) was Hoe 498 > S84 8882 > S86 4065A whereas the ranking according to their antiamnestic activity was S86 4065A > S84 8882 > Hoe 498. Thus, the central ACE-inhibitory effects of these compounds did not correlate with their antiamnestic properties.

60.5

THE NEUROPEPTIDE α-MSH ANTAGONIZES IL-1 INDUCED INCREASES IN CAPILLARY PERMEABILITY. J. M. Lipton, L. B. Deeter* and L. W. Martin*. Dept. of Physiol., The Univ. Tex. Southwestern Med. Ctr. at Dallas, Dallas, TX 75235.

α-MSH, an endogenous peptide, is a potent antipyretic when administered i.v., i.c.v., intragastrically or into CNS sites. Recent research suggests that the peptide can antagonize neutrophilia and other acute phase responses elicited by IL-1. To test the idea that the peptide can antagonize localized cutaneous inflammation caused by IL-1 injections, 30 rabbits with shorn backs were given the peptide (2.5 µg/kg, i.v.) or saline, followed by pontamine sky blue dye (30 mg/kg, i.v.) and intradermal injections of IL-1 (.10 ml, 6-8 sites). One hour later the animals were killed via an overdose of anesthetic and the skins were removed. The intensity and dimensions of subcutaneous staining, an estimate of capillary permeability, were judged by independent observers. α-MSH inhibited the increase in capillary permeability caused by IL-1 ($p = .019$, sign test). Although it is not clear whether the inhibition by α-MSH occurred via peripheral or central actions, the results indicate that the neuropeptide has the capacity to antagonize yet another action of crude IL-1. Supported by NINCDS GRANT NS10046.

60.6

CENTRAL INFUSION OF AMINOPEPTIDASE AND CARBOXYPEPTIDASE INHIBITORS INCREASES BLOOD PRESSURE IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS. L.L. Jensen, J.W. Harding, J.M. Hanesworth*, L.L. Cushing* and J.W. Wright. Dept. of Psychology, Wash. State Univ. WA.99164-4830.

Acute intracerebroventricular (icv) infusion of a cocktail consisting of the aminopeptidase inhibitors, amastatin and bestatin, as well as a carboxypeptidase inhibitor, plummer's maximally elevated blood pressure (BP) in the anesthetized Wistar-Kyoto (WKY: from 100 to 180 mm Hg) and to a greater degree in Spontaneously Hypertensive rat (SHR: from 120 to 274 mm Hg). BP elevations were concomitant with increases in cerebrospinal fluid (CSF) levels of angiotensin in both strains of rat. These increases in BP were blocked by icv infusion with the specific angiotensin receptor antagonist Sar^1,Thr^8-AII (sarthran), suggesting that these inhibitors are acting via the central angiotensinergic system. Icv infusion with leucine aminopeptidase M (LAPM) induced significant drops in BP as well as in CSF levels of angiotensin in both strains (WKY and SHR) of rat.

Cocktail Mean elevations			Sarthran Mean drops			LAP-M		
Strain	n	BP(mm Hg)	Strain	n	BP(mm Hg)	Strain	n	BP(mm Hg)
SHR	8	127→242	SHR	8	185→75	SHR	8	190→116
WKY	8	94→169	WKY	8	143→77	WKY	6	151→104

These data support the notion of a dysfunction of central angiotensinases in the SHR (Wright et al., 1985) contributing to the hypertension in this genetic rat model.

60.7

DSIP MODIFIED THE LEVELS OF BLOOD PRESSURE, HEART RATE, BODY T°, AND PAIN THRESHOLD BEFORE SLEEP ONSET IN RATS. S. Yehuda and R. L. Carasso*. Dept. of Psychol., Bar-Ilan Univ., Ramat-Gan, Israel.

Previous studies showed that the effects of Delta-Sleep-Inducing-Peptide (DSIP) on the levels of blood pressure, heart rate, body T°, and pain threshold are circadian cycle-dependent (Yehuda, S., and Carasso, R.L., Peptides, 1987, submitted). Another study (Yehuda, S. et al., Intern. J. Neurosci., 32, 185-197, 1987,) showed a treatment with DSIP (at 18:00 hr) results in significant changes in the lymphokine system. These changes were found even 10 min after treatment, before the sleep onset (at about 25 min).

In this experiment, groups of male albino rats received a dose of 0.1 mg/kg DSIP at 18:00 hr. In one group, the levels of blood pressure and heart rate were measured 10, 20 and 30 min after treatment. An increase in blood pressure was found after 10 min ($p<.05$) & after 20 & 30 min ($p<.01$) only among DSIP-treated rats which exhibited EEG sleep signs (n=22). Among non-responder rats (n=8) the blood pressure level was the same as in control rats (n=10). A significant decrease in heart rate ($p<.01$) was found even after 10 min only among responder rats. In the 2nd group, DSIP induced significant hyperthermia ($p<.01$) among DSIP responders and non-responders. In the third group, the level of pain threshold was increased in non-responders (n=12) ($p<.05$) & in responders (n=18) ($p<.01$).

60.8

EFFECTS OF NEUROTENSIN AND SOME ANALOGS ON BLOOD PRESSURE IN RAT. E. Donato Di Paola*, D. McCormick*, and E. Richelson. Mayo Foundation, Rochester, MN 55095.

Neurotensin (NT) a tridecapeptide, first found in the CNS, induces hypotension in anaesthetized rats[1]. The effects of NT, NT(8-13), and [D-Lys^8]NT(8-13) (NT2) on DBP are reported below. All three peptides evoked a dose-related decrease in DBP.

Change in Diastolic Blood Pressure (mm Hg)

dose(nmol/kg)	0.1	0.3	1	3	10	30
NT	-5±5	-22±3	-33±1	-44±4		
NT(8-13)		-2±1	-8±2	-18±4	-30±2	-41±2
NT2	-2±1	-12±6	-17±6	-27±4	-42±5	-48±4

These results demostrate that neurotensin was more potent than its analogs in producing hypotension. However, the rank order of potency of these peptides in reducing blood pressure was the opposite of that found in binding studies in human brain or in assays measuring peptide stimulation of cyclic GMP formation in a murine neuroblastoma cell line[2]. These data suggest that subtypes of the neurotensin receptor exist.

[1]Carraway, R., and Leeman, S.E. J.Biol.Chem. 248:6854-6861 (1973).
[2]Gilbert J.A., and Richelson, E. Eur.J.Pharmac. 99:245 (1984).
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60.9

ALTERATIONS IN THE EVOKED RELEASE OF NE AND PERIPHERAL VASCULAR RESISTANCE FOLLOWING INTRATHECAL NPY. X. Chen*, M.M. Knuepfer and T.C. Westfall (SPON: J. Farah). Dept. Pharmacol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Previous studies from our laboratory have shown that the intrathecal injection of neuropeptide Y (NPY) has a depressor effect in anesthetized and unanesthetized rats. We have observed that the depressor effect of NPY involves alpha$_2$ and beta adrenoceptors. The purpose of this study was to further probe the mechanism of action of intrathecal NPY. In the present study, a catheter (PE10) was inserted 10 cm down the spinal subarachnoid space and another catheter (PE20) was placed 0.5-1.0 mm into the cisterna magna in urethane-anesthetized rats. Artificial cerebrospinal fluid (CSF) was infused (100 µl/min) into the lumbar spinal cord and withdrawn from the cistern. We observed that high K^+ CSF (30 mM) increased NE release about twofold. NPY (10^{-7}M) had no effect on basal release of NE, but significantly inhibited high K^+ CSF-evoked NE release. Hindquarter and mesenteric blood flows were measured using miniaturized pulsed Doppler flow probes. Intrathecal injection of 1 nmol of NPY significantly increased hindquarter resistance concomitantly with depressor and bradycardic responses and a small decrease in mesenteric resistance. Our results are consistent with NPY exerting an inhibitory effect on evoked NE release in the spinal cord as well as producing a differential effect on regional vascular blood flow. (Supported by HL26319, HL35202 and HL39299.)

60.10

SUBSTANCE P(SP) ANTAGONISTS CAUSE SPINAL CORD VASOCONSTRICTION THAT IS NOT PREVENTED BY SP OR THYROTROPIN-RELEASING HORMONE (TRH) AGONISTS. C.J. Helke and E.T. Phillips*. Uniformed Services Univ. of the Health Sci., Bethesda, MD.

Intrathecal (i.t.) administration of SP antagonists caused neuropathological changes that were prevented by i.v. pretreatment with TRH (Freedman etal., 1986). We found that SP antagonists, including [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]-SP([D-Arg]-SP), reduced spinal cord blood flow and increased vascular resistance at the i.t. injection site, whereas TRH (i.v.) and a stable TRH analog (MK-771 i.t.) increased thoracic cord blood flow. Thus, the ability of pretreatment with TRH (i.v.) and MK-771 (i.t.) to prevent the [D-Arg]-SP-induced spinal cord vasoconstriction in anesthetized rats was assessed. [D-Arg]-SP (3.3 nmol i.t.) decreased thoracic cord blood flow within 15 min. Neither i.v. TRH (5mg/kg 1 hour, & 2 mg/kg just prior to [D-Arg]-SP) nor i.t. MK-771 (1 ug 8-10 min prior to [D-Arg]-SP) pretreatment prevented the [D-Arg]-SP-induced increase in thoracic vascular resistance. However, TRH (i.v.) but not MK-771 (i.t.) pretreatment partially opposed [D-Arg]-SP-induced reductions in blood pressure and thoracic cord blood flow. Thus, the prevention of SP antagonist-induced neuropathological changes by TRH is not due to prevention of the local spinal cord vasoconstrictor actions of the SP antagonist. In addition, the vasoconstriction is probably not a SP receptor-mediated effect as pretreatment with a SP agonist did not alter the thoracic cord vasoconstriction caused by [D-Arg]-SP.

60.11

DIRECT EVIDENCE FOR AN INVOLVEMENT OF CALCITONIN GENE-RELATED PEPTIDE(CGRP) IN NOCICEPTION AT THE SPINAL DORSAL HORN. M. Satoh, Y. Kuraishi*, R. Oku*, T. Nanayama*. Dept. of Pharmacol., Fac. of Pharm. Sci., Kyoto Univ., Kyoto 606, Japan.

We have reported that CGRP pharmacologically promotes nociceptive transmission at the spinal dorsal horn by potentiating substance P release from the primary afferents. In this study, the role of endogenous CGRP in hyperalgesia of adjuvant arthritic rats was investigated. a)An intrathecal injection of the anti-CGRP antiserum, but not the antiserum absorbed with synthetic CGRP, in arthritic rats significantly elevated nociceptive threshold in the paw-pressure test. b)The content of immunoreactive CGRP (iCGRP) was significantly increased in the dorsal root ganglia (DRG; L4-6), but not in the lumbar dorsal horn. c) An intrathecal injection of colchicine augmented the increase in iCGRP content of the DRG and reduced that of the dorsal horn, only in arthritic rats. d)The amount of capsaicin-evoked release of iCGRP from the lumbar dorsal horn slices was significantly larger in arthritic rats than in control rats. These results provide the direct evidence for the involvement of endogenous CGRP in the primary afferents in nociception at the spinal dorsal horn.

60.12

DIFFERENTIAL EFFECTS OF INTRATHECAL ADMINISTRATION OF SUBSTANCE P, GALANIN AND VIP IN MECHANICAL VS THERMAL STIMULATION TESTS IN THE RAT. Cridland, R.A.* and J.L. Henry (SPON: R.B. Malmo) Depts. Physiol. & Psychiat., McGill Univ., Montreal, H3G 1Y6

Substance P, galanin and VIP have been implicated in thermal and/or mechanical nociception. The present study compares the effects of intrathecal administration of these peptides to the L5 spinal level in a thermal stimulation test (tail withdrawal from a noxious radiant heat source, measured at 5 min intervals) and in a mechanical stimulation test (vocalization in response to application of von Frey hairs at 30 s intervals). In the tail-flick test, VIP (0.65-6.5 nmoles) produced a dose-dependent decrease in reaction time which peaked at 1 min and lasted 6-16 min; 6.5 nmoles decreased reaction time to 37.4 ± 3.8 % (mean ± S.E.M.; n=7) of control values at 1 min. Galanin (.65-6.5 nmoles) produced a dose-dependent increased reaction time at 1 and 6 min; 6.5 nmoles of galanin increased reaction time to 80 ± 10.7 % of the M.P.E. at 1 min (n=9). Adminstratation of CSF was without effect on reaction time (n=5). In the mechanical test, vocalization in response to a previously innocuous pressure stimulus was observed at 30 s in all 5 of the rats given galanin and 1 of the 5 rats given substance P (6.5 nmoles); the effect lasted 4-8 min. VIP and CSF had no effect. These results suggest unique roles of these peptides in the mediation or modulation of nociception at the spinal level. (Supported by MRC Grant MA-5891 to JLH)

60.13

DYNORPHIN AND SOMATOSTATIN INDUCE SPINAL CORD INJURY: NEUROANATOMICAL FINDINGS IN THE RAT. J.M. Petras* and J.B. Long (SPON: C.B.G. Campbell). Walter Reed Army Institute of Research, Washington D.C. 20307-5100.

Dynorphin-A (1-13) and somatostatin (3.1-50 nmoles) induce spinal cord injury in rats when injected, without physical trauma, into the lumbosacral subarachnoid space. Spinal cords of clinically impaired and unimpaired rats show evidence of neuropathology. Paraplegics have widespread bilateral loss of appendicular and axial motor neurons and interneurons, pronounced vascular proliferation and areas of necrosis dispersed in the gray matter. Ischemic or chromatolytic interneurons are plentiful and "surviving" motor neurons may be similarly affected. Axon degeneration is widespread and massive in the gray and white matter. Tracts to the tegmentum, cerebellum and thalamus are degenerated. Neurologically unimpaired low dose rats reveal evidence of less severe injury. Spinal cord interneurons and motoneurons appear to be unaffected when studied by light microscopy, whereas axonal degeneration is clearly evident in the same cases. On the basis of their apparent analgesic effects in mice, rats and human cancer and surgical patients (Chrubasik, J. et al., Lancet, 2: 1208,1984; Han, J-S. and Xie, C-W., Life Sci., 31: 1781, 1984; Nakazawa, T. et al., Peptides 6: 75, 1985; Wen, H.L. et al., Peptides 8: 191, 1987), both peptides have been proposed as potent spinal analgesics at the doses which we have observed to induce injury. More research is required before dynorphin or somatostatin can be considered potent clinical analgesic agents without deleterious effects.

60.14

CONUS PEPTIDES WHICH INDUCE SLEEP IN YOUNG MICE AND HYPERACTIVITY IN ADULT MICE. J. Haack, L. J. Cruz, R. Galyean*, J. Rivier* and B. M. Olivera. Dept. of Biology, University of Utah, Salt Lake City, UT 84112 and Clayton Foundation Laboratories for Peptide Biology, Salk Institute, San Diego, CA 92138.

An unusual heptadecapeptide purified from the venom of the fish-hunting snail Conus geographus induces a sleep-like state in mice under two weeks of age, and a hyperactive state in older mice upon i.c. injection. The unusual change in physiological effects of this peptide are matched by its biochemical properties; out of 17 amino acids, 5 residues are the unusual modified amino acid γ-carboxyglutamate (GLA). We report the isolation and characterization of a homologous sleep-inducing peptide from the venom of Conus tulipa, another fish-hunting cone. This peptide shows the same remarkable developmental switch in physiological effects as the sleep-inducing peptide from Conus geographus. However, the Conus tulipa peptide has 21 amino acids instead of 17; 4 out of the 5 Gla positions are conserved, but only 4 of 12 non-Gla residues. The high degree of conservation of Gla residues suggests that this modification may be important in producing the physiological activity of these peptides, but that there is considerable flexibility in other sequence features. Both sleep-inducing peptides have been synthesized. (Supported by GM22737 and AM26741).

60.15

NEUROTENSIN (NT) APPLIED ON VENTRAL SURFACE OF MEDULLA (VMS) ENHANCES THE PHRENIC NERVE (Ph) RESPONSES TO HYPERCAPNIA AND HYPOXIA. M.A. Haxhiu, E. van Lunteren, E.C. Deal, N.S. Cherniack. Dept. of Medicine, Case Western Reserve University, Cleveland, OH 44106.

NT, a tridecapeptide, fulfills many of the criteria required for a neurotransmitter or a neuromodulator, and NT-like immunoreactive fibers have been described just beneath the surface of the ventral medulla. We determined whether NT applied topically to the intermedio-caudal area of VMS affects Ph activity and its responses to steady state hypercapnia and isocapnic hypoxia. In 16 chloralose anesthetized, paralyzed, artificially ventilated cats application of pledgets containing NT (10^{-5} to 10^{-3}M) to VMS increased phrenic nerve activity, and potentiated the response to hypercapnia and hypoxia. The increases after combination of ventilation with 3% CO_2 in O_2 and VMS application of NT (n=6) were significantly greater ($p<0.05$) than with either one alone (3% CO_2:9.3 ± 1.6; NT:8.2 ± 3; NT + 3% CO_2:24.8 ± 4.5; a.u.). NT also enhanced the response to 7% CO_2, but less than when the animals were ventilated with 3% CO_2. The combination of hypoxia and NT also had significantly greater effects ($p<0.05$) than either stimulus alone (12% O_2:2 ± 1.6; NT:3 ± 2; NT + 12% O_2:12.3 ± 4.1; a.u.). The results suggest that NT excites medullary inspiratory-related neurons possibly by allowing them to fire with less input from central and peripheral chemoreceptors.

SUPPORT: HL-39921; HL-25830; HL-01600.

62

MOLECULAR BIOLOGICAL APPROACHES TO STUDY NEUROPEPTIDE BIOSYNTHESIS. **L.Fricker, Albert Einstein College; S.Watson, Univ. of Michigan; R.Scheller, Stanford Univ., L.Devi, New York Univ., I.Lindberg, Louisiana State Univ.**

This session will focus on the expression, regulation, and proteolytic processing of neuropeptides; with emphasis on both techniques and results. Stanley Watson will discuss the use of in situ hybridization and Northern blot analysis to measure neuropeptide mRNA levels, as an indication of cellular biosynthetic ability. Gene transcription studies will be addressed, with a major focus on the integrated physiology of several endocrine and CNS peptide systems. Richard Scheller will discuss the application of novel approaches to study processing of egg laying hormone in the Bag cells of Aplysia. Lakshmi Devi will discuss the use of gene transfer techniques to introduce foreign neuropeptide genes into heterologous hormonal cell lines in order to study the specificity of the processing enzymes. Iris Lindberg will discuss the use of eukaryotic expression systems as a means to generate neuropeptide precursors, and the use of these precursors to study the substrate specificity of an enkephalin-generating endopeptidase isolated from adrenal chromaffin granules. Lloyd Fricker will present the cloning and sequence analysis of cDNA encoding carboxypeptidase E, a carboxypeptidase B-like enzyme associated with the biosynthesis of numerous neuropeptides. Regulation of carboxypeptidase E mRNA and enzyme activity will also be discussed.

63

WORKSHOP: AFFERENT REGULATION OF THE LOCUS COERULEUS, G. Aston-Jones (Chairman), New York Univ., M.T. Shipley, Univ. Cincinnati College of Medicine, V.A. Pieribone, New York Univ., J.T. Williams, Oregon Hlth. Sci. Cntr., T.H. Svensson, Karolinska Inst., and D.J. Reis, Cornell Univ. Medical School.

Knowledge of afferent mechaisms regulating LC neurons has increased dramatically in the last few years. This workshop will integrate results of experiments in several subdisciplines that converge to yield a markedly revised but coherent view of mechanisms important in controlling this ubiquitous noradrenergic system.

M. Shipley will describe a recent anatomical reinvestigation of afferents to LC revealing that major inputs beyond the LC region are limited to 2 rostral medullary areas, the paragigantocellularis (PGi) and the prepositus hypoglossi (PrH). He will also describe several autonomic and visceral brain areas that send converging projections to PGi areas afferent to LC. **V. Pieribone** will describe double labeling studies revealing that PGi and PrH are sources for several neurotransmitters that innervate LC (incl. epinephrine, 5-HT and GABA). **G. Aston-Jones** will describe physiologic experiments *in vivo* revealing that (i) PGi and PrH have potent effects on LC discharge (ii) PGi uses glutamate and epinephrine, and PrH uses GABA, in projections to LC, (iii) LC sensory responses may be mediated by glutamate inputs from PGi, and (iv) 5-HT has potent modulatory effects on glutamate-evoked responses of LC neurons. **J. Williams** will describe recent *in vitro* intracellular studies revealing that stimulation-evoked synaptic potentials in LC neurons are also mediated largely by glutamate (which is modulated by 5-HT), GABA, and epinephrine. **T. Svensson** will review evidence that many visceral and autonomic stimuli influence LC discharge, and recent studies by G. Engberg indicating that certain drug effects on LC activity may be initiated in the periphery and transmitted to the LC via an excitatory amino acid pathway. These results are consistent with above findings that PGi, a key autonomic and vegetative region, provides prominent glutamate inputs to LC. Finally, **D. Reis** will discuss anatomic and physiologic characteristics of the rostral ventrolateral medulla (PGi area), its role in autonomic control, and functional implications of this area being a major afferent to LC. A general discussion among the speakers and audience will follow.

64.1

PROGESTERONE INDUCES THE DEVELOPMENT OF CALCIUM CURRENTS IN MYOMETRIAL CELLS ISOLATED FROM IMMATURE RAT UTERUS. J. Rendt* and S. D. Erulkar. Departments of Pharmacology and Physiology, University of Pennsylvania, School of Medicine Philadelphia, PA.

We have examined the role of ovarian steroids in modulating the ionic currents of single immature rat myometrial cells in primary tissue culture.

Myometrial cells were isolated from uteri of 23 day old Sprague-Dawley rats as described by McCormick and Glasser (Endocrinology, 106:1634,1980). Steroid treated animals were injected with either progesterone (500 μg/animal/day), or estrogen (1 μg/animal/day) or both for 3 days prior to cell isolation. Treated cells were grown in DMEM with the steroid. Currents were recorded by the whole cell patch-clamp method.

Cells from untreated immature rats show outward K^+ currents, but little or no inward current. Cells from rats injected with either progesterone or progesterone plus estrogen have significantly larger inward currents. Treatment with estrogen alone does not elicit similar inward currents.

The inward current displays time and voltage-dependent inactivation. It is blocked by Co^{++}, Mn^{++}, Cd^{++}, and nifedipine; it is enhanced by Bay-K8644. Total replacement of external Na^+ by Cs^+ does not reduce the inward tail current suggesting that the inward current is carried exclusively by Ca^{++}.Supported by NS-12211

64.2

DEVELOPMENT OF Ca, Na AND K CURRENTS FROM OOCYTE TO NEURULA IN ASCIDIAN EMBRYOS. W.J. Moody, L. Simoncini* and M.I. Block, Dept. of Zoology, Univ. Washington, Seattle, WA

We have studied the ontogeny of voltage-dependent currents in embryos of the ascidian B. villosa using the whole-cell clamp on blastomeres isolated from various embryonic stages from oocyte to neurula. Muscle-lineage blastomeres in this species have an orange pigment that enables them to be identified at any stage. The unfertilized oocyte has three voltage-dependent currents: Na and Ca currents activated by depolarization and an inwardly rectifying K current activated by hyperpolarization. These three currents disappear from all blastomeres during early development, each at a characteristic stage: the Na current by the 2-cell stage, the Ca current by gastrulation, and the inward rectifier by mid-neurula. Just as the inward rectifier disappears, the Ca current reappears, but only in muscle-lineage cells. This is the first stage of development at which cells of different developmental fates have different electrical properties. The appearance of Ca currents in muscle-lineage cells precedes morphological changes, contractility and innervation in these cells. The time between gastrula, when all blastomeres are electrically equivalent, having only inwardly rectifying K currents, and neurula, when muscle-lineage cells, but not other cells, have developed large inward Ca currents, is only about 3 hours. This short time period and cell specificity of the appearance of Ca currents should allow us to study their ontogeny in detail.

64.3

CYCLIC CHANGES IN CHLORIDE CURRENT DENSITY WITH CELL DIVISION IN EARLY BLASTOMERES FROM THE ASCIDIAN BOLTENIA VILLOSA. M. L. Block and W.J. Moody, Dept. of Zoology, U. of Washington, Seattle, WA 98195

The unfertilized eggs and quiescent blastomeres through 3 cell divisions of the ascidian Boltenia villosa have 3 main voltage dependent ionic currents (Block & Moody, 1987, J. Physiol. 393, 619). These are transient inward Na and Ca currents upon depolarization from rest (-70mV) and an inwardly rectifying K current upon hyperpolarization. The Ca and K current densities through the 8-cell stage remain approximately equal to those of the unfertilized oocyte. The Na current density, on the other hand, decays rapidly and steadily after fertilization until the current is no longer detected at the 8-cell stage. This is a report of a 4th current with a completely different behavior in the developing embryo. There is seen in quiescent cells after fertilization a very small inward current with very slow kinetics compared with those of the inward K current when 10Ba-ASW is used to completely block the K inward rectifier. Over the course of about 15-20 min., as the cell prepares for division, this current increases in size, sometimes by a factor of 1000, to a peak just before cell division and then drops rapidly back to its origional level as soon as cell division is complete. Cyclic changes in this current can be seen in the transitions between 1 to 2, 2 to 4 and 4 to 8 cells. This cyclic change can also be observed over at least 3 cycles as a change during nuclear division in cytochalasin B treated embryos (no cell division takes place). Tail current measurements were made in Na free, K free ASW (choline sub.) with varying Cl concentrations (gluconate sub.). The reversal potential of this current follows the Cl concentration. Supported by HD17486 and Research Career Development Award to WJM.

64.4

EFFECTS OF MITOGENS ON SCHWANN CELL ION CHANNELS. G.F.Wilson* and S.Y.Chiu. (SPON: D. Oertel) NeuroScience Program & Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Previously, our lab has shown that increases in potassium current accompany the avid Schwann cell proliferation induced by nerve transection. Since, in Wallerian Degeneration the mitogens initiating proliferation remain enigmatic, we examine the effects of known mitogens on outward current in purified Schwann cell populations from newborn rat sciatic nerve. Primary cultures were stimulated for 12-40 hours using either crude myelin (MF, 150 μg/ml) or axonal fractions (AF, 150 μg/ml), bovine pituitary extract (BPE, 500 μg/ml, Sigma P1167), or a combination of BPE and 2μM forskolin (F, Sigma F6886). Whole cell recordings from unstimulated cells (n=61) revealed a variable outward current averaging 148 ± 14 pA. Mitogen enhancement of this current ranged from 4.8 to 1.0 times control per experiment for MF(n=14), 2.9 to 0.9 for AF(n=17), 2.4 to 1.0 for BPE(n=19), and 1.2 to 1.0 for BPE+F(n=20) when treated and control cells from one culture were compared on the same day. It appears that mitogen induces the largest relative increase in cells having a low pretreatment basal current. These results roughly parallel those observed for mitogen induced Schwann cell proliferation, as measured by scintillation counts of ^{3}H-thymidine incorporation. Preliminary data also suggest 4AP, TEA, and quinine block MF-induced mitosis, functionally linking current to proliferation in developing, as well as adult Schwann cells.

Supported by NS-23375 (NIH), RG-1839 (National Multiple Sclerosis Society) and a PEW Scholar Award in Biomedical Sciences to S.Y.C.

64.5

CONCANAVALIN A INCREASES THE ACTIVITY OF A POTASSIUM CHANNEL IN CULTURED APLYSIA NEURONS VIA AN INTRACELLULAR SECOND MESSENGER. S.S. Lin*, D. Dagan, and I.B. Levitan. Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254

The lectin Concanavalin A (Con A) has been shown to affect a number of properties of *Aplysia* neurons. It alters their response to glutamate (Kehoe, *Nature* 274 (1978) 866-869), and enhances neurite outgrowth and changes the type of synapses formed between cultured neurons (Lin and Levitan, *Science*, 237 (1987) 648-650). The mechanism(s) by which Con A induces these various types of neuronal plasticity is not known. We report here that, in cell-attached membrane patches on cultured medial neurons, the activity of a 100 pS potassium channel increases when the cell is exposed to Con A. This increase in activity is due to a large decrease in the mean closed time between bursts of channel openings with little if any change in the mean open time. Since the lectin is applied in the bathing medium outside the patch electrode and has no direct access to the channel in the patch, it must affect the channel's activity through a second messenger. Preliminary results suggest this effect of Con A is not mediated by voltage or Ca^{2+}. Work is currently under way to determine which intracellular messenger is involved in altering channel activity. Supported by NIH grant NS25366 to I.B.L.

64.6

FMRFamide ANTAGONIZES S CURRENT MODULATION AND PROTEIN PHOSPHORYLATION PRODUCED BY 5-HT and cAMP. A. Volterra, S.A. Siegelbaum, E.R. Kandel and J.D. Sweatt*. HHMI, Ctr. for Neurobiol. & Behavior, Columbia Univ., New York, NY 10032.

5-HT closes the S K^+ channel in Aplysia sensory neurons through cAMP-dependent protein phosphorylation while FMRF-amide increases S channel opening through lipoxygenase metabolites of arachidonic acid. FMRFamide also antagonizes the effects of 5-HT (or cAMP) by reopening S channels closed by 5-HT (or cAMP). At what level in the cAMP cascade does this antagonism occur? Voltage-clamp experiments show that FMRFamide produces a 300 pA increase in S current while cAMP alone produces a 200 pA decrease in S current. However, when FMRFamide is applied on top of cAMP, there is a net increase in S current of 400 pA, confirming a partial reversal of the effects of cAMP. 5-HT or a cAMP analog increase the phosphorylation of 17 spots (by 50-700%) as measured by ^{32}P autoradiography of 2-D gels. By contrast, FMRFamide decreases basal phosphorylation levels in 10 of the 17 spots (40-90% decrease). When FMRFamide is applied 1 min after either 5-HT or cAMP, the normal increase in phosphorylation in all 17 spots is now reversed. In 7 spots, the level of phosphorylation is at control levels. In the 10 spots whose phosphorylation is decreased by FMRFamide alone, the level of phosphorylation is reduced below control values. Since FMRFamide reverses S channel modulation and phosphorylation with cAMP or 5-HT, the antagonism must work either through kinase inhibition or phosphatase activation.

64.7

PROCTOLIN MODULATES K^+ AND Cl^- CURRENTS IN THE CRAB STG. J. Golowasch and E. Marder. Biol. Dept., Brandeis Univ., Waltham, MA 02254.

The peptide proctolin excites the pyloric network of the Stomatogastric Ganglion (STG) of the crab, Cancer borealis. The Lateral Pyloric (LP) neuron is one of the direct targets of proctolin, and its proctolin response is qualitatively the same in the intact ganglion as that seen when it is isolated from presynaptic inputs. Thus LP neurons were studied in minimal isolation conditions (TTX-picrotoxin) in two-electrode current and voltage clamp to determine the currents modulated by proctolin. At hyperpolarized membrane potentials proctolin action is associated with a net conductance decrease, while at depolarized potentials proctolin action is associated with a conductance increase. At least two components can be distinguished in the total current modulated by proctolin: 1) A voltage independent K^+ current that is turned off (or decreased) 2) A voltage dependent Cl^- current that is turned on (or increased) at membrane potentials around and more positive than resting potential. These currents are sufficient to account for most or all of the properties of proctolin's actions on the LP neuron observed in current clamp. Supported by NS 17183.

64.8

FORSKOLIN REDUCES THE POTASSIUM CURRENT I_A BY A cAMP-INDEPENDENT ACTION IN LOBSTER NEURONS. R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

Forskolin directly activates adenylate cyclase and is frequently used as a tool to analyze the effects of cAMP in neurons. However, Watanabe and Gola (Neurosci. Lett. 78:211,1987) demonstrated that forskolin can reduce voltage-dependent potassium currents in snail neurons by a cAMP-independent mechanism. Here I show that forskolin reduces I_A in neurons of the stomatogastric ganglion of the spiny lobster, *Panulirus interruptus*. Using a 2-electrode voltage clamp, the somata of these cells have little or no detectable inward currents. Three voltage-dependent outward currents have been described by Graubard, Hartline and colleagues (Neurosci. Abst. 10:1073,1984; 11:1023,1985): I_A, a transient potassium current activated from hyperpolarized holding potentials; $I_{K(Ca)}$, a calcium-dependent inactivating current; and $I_{K(V)}$, a slowly activating, non-inactivating potassium current. Forskolin (50 μM) reduced I_A, decreasing the peak outward current and accelerating the rate of inactivation. Forskolin had little or no effect on $I_{K(Ca)}$ or $I_{K(V)}$ (seen with measurements from V_{hold} of -50 mV, where I_A is inactivated). Forskolin had strong effects on identified cells with prominent I_A (PD,AB,PY), but very little effect on cells which have small I_A (LP,VD,IC). Several other compounds that enhance cAMP-dependent effects in these cells (8-bromo-cAMP, N,N-dibutyryl cAMP, IBMX: Flamm et al, J. Neurophysiol. 58:1370,1987) had no detectable effect on I_A in any cells tested. Finally, 1,9-dideoxyforskolin, an analog that does not activate adenylate cyclase, mimicked forskolin's reduction of I_A. In conclusion, forskolin reduces I_A in lobster neuronal somata by a mechanism that is independent of cAMP synthesis. Supported by NIH NS17323.

64.9

REDUCTION OF VOLTAGE-ACTIVATED K^+ CURRENTS BY FORSKOLIN IS NOT MEDIATED BY cAMP IN PLEURAL SENSORY NEURONS OF *APLYSIA*. D.A. Baxter & J.H. Byrne Dept., of Neurobiol. & Anat., Univ. of Tex. Med. Sch., Houston, TX 77225.

Forskolin (FSK) is often used in studies of the modulation of ion channels to specifically activate adenylate cyclase. Previously, we found that application of FSK reduced the transient ($I_{K,A}$) and voltage-dependent ($I_{K,V}$) K^+ currents in sensory neurons isolated from pleural ganglia of *Aplysia* (Baxter & Byrne, *Soc Neurosci Abstr* 11:787, 1985). However, we also found that application of cAMP analogues reduced only the serotonin-sensitive ($I_{K,S}$) K^+ current (Baxter & Byrne, *Soc Neurosci Abstr* 13:1440, 1987); suggesting that FSK has actions unrelated to elevation of cAMP. The present study confirms that FSK reduces the amplitudes of $I_{K,A}$ and $I_{K,V}$ in a dose-dependent manner (10-300 μM); apparently without altering the kinetics of the currents. This action of FSK is not mimicked by cAMP, which reduces only $I_{K,S}$. Furthermore, 1,9-dideoxyforskolin, an analogue which does not stimulate the cyclase, also reduces voltage-activated K^+ currents. In contrast, Modified-FSK (Calbiochem), an analogue which stimulates the cyclase, reduces only $I_{K,S}$. While the nature of interaction between FSK and ion channels is not known, the simplest interpretation of these results is that FSK can act as a K^+ channel blocker. These results and those from several other recent studies support the conclusion that, in addition to activating adenylate cyclase, FSK may have a direct affect on ion channels that is not mediated via cAMP (Hoshi *et al.*, *Biophys J* 53:144*a*, 1988; Coombs & Thompson, *J Neurosci* 7:443, 1987; Watanabe & Gola, *Neurosci Let* 78:211, 1987). Supported by grant AFOSR 87-020.

64.10

MODULATION OF A CALCIUM-ACTIVATED POTASSIUM CURRENT BY CYCLIC-AMP. M.D. Womble and W.O. Wickelgren. Dept. of Physiology, Univ. Colorado Health Sci. Ctr., Denver, CO, 80262.

In the presence of Na^+ (TTX) and K^+ (TEA and 3,4 DAP) channel blockers, sensory neurons (dorsal cells) of the isolated lamprey (*Lampetra lamottenii*) spinal cord produce Ca^{+2}-dependent action potentials (Ca AP's). Their duration is prolonged by γ-aminobutyric acid (GABA) which reduces a Ca^{+2}-activated K^+ conductance ($G_{K(Ca)}$) (Leonard and Wickelgren, J. Physiol. 375: 481, 1986). Ca AP's are also broadened by forskolin, an activator of adenylate cyclase (Womble and Wickelgren, Soc. Neurosci. Abstr. 13: 790, 1987). Here, we confirm that forskolin also inhibits a $G_{K(Ca)}$, via activation of adenylate cyclase.

Ca AP's were prolonged by forskolin in a dose-dependent manner, with an EC50 of 55 μM and maximum effective dose of approximately 250 μM. This action is inhibited by dideoxy-adenosine, an inhibitor of adenylate cyclase. Further, Ca AP's are not prolonged by the inactive analogue 1,9-dideoxyfoskolin. We conclude that forskolin is activating adenylate cyclase and does not act by blocking $G_{K(Ca)}$ directly.

In voltage clamp, dorsal cells show a sustained outward current which is decreased by Ba^{+2} or forskolin application and is correlated with an increase in Ca AP duration. We conclude that activation of adenylate cyclase by forskolin results in a prolonged Ca AP by decreasing the size of a repolarizing $G_{K(Ca)}$. Thus, the broadening action of GABA on Ca AP's of lamprey dorsal cells may also be mediated by cyclic AMP.

64.11

ACTIVITY OF PROTEIN KINASE C (PKC)-INDUCED CA^{++} CHANNEL PERSISTS AFTER INHIBITION OF THE KINASE. P.J. Conn, J.S. Strong, and L.K. Kaczmarek. Depts. of Pharmacol. & Cell. Molec. Physiol. Yale Univ. Sch. of Med., New Haven, CT 06510.

Following brief stimulation of an afferent pathway, the bag cell neurons of Aplysia generate a prolonged afterdischarge during which action potentials become enhanced in height and width. In isolated bag cell neurons, PKC activators, such as TPA, enhance the amplitude of action potentials by unmasking a previously covert species of voltage-dependent Ca^{++} channel. We have now shown that PKC inhibitors prevent TPA-induced expression of the covert Ca^{++} channel when added prior to TPA addition, but do not reverse the effects of TPA when added after action potentials have already become enhanced by TPA. This suggests that transient activation of PKC may act as a "trigger" for the appearance of the covert Ca^{++} channels but that continued activation of the enzyme is not required for the maintenance of active channels. We have also shown that, in intact ganglia, PKC inhibitors do not prevent initiation of the afterdischarge but inhibit the enhancement of action potentials during the afterdischarge. These data suggest that the PKC-induced unmasking of the covert Ca^{++} channel contributes to the enhancement of action potentials during the afterdischarge.

64.12

MODULATION OF NEURONAL Ca CHANNELS BY cAMP AND PHORBOL ESTERS. D. Lipscombe, K. Bley & R.W. Tsien. Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06510.

Norepinephrine (NE) has dual actions on whole cell Ca channel currents in frog sympathetic neurons. L-type Ca current is enhanced through ß-adrenergic stimulation while N-type Ca current is inhibited via α-adrenergic stimulation. Inclusion of GTP (0.2-0.3 mM) in the pipette solution is required to see both effects repeatedly. In cell-attached patches, unitary activity of L-type Ca channels is stimulated by NE, isoproterenol or 8-bromo-cAMP added to the bath. The increase in L channel activity is dominated by enhanced opening probability and a clearcut increase in open time. However, the activity of single N-type Ca channels is not increased by ß-adrenergic stimulation. Thus, L and N-type Ca channels can be distinguished by their very different responsiveness to the second messenger cAMP.

α-adrenoceptor mediated inhibition of N-type channel activity is not mediated by a widely diffusible messenger since it is not observed in cell-attached patch recordings when NE is added to the bath. Stimulation of protein kinase C (PKC) has been shown to inhibit Ca channel activity in chick DRG neurons (Rane & Dunlap, *PNAS*, 83:184, 1986). However, in frog sympathetic neurons, PKC activation by either phorbol diacetate or phorbol dibutyrate (0.1-1 μM) does not mimic the α-effect on N-type Ca channels. Stimulation of PKC produces little change in whole cell Ca channel current. In cell-attached patches, L and N-type Ca channel activity both increase within 1-3 min following application of phorbol ester due to a marked enhancement of single channel open probability. This increase of Ca current differs in mechanism from that reported in *Aplysia* bag cell neurons (Strong et al, Nature, 325:714, 1987).

64.13

ROLE OF C KINASE AND cGMP IN REGULATING GUINEA PIG OR RAT HIPPOCAMPAL CA CURRENT: STUDIES WITH KINASE INHIBITORS. D. Doerner, M.P. Walsh* and B.E. Alger, Univ. MD. Sch. Med., Balto, MD 21201; *U. Calgary, Calgary, Alberta T2N 4N1.

We have shown that phorbol esters suppress voltage-dependent Ca currents in hippocampal neurons recorded with Cs filled electrodes under whole cell clamp. To test whether this effect is mediated by C kinase, we used the kinase inhibitors H-7, H-8, a 17 kD peptide (PKCI, McDonald and Walsh, BBRC, 129:603, 1985), and sphingosine, before and after application of phorbol esters.

At $\leq$ 20 μM, H-7 reversed the phorbol-ester-induced suppression of I_{Ba}, but had no direct effects. Other inhibitors, sphingosine and PKCI also blocked phorbol ester effects suggesting these effects are due to C kinase. At $\geq$ 40 μM H-7 directly enhanced I_{Ba}, an effect probably not mediated by inhibition of baseline C kinase activity since other C kinase inhibitors produced no enhancement. Involvement of A kinase in the direct effects of H-7 was excluded as 8-Br-cAMP did not suppress I_{Ba}.

We report for the first time that 8-Br-cGMP reversibly reduces I_{Ba}. Interestingly, this effect was not blocked by H-8, a potent G kinase antagonist, or the other kinase inhibitors, suggesting cGMP effects may be independent of C or G kinase. We also conclude that high dose H-7 does not enhance I_{Ba} by inhibiting ongoing C, A or G (since H-8 did not mimic H-7) kinase activity and may represent a direct effect on the calcium channel.

ALZHEIMER'S DISEASE II

65.1

CALBINDIN D28 NEURONS IN THE HIPPOCAMPAL FORMATION ARE RESISTANT TO DEGENERATION AND DO NOT DEVELOP REGENERATIVE FEATURES IN ALZHEIMER'S DISEASE. S.R. Hoffman*, N.W. Kowall, and A.C. McKee (SPON:Valerie Knowlton) Neurology Service and Dept. Neuropath., Mass. General Hospital, Boston MA 02114.

CA1 neurons in the hippocampal formation are sensitive to ischemic, hypoglycemic and seizure induced insults as well as neurofibrillary tangle (NFT) formation in Alzheimer's disease. The resistance of neurons to seizure induced injury may be related to their enrichment with Calbindin D28 (CaBP). We used an antibody against CaBP (courtesy of C.R. Gerfen) to examine the hippocampus in 6 patients with AD and 6 age-matched controls. In normal dentate gyrus CaBP is prominent in granule cells and scattered stellate cells.A dense band representing mossy fiber terminals is seen in CA 4 and CA 3. Scattered non-pyramidal neurons with extensive dendritic and axonal branches are found in CA 1. In AD dentate stellate cells are more intensely reactive while granule cells are unchanged. Enhanced terminal staining is seen in the outer part of the molecular layer and senile plaques are evident. Non-pyramidal neurons show perikaryal swelling but their dendritic trees are largely unchanged, in stark contrast to changes evident with MAP 2 histochemistry in the same regions. CaBP neurons are less severely affected by degenerative and regenerative responses than pyramidal neurons possibly due to their enhanced calcium buffering capacity.

65.2

THE CALCIUM PUMP AS A POSSIBLE DIAGNOSTIC MARKER FOR ALZHEIMER'S DISEASE. E. RIZOPOULOS, M.J. WAYNER, J.P. CHAMBERS. SPON: (F.C. BARONE). The Brain Research Laboratory of Biochemistry, University of Texas at San Antonio, TX 78285.

Increased intracellular calcium has been correlated with neuronal degeneration. Fibroblasts from SDAT patients have been shown to exhibit high calcium levels suggesting a problem with extrusion of calcium. In an attempt to establish a diagnostic assay for SDAT, we investigated the hydrolytic properties of the calcium pump from SDAT fibroblasts (cell lines AG0364B and AG4402) and their age-matched controls (AG4440A and AG4058). Control homogenates exhibited the well established property of inhibition of ATP hydrolysis at high free calcium (360 uM); whereas SDAT fibroblasts retained at least 50% ATP hydrolytic activity. As assayed, this activity corresponds to ATP hydrolysis by the plasma membrane, calcium pump. Although more SDAT cell lines must be examined, these data suggest that an abnormality in the calcium pump from SDAT patients may be the basis for noninvasive, diagnostic assay. Such abnormalities related to calcium homeostasis could provide insight into the etiology of this memory related neuronal disease. (Supported by the American Health Assistance Foundation)

65.3

CHEMICAL PATHOLOGY OF CEREBRAL VESSELS IN ALZHEIMER'S DISEASE. R.N. Kalaria, M.J. Mitchell* and S.I. Harik Dept. of Neurology, CWRU, Cleveland, Ohio 44106

Increasing evidence suggests there is breakdown of blood-brain barrier (BBB) function in Alzheimer's disease (AD). Cerebral amyloid angiopathy is common in AD and plaques are observed to evolve around brain capillaries. We studied the biochemical and pharmacological properties of cerebral microvessels isolated from the brains of AD subjects and age-matched controls. Microvessels isolated from frontal and occipital lobes were checked for purity by light microscopy and biochemically. Fractions of vessels were also stained with thioflavin-S to assess amyloid deposition. We also assayed several marker enzymes for subcellular fractions of brain endothelium. The activities of γ-glutamyl transpeptidase, alkaline phosphatase, monoamine oxidase, glucose-6 phosphatase, and Na+,K+-ATPase (ouabain binding) were not significantly altered in AD. However, there was a decreased activity of the angiotensin converting enzyme in vessels from AD subjects. Also, BuChE was reduced by ~ 50% in AD. Hydroxyproline levels to assess basement membrane were significantly increased in vessels from AD subjects. These changes along with other alterations in transport proteins such as the glucose transporter suggest functional impairment of the BBB in AD.
Supported by a grant from ADRDA and by grant HL-35617.

65.4

ABNORMAL PROTEIN KINASE C IN ALZHEIMER FIBROBLASTS. T. Saitoh, G. Cole*, T. Huynh*, R. Katzman and M. Sundsmo*. U.C.S.D., Sch. of Med., Neuroscience Dept., M-024, La Jolla, CA 92093.

Protein kinase C (PK-C) cascade is altered in brain of Alzheimer's disease (AD) patients detected as 1) reduced PK-C activity in the particulate fraction (PF), 2) reduced phorbol ester binding in PF and 3) reduced in vitro P86 phosphorylation in the cytosol fraction (CF) (Cole et al., Brain Res. 1988). To further understand the biological meaning of this finding, we asked if these alterations are secondary effects of neuronal degeneration or primary changes involved in AD pathogenesis. If altered PK-C cascade is found in AD fibroblasts which are not involved in the degeneration, the latter possibility is more likely. Upper-arm skin biopsies of AD patients and age-matched controls were trypsinized and dissociated fibroblasts incubated in DMEM. Cells were harvested when 80% confluent, washed, homogenized and fractionated into PF and CF. When PK-C activity was assayed with histone as substrate, AD and control samples were equally active. However, quantification of PK-C with anti-(PK-C) antibody revealed a lower level of enzyme in AD samples in both PF and CF. When AD samples were subdivided into two groups, i.e. sporadic and familial AD, the reduction in the concentration of PK-C was greater in familial cases. In vitro phosphorylation of a major PK-C substrate, P79, in Alzheimer CF was also decreased relative to control. The reduction in the P79 phosphorylation was more pronounced in familial cases than in sporadic cases. From these studies, we conclude that abnormal PK-C cascade reactions that we observed in the brain tissue of AD patients are not the mere consequence of neuronal degeneration but are involved, possibly causally, in the pathogenesis of Alzheimer's disease.

65.5

α_1-ANTICHYMOTRYPSIN IN BRAIN AGING AND DISEASE. H. Potter, D.J. Selkoe[1], D.L. Price[2], L. Cork*[2] and C.R. Abraham. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115; Brigham and Women's Hospital, Boston, MA 02115[1]; Johns Hopkins Medical School, Baltimore, MD 21205[2].

The recent finding (Abraham et al., 1988, Cell 52:487) that the serine protease inhibitor, α_1-antichymotrypsin (ACT), is tightly associated with the amyloid deposits of normal aged and Alzheimer's disease (AD) brains, suggests a role for this inhibitor in the amyloid deposition. We used immunocytochemistry to analyze the presence of ACT in brain amyloid of aging monkeys. The earliest ACT immunoreactivity was found in cortical perivascular cells. The cortical amyloid, both in senile plaques and vasculature, was seen only several years later, and could be stained for both β-protein and ACT. In addition, we examined the distribution of the inhibitor in various human neuropathological states in order to identify cells that contain this protein during brain degeneration. ACT immunoreactivity was found in astrocytes near areas of neuronal or tissue loss, in pericytes and in a few neurons. Lastly, we analyzed the association of ACT with the other types of amyloidoses. ACT antibodies labeled only amyloid deposits that have as their major component the β-protein: normal aging, AD, Down's syndrome and in the hereditary cerebral hemorrhage of Dutch origin. In Familial Amyloidotic Neuropathy, primary amyloidosis and secondary amyloidosis, the ACT staining was not associated with amyloid fibers. In summary, ACT is found in the brain in three cell types. In amyloid deposits it is found only in association with the β-protein, further strengthening its possible role in the processing of the β-protein precursor.

65.6

TAURINE IMMUNOREACTIVE PYRAMIDAL NEURONS DEGENERATE IN ALZHEIMER'S DISEASE HIPPOCAMPAL FORMATION. N.W. Kowall A.C. McKee and M.F. Beal, Neurology Service and Dept. of Neuropathology, Mass. General Hospital, Boston MA 02114.

Taurine is a putative inhibitory neurotransmitter found in high concentrations in animal and human brain. A subset of hippocampal pyramidal and granule cells in rat hippocampus are taurine immunoreactive. Taurine concentrations are not depleted in Alzheimer's disease (AD) cortex, but histochemical studies have not been performed to determine the state of immunoreactive neurons in human brain. An antibody to taurine was raised using colloidal gold adsorbed taurine as the antigen to elicit antibodies in rabbits. Antiserum was characterized with absorption controls for taurine and related amino acids and by its characteristic staining pattern in the brain. Four AD brains and 4 age-matched controls were examined after immersion fixation in neutral buffered formalin or periodate-lysine-paraformaldehyde. Purkinje cell bodies and dendrites were intensely immunoreactive, consistent with reports in other species. In the hippocampal formation a subset of pyramidal neurons were immunoreactive. In AD these neurons degenerated and developed neurofibrillary tangles, as shown with silver and thioflavine S counterstains. Therefore, a second neurotransmitter defined subset of pyramidal neurons, in addition to glutamatergic neurons, is predisposed to neurofibrillary tangle formation in the hippocampus.

65.7

MICROTUBULE DISORGANIZATION AND GROWTH CONE FORMATION CHARACTERIZE DEGENERATION AND REGENERATION IN ALZHEIMER'S DISEASE. A.C. McKee, N.W. Kowall, and K.S. Kosik, Dept. Neuropath. and Neurol., Harvard Med Sch, Boston, Ma 02114

In Alzheimer's disease (AD) widespread neuritic proliferation is associated with cytoskeletal disruption, reorganization of microtubule proteins, and extensive neuronal degeneration. To further define these sequential changes, we examined the hippocampus of 12 AD patients and 10 age-matched controls using immunoperoxidase methods with antibodies against tau (5E2), MAP2 (5F9), ubiquitin and tubulin. Initially, tau and tubulin form granular deposits in normal appearing perikarya. As further tau is deposited, tubulin reactivity diminishes and neurofibrillary tangles (NFT) develop. Mature NFT are ubiquitin positive. MAP2 histochemistry shows distortion and dissolution of apical dendrites. In regions severely affected by NFT, remaining neurons exhibit rampant proliferation of basal dendrites. Fibrillar tau can extend into these MAP2 positive dendrites that demonstrate other features reminiscent of regenerating neurons in culture including lamellipodia and filopodia of growth cone structures. Proliferative tau reactive dystrophic neurites (DN) are evident even in regions with little or no NFT formation. A subset of DN are reactive for tubulin and ubiquitin. The distribution of tau DN suggests a dendritic origin and the presence of filopodia implies a regenerative process. In AD, regeneration is aberrant and largely unsuccessful. Chaotic misconnections may be as important as disconnections in the pathogenesis of cognitive dysfunction in AD

65.8

Enzymatic Properties of Cholinesterases in Normal Human Brain and Alzheimer's Disease. Changiz Geula and Marsel Mesulam, Harvard University and Beth Israel Hospital, Boston, MA.

In the cerebral cortex of the normal human brain, cholinesterase staining is localized primarily within cell bodies and axons and is predominantly of the acetylcholinesterase (AChE) type. This neuronal and axonal AChE is severely depleted in Alzheimer's disease (AD). An analysis of cortical cholinesterase activity in AD reveals 4 additional deviations from the normal pattern: 1) The cerebral cortex in AD does contain considerable AChE activity but much of this is localized within neuritic plaques (NP) and neurofibrillary tangles (NFT). 2) In addition to AChE, many NPs and NFTs also display intense butyrylcholinesterase (BChE) activity while the normal cerebral cortex contains very little BChE in neurons or fibers. 3) Using a modified Karnovsky-Roots procedure, the AChE within normal cells and fibers is stained optimally at pH 8.0. The optimum staining of the NP- and NFT-bound AChE, however, occurs at pH 6.8. 4). In general, the normal AChE activity in cells and fibers is inhibited by 10^{-7}-10^{-6}M of the specific AChE inhibitor BW284C51. The AChE in NPs and NFTs, however, is inhibited at the higher concentration of 10^{-5}-10^{-4}M of BW284C51.

These enzymatic differences may reflect transformations that the premorbid AChE molecule undergoes during NP and NFT formation in AD. The possibility also exists that the cholinesterase activity observed in NP and NFT might reflect the synthesis or accumulation of a novel cholinesterase or perhaps a proteolytic enzyme that also hydrolyzes choline esters.

65.9

NEUROTENSIN AND PHENYL-N-METHYLTRANSFERASE (PNMT) IMMUNOREACTIVITY IN LOCUS COERULEUS OF ALZHEIMER'S DISEASED (AD) BRAINS. H.D. Chung*, W.J.Burke and D.S. Zahm. (SPON: J. Gibbons) VA Med. Ctr., Dept. of Neurol. and Dept. of Anat.& Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104

Sequential retrograde degeneration involving cortex, locus coeruleus (LC) and the C1-C2 group of medullary epinephrine-containing neurons, in that order, has been proposed as one component in the progression of AD (Burke et al., Ann. Neurol.22:278-80, '87). The C1-C2 group, however, does not appear to degenerate or have reduced PNMT levels (Burke et al., Geriatric Clin. Pharmacol., Raven, '87). Because neurotensin (NT) has been reported to coexist within LC-projecting PNMT neurons (Hokfelt et al., JCN 222:543-59, '84), we examined AD brainstems with the intent of exploiting NT immunoreactivity (IR) as another marker of AD-affected axon terminals originating in C1-C2 and projecting to LC. Four AD brainstems were removed between 1 and 3 hours post-mortem, fixed, exposed to antisera against neurotensin (INCstar) or PNMT (Eugene Tech) and processed for light and electron microscopic immunohistochemistry. PNMT-IR, restricted to the central tegmental tract (CTT) in fine fibers, involved LC minimally. NT-IR colocalized with PNMT-IR in the CTT, but was distributed widely, in coarse fibers, throughout other brainstem regions, including the LC, where the plexus was quite dense. NT may accompany PNMT in the C1-C2 projection to LC, but it must originate elsewhere too and therefore is an unacceptable marker for that pathway. Supported by the VA.

65.10

CENTRAL METABOLISM OF SOMATOSTATIN 14 AND 28 IS ALTERED IN ALZHEIMER'S DISEASE. T. P. Davis, P. Davies, A. J. Culling-Berglund*, E. Malek* and T. Gillespie*. Dept. of Pharmacology, Univ. of Arizona Coll. of Med., Tucson, AZ 85724 & Dept. of Neuroscience, Albert Einstein Coll. of Med., The Bronx, New York, 10461.

Senile dementia of the Alzheimer type (SDAT) is a progressive dementia characterized neuropathologically by the presence of neurofibrillary tangles and neuritic plaques in the cerebral cortex. It has been reported that somatostatin (SS) is contained in 20% of all neuritic plaques and RIA-SS is decreased in several brain regions of SDAT patients. The present study was designed to compare the time-course metabolism of SS-14 and SS-28 in specific regions of post mortem brain materials from sex and age matched controls versus SDAT patients and to compare the carboxypeptidase E (CPE) like activity. In vitro SS 14 and 28 metabolism was studied in twice-washed membrane homogenates of hippocampus and temporal cortex (Broadman Area 22). The CPE assay of STACK et al.(Life Science [1984] 34) was performed in the presence and absence of 10mM GEMSA. Previous reports have shown a significant decrease in RIA-SS in SDAT temporal cortex, but little change in hippocampus. Our study resulted in a SS-14 and SS-28 half-life of 140 and 267 min. respectively in SDAT temporal cortex versus 181 and 440 min. in controls. SS-14 and SS-28 half-life in the hippocampus showed no significant difference between SDAT and controls. CPE-like activity was higher in SDAT temporal cortex versus controls (120 vs. 101 pmoles/mg pr.). The order of activity was reversed in the hippocampus with controls greater than SDAT (93.5 vs. 61.5 pmoles/mg pr.). Our data support the hypothesis that alterations in the enzymatic metabolism/processing of SS-14 and 28 is a mechanism leading to decreases in RIA levels in specific SDAT brain regions.

65.11

POSTMORTEM VENTRICULAR FLUID (PVF) GALANIN (GAL) NEUROPEPTIDE Y (NPY) and SOMATOSTATIN (SOM) LEVELS: ALZHEIMER (AD), HUNTINGTON (HD), PARKINSON (PD) DISEASES and CONTROLS (C). N.R. Cutler, W.H. Barrettini, D.M. de Luna and W.W. Tourtellotte. Center for Aging & Alzheimer's, Beverly Hills, CA 90211. GAL, NPY, and SOM have been found to co-exist in hippocampal and cortical cholinergic neurons. In lieu of these associations, we evaluated PVF GAL, NPY, and SOM levels in autopsy-proven AD, HD and PD patients and C. METHODS: Aliquots were withdrawn immediately following death and stored at -70°C. GAL, NPY and SOM levels were measured in duplicate and in a blinded fashion by RIA assay. The subjects were as follows: 14 AD ($\bar{x}$ age=79±6); 5 PD ($\bar{x}$ age=74±9); 5 HD ($\bar{x}$ age=64±6); and 10 C ($\bar{x}$ age=62±16). RESULTS: NPY (pg/ml)--AD ($\bar{x}$=454±294), HD ($\bar{x}$=873±310), PD ($\bar{x}$=606±113) were greater than C ($\bar{x}$=225±113), ($p<0.05$). GAL-HD ($\bar{x}$=42±8) were found greater than C ($\bar{x}$=23.0±18), ($p<0.05$). SOM-PD ($\bar{x}$=202±111) levels were found increased as compared to C ($\bar{x}$=70±40), ($p<0.05$). There was a relationship found between AD SOM and NPY ($r=0.84$; $p<0.01$), but not between GAL and NPY levels ($p>0.05$). CONCLUSIONS: Elevated PVF NPY levels in AD, HD, PD, GAL-HD and SOM-PD will be discussed in relation to postmortem brain tissue, in vivo CSF levels and other neuropeptides.

65.12

THE EFFECTS OF NICOTINE ON ATTENTION, INFORMATION PROCESSING, AND SHORT-TERM MEMORY IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE. G. M. M. Jones*, B. J. Sahakian, R. Levy*, J. A. Gray* and D. Warburton*. Section of Old Age Psychiatry and Dept. of Psychology, Institute of Psychiatry, London SE5 8AF and Department of Psychology, Univ. of Reading, Reading, U.K.

Nicotine (s.c.) in patients with dementia of the Alzheimer type (DAT) produced a significant and marked improvement in performance, as measured by discriminative sensitivity, and faster reaction times on a computerized test of attention and information processing. Nicotine also improved the ability of DAT patients to detect a flickering light in a critical flicker fusion test. These results suggest that nicotine may be acting on cortical mechanisms involved in visual perception and attention and support the hypothesis that acetylcholine transmission modulates vigilance and discrimination. Nicotine may therefore be of some value in treating deficits in attention and information processing in DAT patients.

BASAL GANGLIA AND THALAMUS: MOTOR SYSTEMS II

66.1

HETEROGENEOUS STRIATAL AFFERENT CONNECTIONS FROM DISTINCT REGIONS OF THE DOPAMINE-CONTAINING MIDBRAIN OF THE PRIMATE.
L. A. Feigenbaum and A. M. Graybiel. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

The mesostriatal projections labeled by 35S-methionine injections placed in different parts of the A8-A9-A10 cell complex of the midbrain were analyzed by autoradiography in 4 squirrel monkeys. The locations of injection sites were plotted in relation to subdivisions of the A8-A9-A10 cell complex visible in sections immunostained for tyrosine hydroxylase. Anterograde labeling in the striatum was charted with respect to histochemically identified compartments revealed in serial sections immunostained for met-enkephalin or stained for acetylcholinesterase or butyrylcholinesterase. In all cases labeling appeared from anterior to posterior poles of the striatum, and distinct regional patterns of labeling were also observed. In addition, two strikingly different compartmental patterns of mesostriatal projection were found. There was dense labeling of the matrix compartment in both the caudate nucleus and the putamen with minimal labeling of striosomes in three cases: one with a caudal injection in A8, and two with more rostral injections in A8 involving also A10 and the pars mixta of Francois et. al. (85). By contrast, in the fourth monkey, there was vivid labeling of striosomes in the caudate nucleus and putamen with much weaker labeling of the matrix. The injection site in this monkey was centered in the main horizontal band of the substantia nigra pars compacta. These findings demonstrate that in the A8-A9-A10 cell complex of the primate, there are distinct subdivisions with preferential projections to striosomes and other subdivisions with preferential projections to the matrix. *Supported by The Whitaker Health Sciences Fund, The Seaver Institute, and The McKnight Foundation.*

66.2

ORGANIZATION OF THE EFFERENT PROJECTIONS FROM THE PRIMATE VENTROMEDIAL STRIATUM. E. Lynd, C. Klein, H.J. Groenewegen, and S.N. Haber (SPON: T. Pasternak). Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

The n. accumbens is considered to be the limbic-related portion of the striatum by virtue of its afferent connections from allocortical and mesolimbic areas as well as from the amygdala. However inputs from these regions are not unique to the n. accumbens and project to more rostral and dorsal striatal areas as well. We undertook a study to compare the efferent projections from the n. accumbens with more rostral and dorsal regions of the striatum to determine whether the n. accumbens should be considered a unique striatal region by virtue of its efferent fiber pathways.

Injections of 3H amino acids, WGA-HRP, or PHaL were placed in various striatal regions including, the medial n. accumbens, the lateral n. accumbens, the ventromedial rostral pole of the caudate n., and the centromedial caudate n. Results indicate that regardless of the injection site, terminals were observed in the ventral palidum and in the rostral pole of the external segment of the globus pallidus (GP) and in the inner portion of the rostral internal segment. At this point, fiber bundles head caudalward to the substantia nigra (SN) and are no longer observed in the GP.

In contrast, the entire rostral-caudal extent of the SN receives input from these striatal regions. Rostrally the labeling is concentrated primarily in the medial portion of the SN. In more caudal regions dense terminal labeling was observed more laterally. In all cases terminals to the substantia nigra were not limited to the pars reticulata, but some overlap with the pars compacta was observed. These studies suggest that there is not a significant difference between the efferent projections from the n. accumbens compared to the rostral and medial caudate n. in the monkey.

66.3

COMPARTMENTAL ORIGINS OF THE STRIATOPALLIDAL PROJECTION IN THE PRIMATE. J. M. Gimenez-Amaya* and A. M. Graybiel (SPON: J. Jimenez-Castellanos). Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

In the squirrel monkey we injected horseradish peroxidase-wheatgerm agglutinin (HRP-WGA; 15 cases) or HRP-WGA and fluorescent latex microspheres (FBS; 2 cases) into the globus pallidus (GP). The distribution of retrogradely labeled striatal neurons was studied in relation to striosomal architecture demonstrated histochemically. Deposits involving both pallidal segments (GPe and GPi) and smaller deposits in GPi labeled medium-sized neurons in different parts of the putamen and, often, in the lateral and rostral caudate nucleus. Circumscribed fields of striatal labeling appeared always; in some cases there were also far-flung patches of labeled matrix cells (0.2-0.5mm wide, ca. 1mm apart). The main fields of label were interrupted by pockets of sparse labeling corresponding to striosomes where these could be identified as such. These zones almost always contained some labeled cells: these cells were usually weakly labeled and varied in number from a few to 1/4-1/2 the number per area in the matrix. With FBS in GPe and HRP-WGA in GPi, striatal neurons labeled with each tracer were partly intermingled but FBS cells were striking in some striosomes. Only 1 doubly labeled cell was found of 507 FBS cells checked. Because of variable fibers-of-passage contamination we cannot be sure whether a projection from some striosomal cells to pallidum exists. We can conclude that in the primate (1) a massive striatopallidal projection originates in the extrastriosomal matrix, (2) heterogeneous distributions of these matrix projection cells occur, and (3) GPe and GPi probably receive inputs from different sets of matrix neurons. *Supported by The Seaver Institute and FISSS.*

66.4

PATTERNS OF COEXISTENCE OF NEUROPEPTIDES AND GLUTAMIC ACID DECARBOXYLASE IN NEURONS OF THE FELINE STRIATUM M.-J. Besson*, A. M. Graybiel and B. Quinn. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139; INSERM U114, Paris, FRANCE.

In the striatum of colchicine-treated cats, substance P (SP)-like and dynorphin B (DYN)-like immunoreactivities coexist in ca. 90% of neurons in striosomes (where the SP/DYN cells are concentrated) and neurons in the extrastriosomal matrix (where SP/DYN cells are more sparse). In untreated cats met-enkephalin (mENK)-positive neurons appear mainly in the matrix, but after colchicine many also appear in striosomes, suggesting SP/DYN/mENK coexistence. We report here on the coexistence of these peptides and glutamic acid decarboxylase (GAD) in striatal neurons of colchicine-pretreated cats with methods and controls as previously described. In striosomes we confirm ca. 90% SP/DYN colocalization. In these SP/DYN neurons, mENK was colocalized in 80% and GAD in 60%. Of striosomal mENK neurons, ca. 70% were SP/DYN-positive. In the matrix, mENK cells were more numerous but only 35% expessed SP or DYN. Levels of colocalization of SP and DYN were 85%. About 75% of these SP/DYN neurons expressed mENK. Fully 90% of mENK cells coexpressed GAD, but these mENK cells represented only ca. 60% of GAD-positive neurons. We conclude that (1) there are different populations of peptidergic neurons in striosomes, many expressing GAD and including many SP/DYN cells expressing mENK and fewer SP/DYN-negative mENK cells; (2) in the matrix, GAD neurons expressing SP, DYN, and mENK make up a minority population, mENK/GAD neurons a larger group, and GAD-positive without these peptides a third group. *For antisera we thank Dr. Elde (mENK) and Dr. Wu (GAD). Supported by NSF BNS83-19547 & BNS87-20475 and The Seaver Institute.*

66.5

COMPARTMENTATION OF VENTRAL PALLIDAL EFFERENTS IS REFLECTED IN THE DENSITY OF AFFERENT INPUTS TO PERIKARYA AND PROXIMAL DENDRITES. D.S. Zahm and S.N. Johnson* Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

We reported last year that ventral pallidal efferents projecting to the ventral tegmental area are restricted to a ventromedial part of ventral pallidum (VP) that is rich in neurotensin (NT) immunoreactivity (IR) and that nigropetal axons originate with neurons in dorsolateral, NT-IR-poor parts of VP. Our present results, also generated following small injections of WGA-HRP into the brainstems of rats, indicate that fibers enroute from VP to the subthalamic nucleus also originate from dorsolaterally positioned cells. EM examination of retrogradely labeled cells, which carried the TMB reaction product stabilized with DAB and cobalt, indicated that the cell bodies and proximal dendrites of neurons in the dorsolateral division of VP are contacted by significantly more boutons which exhibit more synapses than are neurons in the ventromedial pallidal division. In ventromedial VP, thin laminae of glia frequently intervene to prevent contact of neurons and afferent boutons. Such glial blockade was not observed in the dorsolateral district of VP. In other respects the projection neurons in the ventromedial and dorsolateral districts of VP are similar, all having fusiform perikarya at the large end of the medium sized range (18-25 µm), with electron lucent cytoplasm and heterochromatic, deeply indented nuclei. Supported by NIH NS-23805 and the American Parkinson Disease Association.

66.6

LIMBIC AND NON-LIMBIC RAT STRIATAL REGIONS DIFFER MARKEDLY IN THEIR HIGH-AFFINITY DOPAMINE TRANSPORT. J.F. Marshall, Dept. of Psychobiology, Univ. Calif. Irvine, CA 92717.

Although the rodent nucleus accumbens septi (NAS) and dorsal striatum (caudate-putamen, CPu) share a common development, architecture, and connectivity, the present experiments reveal large differences in their dopamine (DA) transport systems. The DA content of cylindrical punches from NAS and CPu were similar (102 and 119 ug/mg), whereas the desipramine- resistant, benztropine-inhibited transport of [^{3}H]DA (0.5 uM) into microsomal fractions prepared from these punches differed markedly (0.89 and and 3.38 pmol/mg P, respectively). Saturation analysis of 0.02-1.0 uM [^{3}H]DA transport revealed these regional differences were due to V_{max}, not Km, variations. Compared with CPu, the NAS also had markedly less binding of [^{3}H] mazindol (15 nM) to the recognition site associated with the high-affinity DA transport. In punched tissue samples, the specific binding of this radioligand was three fold lower in NAS relative to CPu (1.12 pmol/mg P vs 3.41 pmol/mg P), which by Scatchard analysis reflected fewer binding sites in NAS. Quantitative autoradiography of [^{3}H]mazindol binding revealed a similar 2.5-3.0 greater ligand binding in dorsal CPu relative to NAS. The relative paucity of high-affinity dopamine transport sites in the NAS may contribute to (i) the observation that in vivo biosynthesis of dopamine in the NAS is higher than that in CPu, and (ii) the tendency for NAS to be spared from neurotoxin-induced DA terminal injury.

66.7

INTERACTION BETWEEN D1 AND D2 DOPAMINE (DA) RECEPTORS REVEALED BY DISCRETE INJECTIONS OF SELECTIVE AGONISTS INTO THE BASAL GANGLIA OF DA-DENERVATED RATS. G.J. LaHoste, J.F. Marshall and R. Navarrete*, *Dept. of Psychobiology, Univ. of California, Irvine 92717.*

Recent studies of dopamine (DA) D1 and D2 receptors have revealed an interaction between the two in intact but not DA-denervated rats. This interaction may vary with neural locus as autoradiographic studies have revealed distinct anatomical distributions of D1 and D2 receptors in rat brain. Using quantitative receptor autoradiography, we confirmed the anatomical distinction between D1 and D2 binding patterns in rats whose ascending DA projections had been unilaterally destroyed by 6-OHDA. In addition, such rats rotated vigorously contralateral to the lesion when 5 ug apomorphine (APO) was injected into the brain at either the substantia nigra pars reticulata (SNr), a site rich in D1 receptors, or the caudate-putamen (CPu). The selective D2 agonist quinpirole (5 ug) induced contralateral rotation when injected into the CPu but was ineffective in the SNr. By contrast, the selective D1 agonist SKF 38393 (15 ug) induced rotation equivalent to APO when injected into the SNr but not the CPu. The effect of SKF 38393 in the SNr was apparently not mediated via the D1 receptor, however, since it was not antagonized by SCH 23390 and since S(-)-SKF 38393, which is inactive at the D1 site, also induced rotation when injected into the SNr. Although these data seem to indicate an exclusive D2 effect in the CPu and a possible D1 effect in the SNr, neither SCH 23390 nor eticlopride, a selective D2 antagonist, could block rotation elicited by APO in either brain site; both antagonists together, however, blocked rotation. Furthermore, a subthreshold dose of quinpirole (1 ug) injected into the CPu in combination with SKF 38393 (15 ug) produced vigorous rotation while neither treatment alone caused rotation. In addition, a subthreshold dose of SKF 38393 (2 ug) injected into the SNr in combination with quinpirole (15 ug) caused rotation while neither treatment alone caused rotation. These data provide evidence for distinct types of D1/D2 synergism that vary as a function of neural locus.

66.8

THE DIFFERENTIAL ROLE OF THE DORSAL AND VENTRAL STRIATUM IN THE REGULATION OF FORELIMB AND HINDLIMB MUSCLE TONE. B.A. Ellenbroek and A.R. Cools, Univ. of Nijmegen, Psychoneuropharmacol. Res. Unit, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands.

Whereas it has become increasingly clear that the dorsal and ventral striatum differ in certain anatomical connections, relatively little is yet known on the functional differences between these two areas.

In the past we have found that the dorsal but not the ventral part of the striatum plays an important role in the regulation of the hindlimb extensor muscle tone (Ellenbroek et al, Brain Res, 345: 132, 1985). Thus, injections of haloperidol (250-750 ng/ 0.5 µl) into the dorsal striatum led to a dose-dependent increase in the tonic EMG activity of the gastrocnemius soleus muscle. Since this haloperidol effect could be antagonised by apomorphine, dopaminergic receptors appear to play a prominent role.

We now report that the ventral but not the dorsal part of the striatum plays an important role in the regulation of the forelimb extensor muscle tone. Thus, injections of haloperidol (500-1000 ng/ 0.5 µl) into the ventral striatum led to a dose-dependent increase in the tonic EMG activity of the triceps muscle. The finding that this effect of haloperidol was insensitive to apomorphine but could be antagonised by phenylephrine suggests that α_1-adrenoceptors might play an important role in these effects of haloperidol.

66.9

REGIONAL DISSOCIATION OF BEHAVIORAL FUNCTIONS IN THE RAT STRIATUM: SPATIAL ALTERNATION, REACHING AND HOARDING. M. Pisa, J.A. Schranz, A. Brooks and S. Shastri. Dept. Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Male Wistar rats with histologically verified, ibotenate-induced lesions of either the medial region or the lateral region of the rostral striatum were compared with vehicle control rats for learning of T maze spatial alternation, forepaw reaching of food pellets inside a tube, and hoarding of food from an alley connected to their home cages. Lesions of the medial striatum produced a statistically significant impairment of spatial alternation performance only. Lesions of the lateral striatum produced a statistically significant impairment of food reaching only. Neither medial nor lateral striatal lesions significantly altered hoarding performance. These results agree with, and extend, previous findings demonstrating distinctive roles of the medial and the lateral striatum in visuospatial cognitive functions and segmental motor control, respectively (Pisa, Neurosci. Abstr., 1987, 13: 980). A role of the rostral striatum in hoarding behavior, reported by other investigators, was not confirmed, however. (Supported by MRC operating grant and Scholarship of the Ontario Mental Health Foundation to M.P., and MRC Studentship to J.A.S.).

66.10

DOPAMINE-MEDIATED CIRCLING BEHAVIOR PRODUCED BY SIGMA LIGANDS. J.M Walker, S.R. Goldstein*, R.L. Patrick and R.R. Matsumoto, Department of Psychology, Brown University, Providence, RI 02912.

Haloperidol-sensitive sigma receptors densely populate many brainstem motor regulatory areas including the facial, hypoglossal and motor trigeminal nuclei, the red nucleus, cerebellum and the substantia nigra pars compacta. Behavioral studies from this laboratory further suggested a role of sigma receptors in the regulation of posture and movement.

Two selective sigma ligands, 1,3-Di-o-tolyl-guanidine (DTG) and (+)-pentazocine, were unilaterally microinjected into the substantia nigra of rats and circling behavior was assessed. Both DTG and (+)-pentazocine (0.7 to 18.6 nmol) produced dose-dependent contralateral rotational behavior; DTG was significantly more potent than (+)-pentazocine.

The role of the ascending nigrostriatal dopamine pathway in these effects was assessed by testing the compounds in animals with unilateral 6-OHDA lesions of the medial forebrain bundle. Lesioned animals showed markedly attenuated circling in response to both DTG and (+)-pentazocine. These data suggest that sigma receptors influence dopamine release from the striatum. Furthermore, it would appear that some dopaminergic effects of neuroleptic drugs may occur indirectly through sigma receptors.

66.11

NEURONAL ACTIVITY IN THE MONKEY STRIATUM PRECEDING SELF-INITIATED ARM MOVEMENTS. W. Schultz* and R. Romo* (SPON: European Neuroscience Association). Institut de Physiologie, Univ. Fribourg, CH-1700 Fribourg, Switzerland.

The inputs from frontal cortex and midbrain dopamine neurons to the striatum (caudate nucleus and putamen) suggest that this structure may be engaged in neuronal processes related to movement initiation.

We recorded the electrical activity of single neurons in the striatum of 2 Macaca fascicularis monkeys conditioned to perform self-initiated arm reaching movements into a covered food box at irregular, self-chosen intervals and without phasic external cues. Electromyograms collected from the arm, shoulder, trunk and leg of both sides during all neuronal recordings served to control for untimely muscle activity.

Neurons in both parts of the striatum were activated 700-3000 ms in advance of self-initiated arm movements (39 of 215 neurons in caudate, 18%, and 38 of 167 neurons in putamen, 23%). Premovement activity clearly began before earliest EMG activity and either ended immediately before the movement or continued during its execution. It only occurred when animals performed a self-initiated reaching movement from the well-defined resting position on an immovable key, and not in advance of occasional postural adjustments.

These data provide evidence for neuronal mechanisms in the striatum related to the initiation of purposive voluntary movements in the absence of phasic external stimuli. The premovement activity may reflect the excitatory input from frontal cortex in which comparable activity in advance of self-initiated movements is found. Alternatively, this activity could be built up by interactions in cortico-basal ganglia-cortical loops.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CORTEX I

67.1

SPATIO-TEMPORAL ACTIVATION OF CORTICAL CIRCUITS: VISUALIZATION WITH MTV/2

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In order to understand better the integrative synaptic mechanisms used by cortical neurons to extract information from afferent inputs, it might be useful to visualize directly the spatio-temporal pattern of stimulus-evoked neuronal activity. To this end we have developed a second generation data acquisition system for multiple site optical recording of transmembrane voltage from intact brains stained with potentiometric probes. Using this MTV/2 system, we have been able to observe the spread of electrical activity in the salamander olfactory bulb following electrical stimulation of the transected olfactory nerve with a temporal resolution better than 1 ms/frame.

The system uses a silicon photodiode array to monitor optically the electrical activity in up to 124 contiguous cortical regions. The optical data is later processed and used to generate Pseudocolor Activity Maps (PAM's) in which the instantaneous amplitude of each diode signal is represented using a 16-level color scale. To correlate changes in activity with specific anatomical regions, the PAM is displayed superpositioned on a computer enhanced video image of the preparation. Animation of the evoked response is achieved through the rapid display of sequentially recorded PAM's. Examples of typical activation patterns observed in the salamander olfactory bulb will be presented in a short movie.

Supported by NSF Grants BNS-8507594, INT-8311534 and NIH Grants R01-DE06904, RR-08194

67.2

VISUAL AND NON-VISUAL MECHANISMS UNDERLYING DISCRIMINATION OF SELF-GENERATED RETINAL SLIP. R.G. Erickson and P. Thier
MIT, USA and University of Tubingen, West Germany.

Visuo-spatial stability requires suppression of retinal image motion caused entirely by voluntary eye movements. To determine if such suppression is evident in the responses of macaque monkey cortical neurons we compared the responses of visual neurons to actively and passively generated stimulus motion. Stimulation consisted of ramp movement of a stimulus during fixation (passive), and pursuit of a target moving in the opposite direction past a stationary stimulus (active). Compilation of an index used to quantify the active/passive comparison showed that many directional visual neurons in the superior temporal sulcus (STS) responded at least twice as well to passive as compared to active stimulation. No non-directional passive-only neurons were found, and few passive-only cells were found outside the STS.

Specific controls verified that at least three separate mechanisms work synergistically to produce this response property. Directional center-surround antagonism accounted for the passive-only responses of some cells. In others, which summed retinal slip across quadrant-sized fields that included the fovea, target retinal slip during tracking suppressed responses to stimulus retinal slip. Finally, some passive-only cells lacking center-surround interactions did not respond to target retinal slip. The responses of these cells to actively induced retinal slip may be suppressed by non-visual mechanisms.

The results imply that conscious perception of movement may be derived primarily from the responses of motion-sensitive cells in the STS, and that visual mechanisms largely supplant the need for oculomotor efference copy.

67.3

INFERIOR PARIETAL CORTEX ACTIVITY DURING PREPARATION OF VISUALLY-CUED ARM MOVEMENTS IN MONKEYS. M. Godschalk.
Dept. of Anatomy, Erasmus Univ., Rotterdam, The Netherlands

Macaque premotor cortex (PM) contains neurons that modulate their activity during preparation for specific movements in a detour reaching task. PM receives inputs from the anterolateral part of the inferior parietal lobule (IPL) and projects into the primary motor cortex (MI). IPL receives input from non-primary visual areas, thus providing access for visual information to the motor system. The present experiment was designed to compare neuronal activity in IPL with that in PM.

Two macaque monkeys were trained to perform a detour reaching task containing a "Visible-phase" of variable length (1.0-2.5 s) during which the direction of the subsequent movement was cued to the animal by the target becoming visible, but the actual movement had to be withheld until a Go-signal occurred. Single unit activity in IPL was recorded on tape and analyzed off-line.

A total of 128 task related units were recorded. Of those, 86 showed cue-related modulation. Most of these units were found in IPL. In 68 units cue-related modulation was related to target position.

Neurons in this area have been reported to be responsive to visual stimuli, and to be involved in visual attention. This reinforces the hypothesis that some neurons in IPL play a role in the preparation for visually-cued arm movements. It is possible that an IPL-PM-MI pathway processes visual information used for these arm movements.

67.4

ACTIVATION OF DIFFERENT NEURONAL ELEMENTS IN CORTEX BY DIFFERENT TYPES OF TRANSCRANIAL ELECTRICAL AND MAGNETIC STIMULATION IN MAN. J.C. Rothwell*, D. Dressler*, B.L. Day*, P.D. Thompson* and C.D. Marsden* (SPON: A. Chapman). Institute of Neurology, London WC1N 3BG, U.K.

Electrical or magnetic stimulation over central areas of scalp can produce short-latency EMG responses in muscles. Here we compare responses in the first dorsal interosseous muscle to anodal and cathodal electrical stimulation and to different orientations of the magnetic stimulator. The latter was achieved with a 10cm diameter coil centered on the vertex, held either so that coil current flowed clockwise or anticlockwise as viewed from above. The latency and form of surface EMG responses depended on the type of stimulation. The shortest latency was observed using anodal or cathodal stimuli, although cathodal shocks had a higher threshold and generally produced more complex, polyphasic responses. Clockwise magnetic stimulation usually evoked responses 1-2ms later, and anticlockwise stimulation often gave responses which began 1-2ms later still. These latency differences were most evident at just-suprathreshold intensities and disappeared at higher intensities. The same latency differences were observed when single motor units were studied using the post-stimulus time histogram technique. This indicates that excitation arrives at the spinal cord at different latencies depending upon the form of stimulation applied at the scalp.

67.5

A SENSE OF MOVEMENT IS ELICITED IN ISCHEMICALLY PARALYZED DISTAL ARM BY FOCAL MAGNETIC COIL STIMULATION OF HUMAN MOTOR CORTEX. V.E. Amassian, R.Q. Cracco and P.J. Maccabee*. Depts. of Physiology and Neurology, SUNY Health Sci. Ctr. at Brooklyn, New York 11203.

Using ourselves as subjects, Cadwell magnetic coils (MCs) of special design were used to stimulate motor cortex transcranially so as to elicit reproduceably movements only of contralateral digits or hand. Movements were tracked with a Videcon-computer system and EMGs were recorded with surface disc electrodes. The arm was made ischemic by a cuff inflated to 180-280 mmHg on the upper arm. After 25-35 min of ischemia, MC elicited movements and EMG responses to stimulation of a given site were abolished, but a clear sense of movement persisted, which was projected even to a single digit. The sensation could be changed in intensity and projected elsewhere if the MC stimulus intensity and location, respectively, were altered. Alternatively, the subject's arm was first ischemically paralyzed. The sensations of distal movement subsequently elicited by MC stimulation at various sites could then be compared with those elicited after the circulation had been restored (they were not necessarily identical).

Our findings probably did not result from fusimotor activation of muscle afferent receptors, because large afferent axons were blocked as evidenced by loss of position sense. Possibly, MC stimulation of motor cortex elicits a "corollary discharge".

67.6

FOCAL MAGNETIC COIL STIMULATION OF HUMAN FRONTAL CORTEX ELICITS SPEECH RELATED MOTOR ACTIVITY. P.J. Maccabee*, V.E. Amassian, R.Q. Cracco, J.B. Cracco* and B.J. Anziska* (SPON: J.B. Ranck jr). Depts. of Neurology and Physiology, SUNY Health Sci. Ctr. at Brooklyn, New York 11203.

Using ourselves as subjects, various sites over left and right frontal cortex were transcranially stimulated with a Cadwell magnetic coil(MC). An X-Y coordinate system measured from the external auditory meatus was used in positioning the MC, which was orientated such that only the edge contacted the scalp, thus enhancing focal activation. Either compound motor action potentials or individual motor units were recorded with bipolar surface electrodes placed paramedianly and contralaterally between thyroid cartilage and sternum. The EMG activity increased markedly during utterances, but insignificantly with head turning. Triggering the MC by voice enhanced the laryngeal EMG response and therefore was routinely used. Latencies of laryngeal muscle responses were: 1) 6-8 ms, often associated with contralateral arm movement. The MC position was consistent with stimulation of precentral gyrus. 2) 13-20 ms, best obtained at more anterior and more medial sites, consistent with stimulation of Broca's area and the supplementary motor area, respectively. 3) 40-60 ms, highly variable in latency and amplitude and often terminating a silent period. Stimulating Broca's area elicited repetitive discharge by some motor units. No clear differences were seen when stimulating the right hemisphere, suggesting the importance of Wernicke's area for left hemisphere dominance.

67.7

NEURONAL ACTIVITY PRECEDING DIRECTIONAL AND NONDIRECTIONAL CUES IN THE PREMOTOR CORTEX OF RHESUS MONKEYS. Steven P. Wise, Kiyoshi Kurata, and Eilon Vaadia*. Lab. of Neurophysiology, N.I.M.H., P. O. Box 289, Poolesville, MD 20837.

Pre-cue activity, a change in neuronal discharge preceding a predictable stimulus, was studied in the premotor cortex of three rhesus monkeys. Two behavioral conditions served as the focus of investigation. In one condition, a directional cue dictated the timing and target of a forelimb movement. In another condition, a nondirectional cue provided only timing information. Of 501 task-related neurons recorded in the premotor cortex, 168 showed pre-cue activity. The onset time of pre-cue activity varied markedly from trial to trial and cell to cell, ranging from trial initiation to 4.8 s later. No pre-cue activity reflected the direction of limb movement; thus, the data argue against a previous suggestion that pre-cue activity reflects the preparation of specific limb movements. A small number of cells showed greater pre-cue activity before directional cues than before nondirectional cues, and this difference may reflect anticipation of the cue's specific directional information. However, the vast majority of units (84%) lacked significant activity differences between the two conditions. We therefore hypothesize that most pre-cue activity reflects or contributes to the main aspect common to the two conditions: anticipation of the time and/or nature of events. The results of the present study indicate supramotor or nonmotor roles for the premotor cortex and serve to undermine the rigid distinction traditionally drawn between the functions of the caudal, "motor" areas of the frontal cortex and the rostral, prefrontal areas.

67.8

CONNECTIONS OF THE PHYSIOLOGICALLY DEFINED SUPPLEMENTARY EYE FIELD. M.F. Huerta and J.H. Kaas. Department of BioStructure and Function, Univ. of Conn. Health Ctr., Farmington, CT 06032, and Department of Psychology, Vanderbilt Univ., Nashville, TN 37240.

In macaque monkeys, cortex medial to the arcuate sulcus has been implicated in oculomotor function, and has been called the Supplementary Eye Field (SEF). In the present study, the connections of the SEF in four M. fascicularis monkeys were determined by: defining the borders of the SEF with intracortical microstimulation, marking these borders with electrolytic lesions, injecting HRP-WGA within the SEF, and subsequently processing the tissue with TMB.

The present data reveal that the SEF has bilateral cortical connections with the SEF, Frontal Eye Field, Supplementary Motor Area, Postarcuate Premotor Cortex, and Frontal Ventral Field. Ipsilateral cortical connections are also made with lateral orbital and periprincipal regions, as well as with cortex in the depths of the intraparietal, lateral and superior temporal sulci. The numerous thalamic connections of the SEF include those made with VA, VAmc, VLp, X, CSL, CL, Pf, Sg-Li nuclei, and all parts of the MD nucleus. The SEF also innervates the subthalamic nucleus, parvocellular red nucleus, central mesencephalic reticular formation, layer VI of the superior colliculus, and several pontine nuclei. (Supported by EY02686 to J.H.K., and EY05743 and UCONN Foundation grants to M.F.H.)

67.9

SACCADE LATENCY AND PREPARATORY NEURONAL ACTIVITY IN THE SUPPLEMENTARY AND FRONTAL EYE FIELDS. **J.D. Schall. Department of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139**

The relationship between preparatory neuronal activity in the supplementary and frontal eye fields and saccade latency was investigated in rhesus monkeys performing a delayed response, go/nogo visual tracking task. The go/nogo cue was given after the target appeared by changing the color of a fixated spot. Saccade latency was negatively correlated with the duration of the delay. After delays over 300-400 msec saccades with latencies under 100 msec were common. Such short latency saccades were observed even though monkeys were required to fixate and attend to the cue spot to successfully withhold saccades on nogo trials. Single units were recorded in rostral supplementary motor area and frontal eye field. Many units were specifically active during the period after the target appeared until the go/nogo cue or the saccade. A prediction of the hypothesis that this neuronal activity is involved in preparing movements is that the level of activity is correlated with reaction time. This prediction was not supported by the results. These results contribute to our understanding of visual attention and motor set.
Supported by NRSA EY05959

67.10

ABLATION OF MI DIGIT AREA ALTERS MOVEMENT RELATION OF MONKEY SUPPLEMENTARY MOTOR AREA. J.TANJI, M.INASE* and H.MUSHIAKE*. Lab. of Neurophysiology, Tohoku Univ. Sch. of Med., Sendai, 980, JAPAN.

In order to study neuronal activity in supplementary motor area (SMA) after localized lesion in primary motor cortex (MI), monkeys were trained to press two small keys with their right or left digits. After a waiting period of 2-5 sec, either a right or left LED in a front panel came on and triggered the right or left digit movement. Microelectrodes were inserted into the anterior and posterior banks of the left central sulcus in order to make detailed receptive field maps and ICMS maps of the digit area of sensory and motor cortex. Subsequently, electrolytic lesions were made in a portion of MI covering most part of digit area. This was achieved by repeatedly passing DC currents of 100-200 uA through the microelectrodes used for neuron recording. About 4 weeks after the lesion, the reaction time and movement time of the key press movement returned to prelesion values. At this stage, premovement activity of SMA ipsilateral to the lesioned MI was analyzed. Both the frequency of occurrence of movement-related neuron and their magnitudes of activity changes were much reduced. In SMA of the opposite hemisphere, in contrast, the occurrences of neurons related to ipsilateral movement and related to ipsi- as well as contralateral movements were greater.

67.11

EFFECTS OF COOLING PARIETAL OR PREFRONTAL CORTEX ON SPATIAL AND NONSPATIAL VISUO-MOTOR TASKS. J. Quintana* and J. M. Fuster (SPON: M. Chase). Department of Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The purpose of this study is to ascertain the importance of spatial and temporal factors in the sensory-motor integrative functions of parietal and prefrontal areas of the primate cortex. Monkeys are trained in color discrimination tasks with delayed and non-delayed motor responses. The stimuli are randomly presented by rear projection on three translucent buttons--one central, for the trial cue, and two lateral, for the discriminanda (choice stimuli) and responses. Some color cues call only for a positional choice (right or left), others call for either a positional choice or a color match. Thus the cue by itself may either connote position or not. Blocks of trials with simultaneous presentation of cue and choice alternate with others with 5-sec or 12-sec delays between the two. In one animal, cooling probes were implanted bilaterally over posterior parietal and dorsolateral prefrontal cortex. Parietal cooling (15° C) induced a reversible impairment of spatial-task performance with delay when the cues connoted exclusively spatial choices; parietal cooling did not impair delayed nonspatial performance or performance guided by cues with ambiguous connotation (spatial and nonspatial). Prefrontal cooling (15° C), on the other hand, induced a reversible deficit in both spatial and nonspatial performance with delay. Neither parietal nor prefrontal cooling impaired tasks without delay. These results suggest that the functional integrity of prefrontal cortex is essential for spatial and nonspatial tasks if they contain temporal discontiguities between cues and responses. The parietal cortex appears important for tasks that depend on the retention of spatial information. These tentative conclusions agree with the concept of a role of both posterior parietal and prefrontal cortex in spatial information processing and a supraordinate role of prefrontal cortex in temporal integration.

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67.12

NEURONAL ACTIVITY IN THE FRONTAL CORTEX DURING ALTERED VISUAL FEEDBACK CONTROL OF MOVEMENT. D.K. Onstott, S.-K. Park and T.J. Ebner. Depts. Neurosurgery and Physiology, Neuroscience Grad. Prog., Univ. of MN, Mpls., MN 55455.

Activity of frontal cortical cells during a visually-guided, voluntary arm movement under conditions of normal and altered feedback was examined. Rhesus monkeys were trained to move a cursor coupled to a manipulandum from a start box to a target box displayed in one of four positions on a video screen. The position of the target box and the gain between the movement of manipulandum (hand) and cursor were randomly varied from trial to trial. Extracellular recordings from single cells in the frontal cortex revealed modulation of activity that included increased discharge rates during or at the end of the movement. The amplitude and/or duration of a large portion of these responses was scaled to the amplitude of the movement gain between hand and cursor. This activity reflected the altered duration of the movement and the direction of corrective movements required by the change in gain. Cells exhibiting increased activity with movement to one target often showed end-of-movement activity or no response to the orthogonal target. For gains > 1.0, which require reversal of movement direction to attain the target, this directional or position sensitivity resulted in an abrupt reduction of activity upon reversal of hand movement direction. Supported by NSF/BNS-8707572.

RETINA II

68.1

EFFECT OF PHOSPHODIESTERASE INHIBITORS ON RODS L. Cervetto*, L. Colombaioni* and G.M. Ratto* (SPON: European Brain & Behavior Society). Ist. Neurofisiol., CNR, 56100 Pisa, ITALY.

Activation of phosphodiesterase (PDE) causes a fall in cytosolic cGMP which in turn close ionic channels and generates the light responses in retinal rods (Stryer,L. Ann.Rev. Neurosci.9:87-119,1986).

We have analyzed the effect of two well known PDE inhibitors (IBMX and zaprinast) on both membrane current and photoresponse of salamander rods.

Application of IBMX (5÷20μM) caused dose-dependent increases in the dark current and a small desensitization to light. By contrast similar amounts of zaprinast had small effect on the dark current and produced large, long lasting, desensitization to light.

These results suggest the existence of two fractions of PDE, one active in darkness and more sensitive to IBMX, the other, activated by light and strongly inhibited by zaprinast.

68.2

L-CIS-DILTIAZEM BLOCKS THE cGMP-GATED CHANNEL IN CONES. L.W. Haynes and K.-W. Yau. Howard Hughes Medical Institute and Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

cGMP-gated channels isolated and reconstituted from retinal photoreceptors by others have not been consistent in their responses to the blocker l-cis-diltiazem, raising the possibility that the unresponsive channels may come from cones[1]. This possibility is ruled out by our experiments. Patches of plasma membrane were excised from catfish cone outer segments and voltage clamped[2]. In isotonic NaCl and low divalent cations, application of diltiazem to the cytoplasmic face of inside-out patches blocked the cGMP-gated channel. The block was enhanced by depolarization, with the resulting current-voltage relation showing inward rectification instead of being nearly linear as in the absence of block. With the channels fully activated, the concentration of diltiazem necessary to reduce the current by half varied from near 10 μM at +30 mV to about 75 μM at -30 mV. These values are in the range found by others for the cGMP-gated channel of the rod outer segment.

1. M.L. Applebury, 1987, Nature 326:546-547.
2. L.W. Haynes and K.-W. Yau, 1985, Nature 317:61-64.

68.3

CALCIUM AND LIGHT ADAPTATION IN RETINAL RODS AND CONES. K.-W. Yau and K. Nakatani* (SPON: J.M. Baraban). Howard Hughes Med. Inst. and Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The role of Ca^{++} in phototransduction as presently conceived is that it provides negative feedback between the light-regulated conductance and cGMP metabolism, consequently down-regulating a cell's sensitivity to light and leading to light adaptation. The question remains, however, as to how much of the light adaptation exhibited by photoreceptors is actually attributed to this Ca^{++} feedback. We have studied this question by recording from a single amphibian (salamander) rod or cone with a suction pipet while exposing its outer segment to a test solution containing low Ca^{++} and with all Na^{+} replaced by guanidinium. This solution essentially removed the Ca^{++} feedback in the cell[1,2]. In normal Ringer, the response of a rod or cone to a step of light rose transiently to a peak but rapidly relaxed to a lower level, indicative of light adaptation. In the test solution, however, the response relaxation was absent, and the steady response levels at different light step intensities could be well predicted by a statistical superposition of invariant single photon responses each assumed to consist of a regional, complete closure of the light-regulated conductance. We therefore conclude that the Ca^{++} feedback basically underlies all light adaptation in rods and cones.

1. Nakatani & Yau (1988) J. Physiol. 395: 695-729.
2. Yau & Nakatani (1988) Biophys. J. 53: 473a.

68.4

NONLINEAR PHOTORECEPTOR PROPERTIES INFLUENCE FLICKER SENSITIVITY: INTRACELLULAR AND PSYCHOPHYSICAL ANALYSIS. T. E. Frumkes, M. Sliwinski*, N. Denny*, and T. Eysteinsson, Dept. Psychol., Queens College CUNY, Flushing, NY 11367

Psychophysical sensitivity to rod-mediated flicker of different waveforms suggests 2 types of nonlinearities. Sensitivity to negative sawteeth (rapid on, slow off) is about 0.3 $\log_{10}$ units less than to sine waves. For frequencies <6 hz, sensitivity to positive and negative sawteeth is equal. For higher frequencies (circa 10 hz), sensitivity to positive sawteeth (rapid off, slow on) is further reduced about 1 $\log_{10}$ unit. No similar phenomenon occurs for cone-flicker. We observe similar nonlinear properties by intracellular recording from rods and horizontal cells in Xenopus and mudpuppy. Responses to relatively high intensity positive sawteeth is often interfered with by the rod-after potential. At most frequencies, sensitivity to sinewaves is generally greater than to either sawtooth. To slow frequencies, the slow ramp of both positive and negative sawteeth elicit a response characterized by two straight line segments: an earlier shorter segment with shallow slope, and a subsequent longer segment with steeper slope.

68.5

CELLULAR MECHANISMS OF SURROUND ANTAGONISM IN TURTLE CONES. Wallace B. Thoreson and Dwight A. Burkhardt. Depts. of Physiology and Psychology, Neuroscience Program, Univ. of Minnesota, Mpls., MN 55455.

Illumination of the receptive field surround of a cone evokes two distinct depolarizing responses: 1) an early, graded depolarization presumably mediated by a reduced release of GABA from horizontal cells and 2) a prolonged depolarization of many seconds in duration which can also be evoked by injecting a brief pulse of depolarizing current.

Both responses, along with the light-evoked response of horizontal cells, are abolished by adding kynurenic acid (1-5 mM) or d-O-phosphoserine (5 mM) to the superfusate but the prolonged depolarization can still be evoked by current injection. These results support the view that the graded depolarization is mediated by synaptic input from horizontal cells and that the prolonged depolarization is normally triggered by the graded depolarization.

The following results indicate that the prolonged depolarization depends on an increased Ca^{++} conductance: 1) The cone's input resistance decreases during the prolonged depolarization. 2) In the presence of 10 mM $CoCl_2$, the light-evoked prolonged depolarization, graded depolarization, and horizontal cell response are all abolished, and the prolonged depolarization cannot be evoked by current. 3) In the presence of 5 mM $CoCl_2$, the light-evoked cone depolarizations and horizontal cell response are abolished, but current can now evoke the prolonged depolarization. 4) The prolonged depolarization is facilitated by 5-10 mM $SrCl_2$ and its duration is increased.

GABA (5-20 mM) and its antagonists, bicuculline (1 mM) and picrotoxin (0.5-2 mM), have little effect on the graded depolarization. This lack of effect suggests that either GABA does not play a role in the surround antagonism of turtle cones or that these agents are denied access to the GABA receptors on cones.

Supported by a Fight for Sight Student Fellowship to W. B. Thoreson and N.I.H. Grant EY 00406 to D. A. Burkhardt.

68.6

EFFECTS OF GABA AND GLYCINE ON THE HORIZONTAL CELL RESPONSE RISE TIME IN THE TIGER SALAMANDER RETINA **Samuel M. Wu and Xiong-Li Yang. Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030**

Our previous studies (Yang and Wu, Neuroscience abstract 12:8, 1987; Wu and Yang, ARVO abstract 102, 1988) have shown that the horizontal cell response rise time (HCRRT) in the tiger salamander retina can be modulated either by background illumination (short-term modulation, STM) or by prolonged light exposure (long-term modulation, LTM). Application of GABA and glycine to the retina blocks the LTM, but not the STM. 100 uM GABA mimicks prolonged darkness and lengthens the time to peak of the HC response. 100 uM bicuculline mimicks prolonged light exposure and expedites the HCRRT. 3mM glycine exerts action on the HCRRT similar to those of the 100 uM GABA, and the glycine action can be reversed by strychnine. In the presence of bicuculline, the glycine action on HCRRT disappears. GABA, glycine and their antagonists do not affect the time course of the photoreceptor light responses. It is possible that GABA regulates the kinetics of the post-synaptic receptors for the photoreceptor transmitters in the HC, and glycine acts either like an agonist of GABA and/or increases GABA release through synapses made perhaps by the interplexiform cells. (Supported by NIH EY04446, the Retina Research Foundation and Research to Prevent Blindness, Inc.)

68.7

LOW IONIC CHANNEL DENSITY OF THE AXON TERMINAL OF TELEOST RETINAL HORIZONTAL CELLS. T. YAGI* and A. KANEKO. Natl. Inst. Physiol. Sci., Okazaki, Aichi 444, Japan.

In the teleost fish horizontal cells light-evoked responses conduct from the soma to the axon terminal (AT) without much decrement through a slender long axon (*ca.* 500 μm long and <1μm thick). To understand the electrotonic conduction through such structure, we studied membrane properties of AT and soma enzymatically dissociated from the goldfish using whole-cell patch-clamp method. Cells were superfused with a solution containing (in mM) NaCl 79, KCl 10, $MgCl_2$ 1, $CaCl_2$ 2.5, Choline-Cl 37, glucose 16 and HEPES 2 (pH 7.4). Pipettes were filled with a solution containing KCl 120, EGTA 5, and HEPES 10 (pH 7.3). Steady state current-voltage relation of most (80 %) ATs was nearly linear in a voltage range of -60 ~ 10 mV, where the slope conductance was *ca.* 5 $\mu S/cm^2$. The sodium current (I_{Na}) was activated by depolarization from a holding potential of -90 mV to more positive than -50 mV, and was maximal (*ca.* 60 pA) at about -20 mV. I_{Na} of AT was <10 % of that of the soma. I_{Ca} was isolated in a small proportion of ATs, with its amplitude being about 5 % of that of the soma. I_K through the anomalous rectifier was induced in AT when the membrane potential was less negative than -60 mV. Its conductance was 15 ~ 20 $\mu S/cm^2$ (*ca.* 1/20 of I_K of the soma). Other types of I_K were not detected. The present results indicate that low conductance of AT minimizes the leakage of signals arriving electorically through the thin connecting axon.

68.8

CALCIUM CHANNEL CURRENTS OF BIPOLAR CELL AXON TERMINALS ARE MODULATED VIA $GABA_B$ RECEPTORS. G. Maguire, B. Maple, P. Lukasiewicz and F. Werblin. Neurobiology Group, University of California at Berkeley. Berkeley, CA 94720.

Calcium channel currents of bipolar cells were recorded under whole cell patch clamp in retinal slices of the tiger salamander. Bipolar cell types were identified with Lucifer yellow staining during the recording. By the criteria of Fox, Nowycky and Tsien J. Physiol. (1987) **394**:173-200, two classes of calcium current were identified:

1) An L-current with slow activation and inactivation when stepped from -50 to 0 mV, that was enhanced by barium substituted for calcium, blocked by nifedipine and cadmium, and was found only in cells with synaptic terminals. Cells with ripped off axon terminals were completely devoid of the L-current.

2) A T-current that was more transient, inactivated at holding potentials more postive than -50 mV, was sensitive to nickel but not cadmium or nifedipine, and was found in most bipolar cells.

The L-type current in barium ringer was reduced by about 50% with 50uM baclofen, a $GABA_B$ receptor agonist, in the bath. When 50 to 500 uM GTP-gamma-S was included in the pipette, bathed baclofen completely eliminated the L-current. This modulation of L-current is limited to classes of bipolar cells with terminals that ramify near the center of the inner plexiform layer (IPL); bipolar cells that ramify near the peripheral margins of the IPL are unaffected by baclofen. The T-current located in the bipolar cell soma, and present in most bipolar cells, is unaffected by baclofen.

Thus, the modulation of calcium channel currents by $GABA_B$ receptors in bipolar cells appears to be: 1) mediated through a G-protein, 2) occurs only at L-type channels in the axon terminal and not at T-type channels in the soma, and 3) is limited to classes of "on" and "off" bipolar cells with processes that ramify in a limited region of the inner plexiform layer.

68.9

DOPAMINE AFFECTS THE OPEN PROBABILITY OF GAP JUNCTION CHANNELS IN WHITE PERCH HORIZONTAL CELLS. D.G. McMahon, A.G. Knapp, and J.E. Dowling, The Biological Laboratories, Harvard University, Cambridge, MA. 02138.

Dopamine, acting through cAMP-dependent protein phosphorylation, reduces the gap junctional conductance between teleost retinal horizontal cells (Lasater and Dowling, 1985, PNAS, 82). We have examined the transjunctional current noise from pairs of voltage-clamped white perch (Roccus americana) H1-type horizontal cells in order to ascertain how dopamine modifies the characteristics of individual gap junction channels.

Cell dissociation, culture, and recording methods were as previously described. Gap junctional gating events were evident as equal and opposite changes in the holding current of the clamp circuits. When individual gating events could be visualized the smallest were of 40-50pS conductance, but most were in the 90-100 and 120-150pS ranges. Analysis of the holding current mean and variance (Sigworth, 1980, J. Physiol., 307) during application of dopamine suggests that the open probability of the channels is dramatically reduced, from p>0.5 to p<0.2, during uncoupling. These experiments yield unitary conductance estimates of 30-47pS and channel numbers of 467-1459 (N=4). These unitary conductance values, while low compared with those reported for other gap junction channels, are consistent with the reported size of subconductance levels of heart cell gap junctions (Veenstra and DeHaan, 1986, Science, 233).

68.10

D_1 AND D_2 DOPAMINE LIGANDS ACT SYNERGISTICALLY TO MODIFY PHOTORECEPTOR TO HORIZONTAL CELL SYNAPTIC TRANSFER IN XENOPUS RETINA. P. Witkovsky and S. Stone. Dept. Ophthalmol., NYU Med. Ctr., NY, NY 10016.

In the mesopic state, rod and cone inputs to the horizontal cell (HC) combine; their relative proportions vary according to the particular stimulus employed. HC responses consist of an early peak (cone and rod input) and a later plateau (rod input). The intensities of red or green flashes were adjusted to give the same HC plateau, the responses were digitized and subtracted to yield the putative cone input. The D_1 agonist, SKF 38393, the D_2 agonist, LY 171555 and the D_2 antagonist, metoclopramide all changed both the amplitude and the kinetics of the cone input to the HC, whereas they altered the amplitude, but not the kinetics of the rod input to the HC. The D_1 agonist induced a larger increase in V_{max} and a faster response time but a smaller HC depolarization than the D_2 agonist. D_1 and D_2 agonist actions were selectively blocked by SCH 23390 and metoclopramide, respectively.

These findings suggest that D_1 and D_2 agonists work through separate but synergistic mechanisms. It appears that the primary action of dopamine ligands is on the cone input, whereas the rod input is scaled by a shunt. A model for a shunt is proposed based on the anatomical finding that axon branchlets enter both rod and cone bases. Using standard values for passive membrane properties and known values for glutamate-sensitive synaptic channels, a resistive network will be described that explains some of the effects observed physiologically. Supported by EY03570 to P.W. and EY06960 to S.S.

68.11

ORGANIZATION OF THE INNER PLEXIFORM LAYER IN THE RABBIT RETINA. J.K. Heussy*, R.F. Dacheux* and E. Raviola, Dept. of Anatomy, Harvard Medical School, Boston, MA 02115.

Amacrine cells (ACs) were impregnated with the Golgi technique or injected with horseradish peroxidase and the distribution of their dendrites in the inner plexiform layer (IPL) was investigated. The IPL is unequally subdivided into a cone bipolar (outer 4/5) and a rod bipolar (inner 1/5) lamina. In turn, four sublaminae (S1-S4) are defined within the cone bipolar lamina by the dendritic arborizations of unistratified ACs. The most scleral sublamina, S1, corresponds to one class of wide-field, unistratified ACs; S2 to outer starburst ACs; S3 to the wide-field, unistratified, ON-OFF ACs and to a class of narrow-field, unistratified ACs; S4 to inner starburst ACs and to another class of wide-field, unistratified ACs. The rod bipolar lamina (S5) contains the dendrites of two classes of rod ACs: indoleamine-accumulating ACs and bistratified ACs (AII). The vitreal dendrites of the two types of AII amacrines, narrow- and wide-field, spread tangentially in S5, whereas their lobular appendages occupy S1 and S2. Four classes of bushy, varicose ACs were observed: a narrow- and a wide-field type distributed throughout the IPL (S1 through S5); a second class limited to the cone bipolar lamina (S1 through S4); a third class confined to the outer IPL (S1 and S2) and a fourth to the inner IPL (S4 and S5). There is a precise correlation between layering of AC dendrites and distribution of the axon terminals of bipolar cells. (Grants EY03011 and EY01344).

68.12

CO-RELEASE OF GABA AND ACETYLCHOLINE BY AN AMACRINE CELL. R. H. Masland and D. M. O'Malley. Department of Physiology, Harvard Medical School, Boston, MA 02115.

The cholinergic amacrine cells of the rabbit retina contain GABA as well as acetylcholine (Brecha et al., 1988; Vaney and Young, 1988). We have developed methods by which the release of both compounds may be studied simultaneously. Isolated retinas are incubated in the presence of 3H-choline and 14C-GABA. The retinas, which then contain 3H-acetylcholine and 14C-GABA, are superfused and the compounds released into the medium are analyzed by electrophoresis and differential scintillation counting.

Stimulation of the retina by various flashing or moving lights triggers an increased secretion of 3H-acetylcholine. The simultaneously measured release of 14C-GABA is unaffected by any photic stimulation thus far tested. GABA is readily released by elevated K+, and the release can be shown autoradiographically to occur from the cholinergic cells. Three different blockers of GABA reuptake increased the basal release of GABA but did not unmask a light--induced release. 14C-GABA endogenously synthesized from 14C-glutamate behaved the same way as 14C-GABA accumulated from the medium. Under our conditions a 2-3% change in GABA release would have been detectable.

Flashing light depolarizes the cholinergic cell enough to more than double its rate of release of acetylcholine, and yet that same depolarization does not measurably change the rate of release of GABA by the cell. The two releases appear to be controlled differently.

68.13

NON-VESICULAR STORAGE OF GABA IN THE RETINA. D. M. O'Malley and R. H. Masland, Department of Physiology, Harvard Medical School, Boston, MA 02114.

GABA and acetylcholine, which are co-localized in one type of amacrine cell, are released differently: most of the release of acetylcholine is Ca++-dependent and appears to occur by exocytosis of synaptic vesicles, while GABA release appears in these cells (and some others; Schwartz, 1986) to be carrier-mediated. If so, one would expect their sites of storage to be different.

Isolated rabbit retinas were labeled to contain 14C-GABA and 3H-acetylcholine. Subsequent incubation in the presence of black widow spider venom caused a large increase in the release of 3H-acetylcholine but no increase in the release of 14C-GABA. Disrupting the retina's plasma membranes by freezing and thawing, which in synaptosomes is reported to release cytoplasmic contents but retain vesicles (Israel, 1981), retained 40% of the retina's 3H-acetylcholine but only 6% of its 14C-GABA. Immunobeads labeled with an antibody to synaptic vesicle antigen P38 (synaptophysin) were used to precipitate synaptic vesicles (Buckley and Kelly, 1985) from retinal homogenates. The beads brought down 25% of the total acetylcholine initially present, but essentially no GABA.

These are the results expected if the cell releases acetylcholine by exocytosis of vesicles and GABA by transport from the cytoplasm. Because the mechanisms are distinct, the same amacrine cell is capable of releasing acetylcholine without releasing GABA, and vice versa.

DIFFERENTIATION AND DEVELOPMENT II

69.1

IN VITRO ANALYSIS OF NEUROGENESIS IN A MAMMALIAN NEUROEPITHELIUM. A.L. Calof & D.M. Chikaraishi. Neuroscience Program, Tufts University School of Medicine, Boston, MA 02111.

A primary event in the formation of the vertebrate nervous system is the generation of neurons from the neuroepithelium of the neural tube and neurogenic placodes. Analysis of the molecular events involved in neurogenesis would be greatly facilitated by a system in which this process could be studied *in vitro*. Such an *in vitro* system has been developed using the olfactory epithelium (OE), an ideal tissue in which to study neurogenesis: the OE contains a limited number of cell types, all identifiable; these cells participate in neurogenesis; and neurogenesis is an ongoing process in this tissue throughout life, reducing the temporal constraints associated with studying this process elsewhere in the nervous system.

Olfactory epithelium from mouse or rat embryos is separated from underlying stroma and placed into culture. Within 24 hours, three cell types appear: an epithelial cell, an immature neuron, and a third cell type; ^{3}H-thymidine labeling and immunocytochemical studies indicate that this third cell type, which divides in culture, may be the immediate precursor of the immature neuron. In serum-free, defined culture conditions, Epidermal Growth Factor enhances the growth of the epithelial cells, but not the generation of immature neurons or their subsequent survival. Nerve Growth Factor, Acidic Fibroblast Growth Factor, and Basic Fibroblast Growth Factor also do not appear to enhance neuronal survival in these cultures. Conditions necessary for the long-term survival of the immature neurons, as well as for their continued generation in primary culture, are being investigated. The derivation of cell lines from these cultures by means of retrovirus-mediated oncogene transfer is also being pursued. *Supported by awards from the Alzheimer's Disease and Related Disorders Association and the Muscular Dystrophy Association to A.L.C.*

69.2

MITOGENIC EFFECT OF EMBRYONIC GUT AND STRIATED MUSCLE ON THE NEUROEPITHELIUM OF AVIAN EMBRYOS. T. P. Rothman, J. Fontaine-Pérus*, M. D. Gershon and N. M. Le Douarin. Columbia Univ., P & S, NY, NY 10032 and Institut d'Embryologie, 94736 Nogent-sur-Marne, France.

We have previously shown that neuromuscular tissue of quail or murine gut, back-grafted between the neural tube and somites of younger chick embryos, secretes a short-range diffusible factor that induces unilateral enlargement of the spinal cord [Rothman et al., *Dev. Biol.* 124(1987)331-346]. Experiments were done to determine whether the factor affects proliferation, the timing of its action, and what types of muscle produce it. Embryonic quail foregut (E4) or striated muscle (breast or epaxial; E8-9) or murine bowel or skeletal muscle (triceps or epaxial; E14-15) was backtransplanted between the neural tube and somites of avian embryos (E2). Host embryos were incubated with ^{3}H-thymidine (^{3}H-TdR; 10 μCi). Feulgen stained sections, which permitted quail, chick, or murine cells to be distinguished, were then processed for radioautography. All cells of the neuroepithelium (NE) of host embryos exposed for 24 hrs to ^{3}H-TdR were labeled, although the number of proliferating cells on the side adjacent to grafts greatly exceeded the number on the non-operated side (control). In order to determine when the mitogenic effect first became manifest, NE cells in the S phase of the cell cycle were visualized in host embryos incubated for 2 hrs with ^{3}H-TdR, 6-8, 15 or 22-26 hrs after grafting. A significant increase in the number of cells in S on the operated side was apparent by 15-22, but not by 8 hrs following backtransplantation of any of the investigated muscle. These experiments demonstrate that skeletal muscle as well as enteric smooth muscle is capable of enhancing the rate of proliferation of NE cells after they are experimentally placed together. An effect can be detected as early as 15 hrs after grafting. The muscle derived growth-promoting factor, which is not species specific, must still be identified. Supported by NIH, HD20470, HD21032, NS15547; MODBDF 1-747, 1-866; CNRS; Fdn. Rech. Méd. Française; Ligue Française contre le Cancer.

69.3

AN AUTORADIOGRAPHIC ANALYSIS OF NEURONAL PROLIFERATION IN JUVENILE *APLYSIA*. P. W. Hickmott* and T. J. Carew (SPON: M. J. Cohen). Dept. of Biology, Yale Univ., New Haven, CT 06520.

During late juvenile development in *Aplysia* there is a dramatic proliferation of neurons throughout the CNS (Cash and Carew, 1987). To investigate the source of these neurons, we used ^{3}H-thymidine labelling in two different juvenile stages: Stage 11 (before proliferation), and Stage 12 (when proliferation occurs). Previous results (McAllister, et. al., 1983; Jacob, 1984), indicated that some neuronal precursor cells in *Aplysia* originate in body wall ectoderm and migrate into their target ganglia.

We focussed our attention on the abdominal ganglion and its adjacent body wall. In Stage 11, there was uniform labelling of a fraction of cells in the body wall. However, in Stage 12, there was a significant increase in labelling in the region of body wall immediately opposite the ganglion (compared to adjacent control regions) (mean increase=44%; p<.001). Thus, at the time of neuronal proliferation, body wall sites immediately opposite the ganglion show a marked increase in cell division.

We next examined the distribution of labelled cells in the abdominal ganglion at survival times of 1 and 7 days after injection to determine whether cell migration occurs in the CNS. In both stages, the fraction of labelled cells on the outside of the ganglion significantly decreased over time, with a corresponding increase on the inside. Both processes were significantly accelerated in Stage 12 [outside decreases: Stage 11 (p<.05), Stage 12 (p<.001); inside increases: Stage 11 (p<.05), Stage 12 (p<.001)].

Our results suggest: 1) proliferative zones in the body wall are significantly up-regulated late in juvenile development to give rise to widespread proliferation; and 2) cells arriving at their target ganglion continue migrating deeper into the ganglion.

69.4

DEVELOPMENTAL EXPRESSION OF FACILITATORY NEUROPEPTIDES IN *APLYSIA*. E.W.Harkins*, B.B.Worrall*, E.A.Marcus, T.G.Nolen[1], and T.J.Carew. Dept. of Biology, Yale Univ., New Haven, CT 06520, and [1]Dept. of Biology, Univ. of Miami, Coral Gables, FL 33124.

Small cardioactive peptide B has been implicated in synaptic facilitation contributing to sensitization in *Aplysia* (Abrams et al, 1984). With an interest in SCP-B's possible role in the development of learning, we tested SCP immunoreactivity (SCP-IR) in two juvenile stages: Stage 11, when sensitization is absent, and Stage12, when sensitizaiton first emerges.

In Stage 12 animals (5-12 mm, n=19), SCP-IR was found in all central ganglia (total cells, $\bar{x}$=46.5); it was greatest in the buccal ganglia, moderate in the cerebral, pedal, and abdominal ganglia, and least in the pleural ganglia. In most ganglia, specific cells were repeatedly stained. These include B1 and/or B2 candidates in the buccals, two small cells in the pleurals, and a large L10 candidate in the left abdominal ganglion. All SCP-IR showed bilateral symmetry except in the abdominal ganglia. In Stage 11 (1-4 mm, n=25), significantly fewer SCP-IR cells were present, compared to Stage 12 ($\bar{x}$=8.8, p<.002), though the overall staining pattern represented a subset of that seen at Stage 12. Interestingly, Stage 12 not only had more SCP-IR cells than Stage 11, it also had SCP-IR cells that are not observed in adults (Lloyd et al, 1988), including specific cells in the abdominal and pleural ganglia, suggesting that some peptidergic expression may be transient during development.

The 5-fold increase in SCP-IR between Stages 11 and 12 coincides with a 6-fold increase in total cell number (Cash & Carew, 1987) as well as the onset of sensitization and its cellular analog (Rankin &Carew, 1988; Nolen & Carew, 1988). It will now be of interest try to relate the expression of peptides in specific cells to the emergence of specific forms of learning.

69.5

OVERALL SEGMENTAL PLAN OF THE EMBRYONIC BRAIN OF A SIMPLE VERTEBRATE, THE ZEBRAFISH B.W.Trevarrow Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403; present address Div. of Biol. 216-76, Cal. Inst. of Tech., Pasadena, Ca 91125

Monoclonal antibodies have been used to analyze segmentation of the brain of the embryonic zebrafish. Hindbrain segments are present as a series of neuromeres containing segmentally organized neurons, glial, fiber tracts, and immunoreactive regions of neuropil. Each segment contains two separate regions, termed neuromeric centers and interneuromeric zones, composed of structures binding different specific antibodies. These two regions form an alternating series along the neuraxis.

Labeling with these antibodies reveals a similar alternating pattern more rostrally. The forebrain and midbrain each behave as single segments, albeit longer than those of the hindbrain. The telencephalon and the major part of the mesencephalon correspond to the neuromeric centers of these segments. The diencephalon and a region including the caudal mesencephalon and cerebellum correspond to the interneuromeric zones.

Throughout the brain the first neurons to develop are located in the neuromeric centers and include cells that project longitudinally. Neurons in the interneuromeric zones develop later, form axons that decussate in ventral fiber tracts, and receive special sensory projections. This simple iterated pattern may reflect a highly conserved plan of organization of the chordate brain. **(supported by NIH grants NS17963, HD22486, and 5 T32 GM0 7413 [Genetics Training Grant at the Univ. of Oregon])**

69.6

DEVELOPMENTAL ACQUISITION OF GABA-LIKE IMMUNOREACTIVITY BY AMPHIBIAN SPINAL NEURONS DIFFERENTIATING *IN VITRO*. N.C.Spitzer, Rosario C.de Baca* and Janet Holliday. Dept. of Biology, UCSD.

The development of membrane properties of embryonic *Xenopus* spinal neurons in culture parallels that seen *in vivo*. We have studied the development of cytoplasmic properties such as neurotransmitter phenotype by examining the appearance of GABA-like immunoreactivity (GLIR) *in vitro*. Cells cultured from neural plate stage embryos differentiate into neurons that can be labeled with antiserum raised against GABA conjugated to BSA . Most immunoreactive neurons display reaction product through-out the cell but a few have only heavily labeled processes and growth cones.

Neurons first display detectable GLIR at 10 hrs in culture. The number of labeled cells, as a per cent of morphologically differentiated neurons, increases steadily over time with the greatest increase (19%) occurring between 12 and 16 hrs. The time course of acquisition of GLIR *in vitro* parallels that of neurons developing *in vivo* (Roberts et al.,1987). By one day *in vitro*, 35% of neurons are labeled when grown in culture medium containing calcium (10 mM) . The percent of immunoreactive neurons at this time is similar to that previously shown to acquire a high affinity GABA uptake system (26%, Lamborghini & Iles, 1985) and the time course of acquisition of these two properties is also similar.

Long duration Ca-dependent action potentials are evoked from these neurons and they also exhibit spontaneous elevations of internal Ca at early stages of development (Holliday and Spitzer,1988). Since Ca influx can influence neurotransmitter synthesis (Walicke and Patterson, 1981), cells were allowed to differentiate in a medium in which Ca was replaced with Mg (10 mM) and EGTA. Only 5% of neurons grown under this condition acquire GLIR. This culture condition does not inhibit the development of the uptake system. The roles of synthesis and uptake in the development of GLIR are under investigation.

Supported by NS15918 to NCS; JH is a fellow of the USPHS.

69.7

THE DEVELOPMENT OF CHOLINERGIC NEURONS IN EMBRYONIC RAT SPINAL CORD. P.E.Phelps, R.P.Barber,* L.A.Brennan,* P.M.Salvaterra, and J.E.Vaughn. Div. of Neurosciences, Beckman Res.Inst.of the City of Hope, Duarte, CA 91010.

Using a monoclonal antibody against choline acetyltransferase (ChAT), we have previously defined 4 groups of cholinergic neurons in cervical spinal cord; i.e., motoneurons (MNs), partition cells, and small neurons around the central canal as well as in the dorsal horn. We have also described their times of origin, with MNs being the first born (E11-12). In this study, we have used both immunocytochemical and biochemical methods to study ChAT expression during embryonic development. ChAT was first detected immunocytochemically in MNs on E13, and all 4 types of ChAT+ cells were identifiable by E17. Immature ChAT+ neurons were found on E14-16 adjacent to, and within, the germinal epithelium thereby demonstrating that the cholinergic phenotype is already expressed by cells during their migratory phase of development. Biochemical assays demonstrated the first detectable levels of ChAT activity at E13 and a 9-fold increase in activity by E17. In combination with our previous results, these findings suggest that 1-2 days are necessary after a spinal cholinergic neuron is born before its neurotransmitter phenotype is detectable. Supported by NIH grant NS18858.

69.8

FIBER TYPE DIFFERENTIATION DURING RAT HINDLIMB MYOGENESIS DOES NOT DEPEND UPON INNERVATION. K. Condon, W. J. Thompson, L. Silberstein, & H. M. Blau*. Dept. of Zoology, Univ. of Texas, Austin, TX 78712 and Dept. of Pharmacology, Stanford Univ., Stanford, CA 94305.

Recent work in chick limb myogenesis has demonstrated that muscle fiber types can differentiate in the absence of innervation. To determine if innervation is necessary for mammalian fiber type differentiation, aneural limbs were produced by injection of the neurotoxin β-bungarotoxin (β-BTX) into rat embryos, a technique developed by A. J. Harris. β-BTX was given at or prior to the time of the initial innervation of muscle fibers in the lower hindlimb (embryonic days 14-15). By several criteria such treatment destroyed the innervation of the hindlimb muscles in these embryos: no nerve processes could be detected with silver stains, no nerve terminals were detected using antibody to a synaptic vesicle protein, and few if any motor neurons were found in the lateral motor column of the lumbar spinal cord. Following injection, development was allowed to continue until embryonic day 19 or 20. In control animals and non-injected littermates, a characteristic and complementary distribution of fast and slow class myofibers (as determined by immunohistochemistry with monoclonal antibodies) is present in the tibialis anterior, extensor digitorum longus and soleus muscles by embryonic day 19 (E19). While aneural embryos show a marked reduction in the number of fibers in these muscles, both fast and slow class fibers are present in their characteristic intramuscular positions. Thus, fiber type differentiation in rat myogenesis appears to occur in the absence of innervation, suggesting that nerves do not instruct the initial differentiation of mammalian muscle fiber types.

69.9

THE APPEARANCE OF POSTEMBRYONIC NEURONS IN THE LEECH *HIRUDO MEDICINALIS* IS CONTROLLED BY THE INNERVATION OF THE MALE ORGANS. C.A. Baptista* and E.R. Macagno. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

In adult *Hirudo medicinalis*, the segmental ganglia of the fifth and sixth body segments (SG5 and SG6, also known as the sex SG) contain a few hundred more cells than the other segmental ganglia of the body (Macagno, E., *J. Comp. Neurol.*, 190:2301, 1980). Both SG5 and SG6 innervate the male organs, while the female organs are exclusively innervated by SG6. The extra cells appear to be neurons since they can be stained by two neuron-specific antibodies. The extra cells are added exclusively to the sex SG, begining at about the end of embryogenesis, 30 days after egg-laying, and continuing post-embryonically. If the male organs are ablated before 16 days of development, extra cells do not develop in either SG5 or SG6. In addition, preventing the innervation of the male organs by SG6, up to day 16, again results in a SG6 without extra cells. From 16 days onward, the generation of the extra cells is independent of the male organs. No extra cells appear in non-sex SG that innervate male organs transplanted to ectopic locations at 10 days or later. In contrast, control transplants of male organs done at the same ages are still competent to elicit extra cells in either of the sex SG. These data suggest that the extra cells are generated within the sex SG, by a process that is triggered by an interaction with the male organs during a critical period and mediated through the innervation of the organs by these ganglia.

69.10

SYNAPTIC CONNECTIVITY OF IDENTIFIED LEECH NEURONS IS SPECIFIED BY PERIPHERAL TARGETS DURING EMBRYOGENESIS. C. M. Loer and W. B. Kristan, Jr., Dept. of Biology, B-022, UCSD., La Jolla, CA 92093.

A pair of serotonergic Retzius (Rz) cells is found in each segment of the leech nervous system. Whereas most Rz cells innervate the body wall in their own and adjacent segments, Rz cells in segments 5 and 6 [Rz (5,6)] instead innervate the reproductive tissue, which is found only in those segments. Standard Rz cells also have a denser central arbor than do Rz (5,6) and receive a number of excitatory synaptic inputs which are lacking in Rz (5,6). Early in development, however, all Rz cells grow similar central and peripheral projections. Rz (5,6) only become different from their segmental homologs after contact with their unique target, the reproductive tissue. Early ablation of reproductive tissue causes Rz (5,6) to innervate the body wall and elaborate a central arbor like that of standard Rz cells. Here we report that ablation of reproductive tissue also causes Rz (5,6) to acquire synaptic connections that are appropriate for standard Rz cells.

Ablations or sham operations were performed by removing either reproductive primordia or nearby tissue from early 10 day embryos. Responses of Rz cells in ganglia 3 - 9 to sensory stimuli were recorded in juvenile leeches after completion of embryogenesis. In unoperated control juveniles, as in the adult, standard Rz cells were depolarized by a number of stimuli: pinching the tail, swimming of the leech, and stimulation of pressure mechanosensory neurons (P cells). Rz (5,6) were not excited by any of these stimuli. In reproductive tissue-ablated animals, Rz (5,6) frequently received synaptic input appropriate for standard Rz cells: Rz (5,6) were depolarized by pinching the tail (12/17 cells, 8 leeches), during episodes of swimming (5/11 cells, 6 leeches), and by P cell stimulation (11/13 cells, 8 leeches). Rz (5,6) in sham-operated controls were not different from those in unoperated controls: they were never excited by tail pinching (0/10 cells, 4 leeches), during episodes of swimming (0/5 cells, 3 leeches) or by P cell stimulation (0/8 cells, 4 leeches). These results demonstrate that both structure and function of Rz cells is profoundly affected by the target they contact during embryogenesis.

Supported by NIH grants NS20746 (WBK) and GM07313 (CML).

69.11

SEGMENT-SPECIFIC DIFFERENCES IN ACh RECEPTORS ON LEECH RETZIUS NEURONS. W.B. Kristan, Jr. & K. A. French, Dept. of Biology, UCSD, La Jolla, CA, 92093.

The pair of Retzius cells in leech segments 5 and 6 [Rz(5,6)] differ physiologically and morphologically from their homologs in the 19 other midbody segments. These differences depend at least in part upon the growth of particular peripheral processes of Rz(5,6) into the region of the primordial reproductive organs. We report here that Rz(5,6) also differ from all the other Rz cells [Rz(X)] in the type of ACh receptors they have on their somata.

Responses of Rz cells were recorded from the somata while solutions of ACh (10^{-6} to 10^{-4} M) were pressure ejected onto the somata perfused by leech saline solution containing 20 mM Mg^{+2} to block chemical synaptic transmission. The ACh depolarized all Rz(X) cells, but produced a mixed depolarization and hyperpolarization of Rz(5,6). The mixed response always occurred in Rz(5); the depolarizing response in Rz(6) could be seen only after the much stronger hyperpolarizing response was blocked. Depolarizing responses were blocked by *d*-tubocurarine (10^{-5} M), and hyperpolarizing responses were blocked by α–bungarotoxin (10^{-7} M). All the responses were accentuated by eserine (10^{-5} M). These results indicate that there are two kinds of ACh receptors on the somata of Rz cells: all Rz cells have a depolarizing, curare-blockable receptor, and Rz(5,6) have in addition a hyperpolarizing, α–bungarotoxin-blockable receptor. We will determine whether these differences in ACh receptors depend upon the presence of reproductive organs during development, as do other properties of Rz(5,6). [This work was supported by research grants from NIH (NS14410) and the March of Dimes.]

69.12

TWO DISTINCT TYPES OF DEVELOPMENTAL INTERACTIONS BETWEEN IDENTIFIED NEURONS AND CELLS IN THE PERIPHERY OF THE LEECH, *Hirudo*. K. A. French, S. M. Furgal*, and W. B. Kristan, Jr., Dept. of Biology, UCSD, La Jolla, CA 92093.

In the ventral nerve cord of the medicinal leech, the paired Retzius (Rz) neurons in each of the two segments containing the reproductive structures (segments 5 and 6) are different from Rz neurons in all other segments. The two types of Rz neurons differ in target choice, peripheral and central morphology, synaptic input, and somatic ACh receptors. Because many of these differences fail to develop when the embryonic reproductive structures are ablated prior to neurite outgrowth, the distinctive phenotype of the reproductive segment Rz neurons [Rz(5,6)] appears to be induced by an interaction between the developing neurons and some structure in the periphery. Growing Rz(5,6) neurites contact two possible sources of the signal: the primordial reproductive ducts (PRD) and a population of distinctive cells (V-cells) that surround the PRD and lie along the pathway followed by developing Rz(5,6) neurites.

Rz(5,6) neurites initially contact V-cells on the 10th day of development, in an apparently transient association that closely resembles pathfinding interactions seen in other species. Only later, on the 12th day of development, do they first contact and begin to arborize and form synaptic varicosities in the PRD, starting near the genital pore. Because the shift in phenotype from that of standard Rz cells to Rz(5,6) begins on the 10th day, contact with the V-cells, rather than the more conventional contact with the PRD, is the most likely source of the inductive signal. [This work was supported by an NIH Research Grant (NS20746) and a March of Dimes Grant to WBK and a University of California, San Diego OGSR grant to KAF.]

REGENERATION: PNS

70.1

AXONAL GROWTH AND SCHWANN CELL MIGRATION INTO BASAL LAMINA GRAFTS TO THE RAT SCIATIC NERVE. J.W. Fawcett* and R.J. Keynes. Physiological laboratory and Dept. Anatomy, Downing St., Cambridge CB2 3EG, England.

We have previously shown that grafts of striated muscle extra-cellular matrix, made by extruding the myoplasm from lengths of muscle, are extremely effective in repairing cut peripheral nerves, performing as well as nerve autografts in lengths up to 4cm (J. Neurosurg. 65:354-363).

We have studied the early events following the insertion of a graft into rat sciatic nerves, by immunohistochemistry using antibodies to neurofilament and S-100, which stain axons and Schwann cells (SC) respectively, and by electron microscopy.

7 days after insertion of the graft many axons and SC's are seen growing into the graft from the proximal stump, becoming sparser with distance into the graft, the maximum distance of migration being 0.3cm. All axons were associated with SC's, but some SC's were not visibly accompanied by axons; some of these SC's were in advance of the front of regenerating axons. A few SC's migrated from the distal stump into the graft, but very much fewer than from the proximal stump. By 10 days many axons had crossed the 0.5cm grafts, and many more SC's were present. Later time points showed increases in axon number,size and myelination.

We conclude that Schwann cells precede axons into the grafts, and that factors present in the proximal but not the distal stump exert a strong mitogenic effect on Schwann cells.

70.2

SYNTHETIC GUIDANCE CHANNELS RELEASING BASIC FIBROBLAST GROWTH FACTOR ALLOW BRIDGING OF A 15 MM LONG GAP IN THE RAT SCIATIC NERVE MODEL. A.N. Salessiotis *, S.R. Winn *, P. Aebischer * (SPON: J. T. McIllwain). Artificial Organ Laboratory, Brown University, Providence, RI 02912.

The maximum gap that can be bridged by an impermeable synthetic tube in the rat sciatic nerve model is 10 mm. In the present study we investigated the ability of synthetic nerve guidance channels which release controlled amounts of b-FGF to bridge a 15 mm long gap. Eighteen mm long guidance channels were fabricated by dipping a stainless steel mandril in a polymer solution of poly[ethylene-vinyl acetate] (EVA) containing bovine serum albumin (BSA) and b-FGF. In vitro kinetic studies of protein release from these channels showed an initial burst during the first three days followed by linear release for at least two weeks thereafter. Further in vitro studies indicated that the b-FGF released from the polymer was biologically active as assessed by the ability of channels containing b-FGF to induce neurite extension from PC12 cells in culture. For in vivo studies, 8 mm of rat sciatic nerve was resected, and the proximal and distal nerve stumps were secured 15 mm apart within the guidance channels. Cohorts of 6 animals were implanted for 4 weeks with channels made of pure EVA, EVA/BSA, or EVA/BSA/bFGF. Upon retrieval, 4 EVA/BSA/bFGF channels had cables bridging both nerve stumps whereas none of the pure EVA or EVA/BSA bridged the nerve gap. Two of the nerve cables regenerated in b-FGF releasing tubes contained more than a thousand myelinated axons at the channel's midpoint. We conclude that b-FGF enhances peripheral nerve regeneration and that polymeric tubes releasing macromolecules provide a powerful tool for the study of nerve regeneration.

70.3

CONDUCTION PROPERTIES OF PERIPHERAL NERVES REGENERATED BY USE OF COPOLYMER MATRICES WITH DIFFERENT BIODEGRADATION RATES. I.V. Yannas*, A.S. Chang*, C. Krarup*, R. Sethi*, T.V. Norregaard* and N.T. Zervas. Fibers and Polymers Lab., Mass. Inst. of Tech., Cambridge, MA 02139, Division of Neurology, Brigham and Women's Hospital, Boston, MA 02115 and Division of Neurosurgery, Mass. General Hospital, Boston, MA 02115.

Collagen-glycosaminoglycan (CG) matrices ensheathed in silicone tubes were used to bridge 10-mm gaps in transected rat sciatic nerves. Recovery was monitored electrophysiologically by stimulating the nerve at the sciatic notch and, separately, on the tibial branch and recording the evoked CMAP's at the plantar flexor muscles in the foot. We studied the effect of the average molecular weight between crosslinks $\bar{M}_c$, of CG matrices on the degree of physiological recovery. Matrices with $\bar{M}_c$ = 45 kD ± 20% (low crosslink density, rapid degradation rate) and $\bar{M}_c$ = 12 kD ± 20% (high crosslink density, slow degradation rate) were studied. The average pore diameter was 10 ± 5 μm in both groups of matrices. The results obtained 28 weeks past grafting are shown in Table I. The frequency of recovery of physiological function is greatly enhanced when the silicone tube contains either of the two CG matrices rather than saline. These results support our previous histological observations on the same model. The results also show that a lower crosslink density (higher $\bar{M}_c$) leads to superior recovery. We suggest that the diminished recovery which is observed with the highly crosslinked matrices is due to their significantly lower degradation rate in collagenase. This observation underscores the importance of the detailed physicochemical structure of the CG matrix in the mechanism of nerve regeneration.

Table I. ELECTROPHYSIOLOGICAL EVIDENCE OF REGENERATION (28 wks)

Graft Type	Regeneration Rate	Distal Motor Latency (msec) From Tibial Branch	From Sciatic Notch
CG, M_c = 45 kD	(6/6) 100%	1.84 ± 0.20	3.43 ± 0.49
CG, M_c = 12 kD	(8/8) 100%	2.26 ± 0.20	4.38 ± 0.38
Empty Tube	(1/6) 17%	2.36 ± 0.21	4.08 ± 0.40
Saline-filled Tube	(5/15) 33%	2.47 ± 0.57	4.84 ± 0.73
Normal Nerve	———	1.58 ± 0.17	2.47 ± 0.17

70.4

NERVE GROWTH FACTOR ENHANCES AXONAL GROWTH THROUGH SILICONE CHAMBERS IN MATURE RATS. J.Pryor*, T.Alexander* and K.M.Rich. Department of Neurosurgery, Washington University School of Medicine, St. Louis, MO 63110.

The effect of NGF on axonal growth within a chamber was examined in adult Sprague-Davley rats. After sciatic nerve section and surgical implantation of a silicone chamber with a 7 mm. gap between proximal and distal nerve ends, chambers were filled with either a 1mg/ml NGF-saline solution (experimental) or a normal saline solution (control). Four weeks after the surgery semi-thin cross-sections were prepared and stained with toluidine blue. Total myelinated axonal counts were done at both the extreme proximal and distal segments of the regenerated nerve segment within the chamber. Morphometric analysis of the distal myelinated axons was done with a computer-assisted digital analyzer.

NGF significantly increased the number of myelinated axons growing into the distal end of chamber (2126±437 NGF-saline; 1064±268 saline; P<0.05 Student's t-test). Counts of myelinated axons from the extreme proximal portion of the nerve within the chamber showed fewer myelinated axons in the NGF-treated nerves (3326±562 NGF-saline; 5110±661 saline P<0.10). The ratio of total myelinated axonal counts from distal vs. proximal segments from the same nerves showed a significantly higher number in the NGF-treated group (.13 saline; .62 NGF-saline). There was no difference in mean axonal diameters between groups (2.8μ saline; 2.7μ NGF-saline).

70.5

NERVE REGENERATION ACROSS A 16MM GAP IS ENHANCED BY A FROZEN NERVE AND A "SLOT" TUBE GUIDE IN RATS. C.-B. Jeng & L.-L. Jeng*. Dept. Anat. & Neurosci., Univ. Texas Med. Br., Galveston, TX 77550 & Dept. Anat., Chang Gung Med. College, Tao-Yuan, Taiwan, R.O.C.

When nerve stumps are sutured 16mm apart in an empty silicone tube, nerve regeneration fails. However, some regeneration occurs if cells from the connective tissue have access to the tube lumen through holes cut in the tube wall (holey tube). Here, we ask if regeneration is further improved by a greater access of connective tissue or a frozen-thawn nerve placed at the gap. Under deep Nembutal anesthesia, a 16mm gap between rat sciatic nerve stumps was maintained by a tube with a 1mm x 10mm slot at the middle (slot tube). The gap was either left empty or filled with an autologous fresh sciatic nerve segment or a nerve that had been frozen-thawn three times. Six weeks later, regenerated myelinated axons were counted in the distal sciatic nerve stumps. The myelinated axon numbers are 86 ± 123 with holey tube (Brain Res., 408:239, 1987), 2330 ± 1408 with slot tube, 6111 ± 1185 after frozen nerve transplant and 8508 ± 1445 after fresh nerve transplant. Thus, compared to holey tubes, the slot tubes greatly improve nerve regeneration. A frozen nerve which presumably contains no live cells, is able to enhance nerve regeneration but not as effective as a fresh nerve transplant. (Supported by the American Muscular Dystrophy Association and the Chang Gung Medical Research Fund).

70.6

FACIAL NERVE REGENERATION IN THE SILICONE CHAMBER: INFLUENCE OF NERVE GROWTH FACTOR. Y.-S. Chen*, L.T. Wang-Bennett and N.J. Coker*. Dept. of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas 77030.

The role of nerve growth factor (NGF) was examined in the neural repair of adult rabbit facial nerve using an in vivo preparation. A 35 μl nerve growth chamber was created by suturing the proximal and distal ends of a transected facial nerve (buccal branch) into a silicone tube. A gap of 8 mm in the chamber remained after removal of a 5 mm piece of nerve. Animals were operated bilaterally; one side of the chamber was filled with NGF and the contralateral side was filled with Ringer's solution. Regeneration of the nerves was examined 1 wk to 5 wk following the surgery. The caliber of the nerve bundle, the distribution pattern of regenerating axons, axon number per fascicle, size distribution, and the total number of axons were compared to the preoperative morphology pattern found for that animal. Each buccal branch served as its own control.

The NGF-filled chambers demonstrated an overall larger caliber at 5 wk and a higher density distribution of axon growth at 3 wk and 5 wk. In the early regeneration cases (3 wk), the axon growth profile exhibited more fascicles and less axons while in the 5 wk cases, the intrafascicular axon density increased. Axon size at 5 wk was 80% that of preoperative controls. The histogram of the size distribution revealed the same distribution as in the preoperative control section.

70.7

RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE DURING FACIAL NERVE REGENERATION. L.T. Wang-Bennett, Y.-S. Chen* and N.J. Coker*(SPON: C.D. Fermin). Dept. of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas 77030.

In the companion abstract (Chen, Wang-Bennett and Coker) we reported that adult rabbit facial nerve (buccal branch) regenerated in a silicone chamber sutured to the stumps of the transected nerve. We then quantified the regenerating axons in the silicone chamber for their ability to uptake and exhibit somatopetal transport of horseradish peroxidase (HRP). We also examined if the somatotopic connection was altered by the regeneration process at 3 wk and 5 wk PO.

Upon resection of the distal end of the regenerating nerve, a 30% HRP solution (35 μl) was injected through the tube wall into a second chamber sutured to the distal end of the nerve stump. Using the histochemical method of Mesulam(1978), the number of labelled cell bodies in the facial motor nucleus (FMN) and the distribution in the subnuclei: lateral, intermediate and medial were analyzed.

In normal acute preparations, most of the HRP-labelled soma were localized in intermediate and lateral subnuclei and few in medial subnucleus. This distribution pattern remained in the 3 wk and 5 wk regeneration groups. However, HRP labelled cell bodies that survived the nerve transection surgery reflected only a small percentage of the axons seen in the center cross section of the regeneration chamber and the density of the HRP reaction product was low and indicated few dendritic processes.

70.8

ACETYLCHOLINESTERASE PROVIDED BY MOTOR AXONS INNERVATING MYOFIBER BASAL LAMINA SHEATHS. L. Anglister and B. Haesaert* (SPON: A. Reches). Anatomy Dept., Hebrew Univ. Med. Sch., Jerusalem 91010, Israel.

Acetylcholinesterase (AChE) consists of asymmetric or globular molecular forms that can be distinguished by their sedimentation coefficients. In frog muscle the major asymmetric form is a 17.5S AChE, while most globular forms are 4-6S AChE. Muscle AChE is highly concentrated in the synaptic clefts at neuromuscular junctions (NMJs). We have shown that motor nerve is capable of producing synaptic AChE and some of it becomes adherent to synaptic basal lamina (Anglister, Soc.Neurosci.Abstr.13:1211, 1987). This study was aimed to determine which molecular forms of AChE the nerve may contribute to NMJs.

Experiments were carried out, as before, on operated cutaneus pectoris (CP) muscles of frog, in which myofibers had been removed from their basal lamina sheaths, while leaving behind intact motor axons, nerve terminals and synaptic basal laminae, but irreversibly blocking all original AChE. At 30 days all synaptic sites were occupied by nerve terminals and showed histochemically the presence of AChE, newly formed by nerve. Biochemical analysis revealed that 30-50% of this AChE was a 17.5S species; an equal fraction migrated as 4-6S molecules. In innervated region of intact frog muscle only 15-20% AChE consisted of 17.5S species, while most (60-70%) were 4-6S forms. The high 17.5S content in the innervated CP-sheaths originated from the motor axons: In the nerve bundle to CP most of the enzyme (50-70%) was 17.5S AChE, while 4-6S species were a minor component (10-20%). Similar composition was found in the brachial nerve from which the nerve to CP branched.

The results show that the motor nerve to CP, which produces all forms of AChE, carries high concentrations of 17.5S AChE. This is also reflected in the high content of this form in the innervated basal lamina preparation, in the absence of myofibers. 17.5S AChE may be destined for synaptic localization or for neurotrophic purposes. (Supported by grants from BSF and Israel Acad. Sci. to L.A.).

70.9

FOCAL REINNERVATION OF MOTOR ENDPLATES IS FASTER BUT LESS PRECISE IN OLD THAN IN YOUNG MOUSE MUSCLE. N. Robbins, M. Kuchynski* & A. Grasso*. Div. Neuroscience, Case Western Reserve Sch. Med., Cleveland, OH 44106.

Slower recovery of function after nerve injury in old animals (Drahota & Gutmann, 1961) might be multifactorial. In order to study age effects on latency of recovery and endplate reoccupation, motor nerve terminals (+/- a few preterminal internodes) were focally destroyed by intramuscular injection of black widow spider venom (BWSV) (Duchen et al., 1981). Soleus muscles of CBF1 mice, aged 5-7 mo. (young) or 25-28 mo. (old), were injected with sufficient BWSV to produce loss of transmission and synaptic destruction within 30 mins.

Two days after BWSV, electron micrographs showed post-synaptic folds with virtually no nerve terminals or with terminal debris. At 3 days, the indirect twitch first returned in 8 out of 9 old muscles but in none of 7 young muscles. One day later, both young and old treated muscles responded, and by 5 days, the ratio, indirect/direct twitch, approached control levels. Similarly, the low calcium/normal calcium twitch ratio recovered earlier in old muscles. In ZIO preparations, regenerated terminals were first found at 3 days in old and at 4 days in young muscle. Both ZIO preparations and whole mounts stained with fluorescent tetanus toxin c fragment and alpha-bungarotoxin (Robbins & Polak, 1988) showed considerably more overgrowth of regenerating terminals beyond the borders of the previous endplate (measured by terminal length or by protrusion beyond bungarotoxin sites) in many old junctions. Thus, nerve recovery from injury and endplate reoccupation do not account for slow functional recovery after denervation in old animals. The latency of recovery in old animals may reflect "conditioning" of neurons (McQuarrie, 1984) by pre-existent increased synaptic structural turnover or pre-existing excess of sprouting factors. The reduced recognition of the synaptic matrix may indicate a loss of synaptic adhesivity in aging neuromuscular junctions. (Support, NIH AG00795, AG06641, and M.D.A.)

70.10

PREFERENTIAL MOTOR REINNERVATION:A SEQUENTIAL DOUBLE LABELING STUDY. T.M.Brushart. Depts. of Orthopaedics & Neurology Johns Hopkins Hospital, & The Raymond M.Curtis Hand Center, Baltimore, Md. 21218.

Previous experiments have shown that when regenerating motor axons are given equal access to terminal sensory & motor branches of the rat femoral nerve, they will preferentially reinnervate the motor branch (J.Neurosci.8:1026)

Both femoral nerves of 30 3 wk.old female Sprague Dawley rats were severed proximally, where sensory & motor axons intermingle, and the stumps were separated by ½ mm. within silicone tubing. Regeneration was evaluated at 2 wks,3 wks, & 2 mos. HRP was applied to one terminal branch of each femoral nerve & Fluoro Gold was applied to the other branch. 48 hrs. were allowed for proximal transport of the tracers, after which the animals were sacrificed & the lumbar spinal cords processed for demonstration of HRP & Fluoro Gold.

The mean number of motor neurons projecting axons into motor & sensory branches was not significantly different at 2 wks (motor 117, sensory 110), but was significantly different (P=.001) at 3 wks (motor 206, sensory 101) & at 2 mos. (motor 219, sensory 78). A mean of 82 motor neurons was double labeled at 2 wks., while significantly fewer neurons were double labeled at 3 wks (47) & at 2 mos.(32).

Regenerating motor axons demonstrated no preference for motor or sensory Schwann cell tubes at early time periods. Neurotropic or contact guidance mechanisms could thus not have influenced their behavior. Subsequent generation of specificity could partially reflect pruning of motor axon collaterals in the sensory branch, though other mechanisms must also be responsible.

70.11

Quantitation of Sensibility in Full Thickness Skin Grafts To The Hand Dr. Julia Terzis & Dr. C. Scilley* E.V.M.S., M.R.C., Norfolk, VA. 23507

Full thickness glabrous and hairy skin grafts were exchanged between glabrous and hairy recipient sites in the upper and lower extremities of nine female Papio Anubis baboons. At the appropriate time intervals ranging from five months to one year, a detailed electrophysiological assessment was carried out of the mechanoreceptive fibers reinnervating the grafts. Subsequently full thickness biopsies of the functionally tested skin areas and adjacent normal skin were taken for study. Detailed light microscopic examination was carried out using Masson's trichrome, Munger's silver stain and Bodian silver stain. From the Masson's stained sections the architecture of the dermis and epidermis in grafted and normal tissue was examined, as was the appearance, location and number of Meissner's corpuscles. In the silver stained sections of glabrous tissue the innervation of the grafted and normal tissue was compared by recording the number of Meissner's corpuscles as well as the number of individual nerve fibers present in the papillary dermis per centimeter of specimen. In the hairy skin, the number of hair follicles present in the dermis as well as the number of nerve bundles per centimeter were compared in the grafted and normal tissue. All grafts maintained their basic architecture independent of their recipient site. No Meissner's corpuscles survived any of the tissue transfers. All grafts showed a decrease in the density of innervation. The innervation was best maintained in the transfer of full thickness glabrous skin grafts to the hand. The morphological parameters will be correlated with the electrophysiological data. This study attempts to provide for the first time a rational basis for the type of sensibility we obtain in full thickness skin grafts by considering the quality of reinnervation at the level of individual nerve fibers.

AMINO ACIDS: GABA AND BENZODIAZEPINES I

71.1

REGIONAL DISTRIBUTION OF BENZODIAZEPINE/GABA RECEPTOR mRNA (α SUBUNIT) IN RAT BRAIN. P. Montpied, B.M. Martin, S. Cottingham, B.K. Stubblefield, E.I. Ginns, S.M. Paul SPON: J. Kelsoe. Sections on Molecular Pharmacology and Neurogenetics, NSB, NIMH, Bethesda, MD 20892.

Oligodeoxynucleotides derived from the reported nucleotide sequence of the bovine brain $GABA_A$ receptor α subunit were used to isolate cDNA clones from a human brain cortex cDNA library constructed in gt11 (Martin et al., in preparation). Using a human 5' cDNA clone (900 bp), whose deduced amino acid sequence is identical to that of the previously reported bovine cDNA (nucleotide sequence is 95% homologous to the 5' bovine cDNA), we have studied the regional distribution of rat brain α subunit $GABA_A$ mRNA's. Total and $polyA^+$ RNA was purified from different brain regions and RNA levels quantitated by Northern analysis using a ^{32}P-labelled cRNA probe. At least 3 species of mRNA (4.8, 4.4, and 3 kb) were observed. The level of $GABA_A$ α subunit mRNA was highest in the cerebellum > thalamus > frontal cortex > hippocampus > parietal cortex > hypothalamus > striatum > medulla/pons. The 3kb mRNA species was present predominantly in the cerebellum and hippocampus and was not detected in several brain regions. In situ hybridization using the same antisense probe revealed a similar regional distribution of mRNA. Studies on the effect(s) of pharmacological agents and environmental stimuli on the level $GABA_A$ receptor mRNA in discrete brain regions are in progress.

71.2

$GABA_A$ RECEPTOR: SITES OF SYNTHESIS MAPPED IN RAT BRAIN BY IN SITU HYBRIDIZATION. H. Mohler, J.M. Séquier, P. Malherbe and J.G. Richards. Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basle, Switzerland

Neurons which express the $GABA_A$ receptor are of particular interest as recipient neurons for GABAergic inhibition and as target cells for drugs acting at the benzodiazepine receptor. These neurons have now been mapped by determining the distribution of RNA homologous to cDNA clones encoding the α- and β-subunit of the rat brain $GABA_A$ receptor. The receptor proteins were mapped in adjacent sections autoradiographically and immunohistochemically. Many brain areas containing high densities of $GABA_A$ receptors showed strong hybridization signals with both the α- and β-subunit antisense RNA probes e.g. cerebral cortex. On a cellular level, a dense dendritic localization of $GABA_A$ receptors was correlated with a strong hybridization in the corresponding somata e.g. in mitral cells of the olfactory bulb, pyramidal cells of hippocampus, granule cells of the dentate gyrus as well as Purkinje and granule cells of the cerebellum.

In some brain areas, the intensity of the β-subunit probe was much weaker than that of the α-probe e.g. substantia nigra, while the inverse ratio of hybridization intensity was found e.g. in bed nucleus. This regional heterogeneity in the hybridization pattern may reflect regional differences in RNA stability, transcription rate or subunit composition. The results open the way for studies on the regulation of $GABA_A$ receptor gene expression in normal and pathological brain in situ.

71.3

NEURONAL LOCATION OF ^{3}H-PK11195 AND ^{3}H-RO 5-4864 BINDING SITES LINKED TO $GABA_A$ RECEPTOR.

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^{3}H-PK 11195 and ^{3}H-Ro 5-4864 binding was studied in synaptosomes of rat brain and in primary culture of neonatal rat cerebellar neurons and glial cells. In purified synaptosomes, the density of PK-11195 binding sites is 0.1 pmol/mg prot (5 times lower than that found in mitochondria) PK-11195 or Ro 5-4864 binding in synaptosomes is noncompetetively displaced by picrotoxinin, TBPS, bicuculline, but not protoporphyrin. In contrast, in mitochondria, PK-11195 binding is displaced competetively by protoporphyrin, but not by picrotoxinin, TBPS or bicuculline. In neurons, ^{3}H-PK-11195 binds with high affinity to a small number of binding sites (K_d ~ 10 uM, Bmax 300 fmol/mg prot). These binding sites are associated with GABA binding sites in the same subcellular fraction. In glial cells, PK-11195 or Ro 5-4864 binds with high affinity to mitochondria (Bmax 15-20 pmol/mg prot). The binding of ^{3}H-PK-11195 to neurons and glial in primary culture is displaced by DBI (1-86) and one of its natural processing products, TTN (DBI 17-50) with an affinity of 10 µM. TTN also enhances the TBPS-induced inhibition of ^{3}H-flunitrazepam binding in a manner resembling Ro 5-4864.

71.4

3D RECONSTRUCTION OF FLUNITRAZEPAM BINDING ON C6 GLIOMA

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Previous work has shown selective binding of peripheral benzodiazepine receptor ligands to glial tumors. We sought to determine the heterogeneity of binding sites within the tumor and to acquire spatial statistics on their volumes.

Rats were implanted with C6 glioma cells ($5x10^5$) into the parenchyma of the left cerebral hemisphere. [^{3}H] Flunitrazepam (250µCi/rat) was administered i.v. Animals were decapitated 5 min. after radioligand injection, brains were removed and frozen. Twenty micron coronal sections were then processed for autoradiography.

Quantitative autoradiographic and histologic data (100 coronal sections) were digitized and aligned. The geometry was reconstructed by assigning z-values to each coronal section based upon its rostral-caudal position. The cortical surface of the brain was modelled and rendered to provide visual frame of reference. Tumor reconstructions were performed in one of two ways. In the first, outlines were drawn using a digitizing tablet. In the second, the tumor was circumscribed automatically by generating isodensity contours. Statistics were computed for the volume and binding densities. Three dimensional models illustrating the reconstructions of the tumor within the brain were synthesized.

71.5

β-CARBOLINE INTERACTIONS AT THE BZ-GABA RECEPTOR CHLORIDE-IONOPHORE COMPLEX IN THE RAT CEREBRAL CORTEX.

E. Malatynska*[1], R. Knapp[2], M. Ikeda*[2], and H.I. Yamamura[2] (SPON: J. Coffman[1]). [1]Dept. of Psychiatry, Neuroscience Program, Ohio State Univ., Columbus, OH 43210-1228, and [2]Dept. of Pharmacol., Coll. of Med., Univ. of Arizona, Tucson, AZ 85724.

We measured the effects of the benzodiazepine (BZ)-related β-carbolines ZK 93423, ZK 91296, ZK 93426, and DMCM on GABA-stimulated Cl^- conductance into rat cortical vesicles. The addition of 1.0 µM ZK 93423 or ZK 91296 reduced the GABA EC-50 1.8-fold and 1.3-fold, respectively, which is consistent with previous studies (Malatynska et al., Brain Res., 443: 395, 1988) showing that ZK 93423 is a full agonist at the BZ receptor while ZK 91296 is a partial agonist. DMCM inhibits Cl^- conductance in this assay and is thus an inverse-agonist (Malatynska et al., ibid). ZK 93426 (0.01-1.0 µM) antagonizes both ZK 93423 and ZK 91296 in a concentration-dependent manner with full reversal occurring at 1.0 µM for both drugs. However, there is an inhibition of Cl^- conductance at higher ZK 93426 concentrations that is not observed with ZK 93426 in the absence of the agonists. ZK 93426 also antagonizes DMCM but in an incomplete and multiphasic manner. These studies suggest that ZK 93426 may antagonize the effects of the other β-carbolines through a non-competitive mechanism.

71.6

ACUTE BENZODIAZEPINE ADMINISTRATION ALTERS $GABA_A$ RECEPTOR GENE EXPRESSION.

L.G. Miller, W.R. Smith* and W.C. Claycomb*. Depts. of Medicine, Pharmacology, and Biochemistry, LSU Medical Ctr., New Orleans, LA 70112.

The recent cloning and sequencing of the $GABA_A$ receptor has enabled analysis of expression of its gene. Since the "central" benzodiazepine binding site is located on this receptor, we examined expression of alpha-subunit mRNA in cerebral cortex of mice treated with a single dose of lorazepam(10 mg/kg). Total RNA was isolated and transferred to nylon membranes. Hybridization was performed using a radiolabeled synthetic 33-base oligonucleotide complementary to the alpha subunit mRNA. Specific hybridization was observed at 4.3 and 4.8 kb, similar to that reported for alpha subunit mRNA. Autoradiographs were then quantitated by densitometry. In mice treated with vehicle alone, mRNA levels were similar to an untreated control group at 0, 2, 4 and 24 hours after injection(8 AM), but mRNA levels were increased after 8 hours. In lorazepam-treated mice, mRNA levels were similar to controls at 2 and 24 hours, but were increased at 4 and 8 hours. These results suggest that there is a diurnal variation in the expression of alpha subunit mRNA which corresponds to reported diurnal variations in benzodiazepine binding, and that acute lorazepam administration augments mRNA synthesis after 4 hours.

71.7

GABA DOWN-REGULATES GABA/BENZODIAZEPINE RECEPTORS IN CHICK CEREBRAL NEURONS.

M.H. Jalilian Tehrani, J.J. Hablitz and E.M. Barnes, Jr. Depts. of Biochem. and Neurology, Baylor Col. of Med., Houston, TX 77030.

Exposure of cultured cortical neurons (3 h after plating) to 100 µM GABA for 4 days resulted in a 50% reduction in the level of [^{3}H]flunitrazepam binding to intact cells. A 7-day exposure reduced [^{3}H]Flu binding by 70% compared to controls cultured without exogenous GABA. Scatchard analysis indicated that the reduction in [^{3}H]Flu binding was due to a decrease in receptor density rather than affinity. The chronic effect of GABA was dose-dependent with an IC_{50} value of 70 µM. The loss of receptors could be prevented by concomitant exposure of developing neurons to 1 µM R 5135. The association of this labile receptor pool with functional channels was demonstrated by GABA-gated ^{36}Cl flux measurements and patch-clamp recordings. A 7-day exposure to 100 µM GABA reduced the rate of GABA-gated ^{36}Cl uptake by washed cells to 25% of that for untreated controls. Whole-cell recordings of responses to 1-100 µM GABA revealed that currents were reduced by 60-70% in neurons treated for 7 days relative to controls. Prolonged agonist exposure led to a fading of the GABA response, but the rate of this rapid desensitization was not affected by chronic GABA exposure.

Supported by NIH grants DK 17436 and NS 11535.

71.8

CHARACTERIZATION OF SOLUBILIZED MITOCHONDRIAL BENZODIAZEPINE RECOGNITION SITE COMPLEXES.

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A protein of 17 kilodaltons associated with peripheral-type benzodiazepine binding sites has been purified in this laboratory. To investigate the probable association of this protein in a larger macromolecular complex adrenal mitochondrial membranes were photolabeled with [^{3}H]PK 14105, solubilized in 1% digitonin, and fractionated under nondenaturing conditions. Anion-exchange chromatography resolved two components each containing the 17 kilodalton photolabeled protein. Molecular exclusion chromatography of both of these species revealed that they also differed in size, one component being 75-100 kilodaltons and the other about 150-200 kilodaltons in the presence of 0.1% digitonin. Specific binding of [^{3}H]PK 11195 exhibited chromatographic profiles matching those obtained by [^{3}H]PK 14105-photolabeled adducts. These results demonstrate that at least two distinct molecular forms of peripheral-type benzodiazepine binding sites are generated following digitonin solubilization and could be indicative of heterogeneity in these receptor protein complexes. Additional studies are in progress to identify and characterize the proteins comprising these complexes.

71.9

IMMUNOAFFINITY PURIFICATION OF BENZODIAZEPINE RECEPTOR. J.-Y. Wu*, C.C. Liao, J.-Y. Liu, M.M. Fieo, and G.H. Yan. Dept. of Physiology, Hershey Medical Center, Penn State University, Hershey, PA 17033

Previously we reported an immunoaffinity procedure for the purification of L-glutamate decarboxylase and choline acetyltransferase [J. Neurochem., 48, S147 (1987)]. Similar approaches were used to purify both the native benzodiazepine receptor (BZR) and its subunits. Briefly, the specificity of monoclonal antibody was first established by ELISA, immunoblotting and immunoprecipitation tests. IgG was then isolated from the ascites and about 5 mg of the well characterized IgG was coupled to Sepharose 4B as immunoaffinity ligand. About 450 mg of Triton solubilized and partially purified native BZR was incubated with the immunoaffinity ligand at 4°C for 4 days. The entire mixture was packed to a column. The column was first washed with 50 mM Tris-citrate buffer, pH 7.2, followed by a specific elution using 0.1 M Na-citrate/citric acid, pH 3. The BZR thus obtained appeared to be homogenous since in SDS-PAGE only two protein bands corresponding to the α and β subunit of BZR were obtained. It is concluded that monoclonal antibody affinity chromatography provides an efficient method for the purification of neuroreceptors such as BZR. (Supported in part by PHS grants NS20978, NS20922 and EY05385.)

71.10

[^{3}H]MUSCIMOL AND [^{3}H]FLUNITRAZEPAM PHOTOAFFINITY LABEL THE SAME MOLECULAR WEIGHT BAND IN CODFISH BRAIN GABA/BZ RECEPTOR. L. Deng*, R.W. Olsen, and M. Nielsen† (SPON: J.P. Segundo). Dept. Pharmacology and Brain Research Institute, Univ. California, Los Angeles, CA 90024, and †Psychopharmacology Lab., Sct. Hans Hospital, Roskilde, Denmark.

Codfish brain GABA/benzodiazepine (BZ) receptor was characterized biochemically and pharmacologically by radioactive ligand binding assays, affinity chromatography, sodium dodecylsulfate polyacrylamide gel electrophoresis, and photoaffinity labeling. Scatchard plot analysis of [^{3}H]muscimol binding in extensively washed frozen membrane homogenates indicated one population of binding sites with K_D = 14 nM, Bmax = 2.7 pmol/mg protein. [^{3}H]Flunitrazepam (Flu) binding was exclusively central type (insensitive to Ro 5-4864), showed K_D = 4 nM and Bmax = 1.4 pmol/mg, and was enhanced by GABA (25 % at 1 μM), as in mammalian brain. Codfish brain lipid-enriched fractions significantly increased BZ binding.The receptor was purified 700 fold on a BZ affinity column, giving a single Coomassie blue stained band on SDS gels at 57-58 kD. This single band was specifically photoaffinity labeled by both [^{3}H]muscimol and [^{3}H]Flu, showing that codfish GABA/BZ receptor is a single subunit carrying both GABA and BZ binding sites.

Supported by US Army Research Office Contract DAAL03-87-K-0020.

71.11

IS THERE AN ADDITIONAL AGONIST SITE WITHIN THE $GABA_A$ RECEPTOR SUPRAMOLECULAR COMPLEX(GRSC): EVIDENCE USING THE NEW ANTIDEPRESSANT FENGABINE. K.G. Lloyd and F. Thuret*.Lab.Etud.Rech.Synthélabo(LERS), Bagneux,F92220.

Fengabine is a novel antidepressant with the pharmacological spectrum of a GABA agonist (Lloyd et al, J.P.E.T., 241:245, 1987). "In vitro" fengabine is inactive at $GABA_A$ receptors or on GABA metabolism; however it stimulates ^{3}H-diazepam binding and "in vivo" decreases cerebellar cGMP levels (Scatton et al, J.P.E.T., 241:251,1987). In order to elucidate its action within the GRSC we studied the effect of fengabine on ^{35}S-TBPS binding to the $GABA_A$ receptor modulated chloride ionophore. With the experimental conditions used (well-washed rat cerebral membranes, NaCl) GABA agonists enhance ^{35}S-TBPS binding. Fengabine and several of its metabolites increased ^{35}S-TBPS binding (Table) with fengabine being the most active. Under these conditions GABA enhanced ^{35}S-TBPS binding with an EC_{50} of 0.5μM and a maximal effect of 29%. Bicuculline (10^{-3}M) completely blocked the enhancement of ^{35}S-TBPS binding by fengabine and its metabolites. Flumazenil (10^{-6}M) did not alter the action of SL79.781, SL81.0013 or SL 81.0048 whereas it moderated the effect of fengabine.

These results demonstrate a $GABA_A$ agonist activity of fengabine and certain of its metabolites. The site of action appears to be close to, but not identical with, the $GABA_A$ recognition site as none of these compounds displace ^{3}H-GABA binding. Thus, it is possible that another site exists within the GRSC which permits a $GABA_A$ agonist effect, but has a different pharmacological profile.

Compound (n=3)	Fengabine	SL79.781	SL81.0013	SL81.0048
EC_{50}(μM)	3.3±0.8	3.5±0.6	1.4±0.2	2.9±0.2
Maximal eff. (%) Increase	47.3±3.8	26.3±3.2	23.7±1.2	39.3±7.5

71.12

OPPOSITE MODULATION OF [^{35}S]t-BUTYLBICYCLOPHOSPHOROTHIONATE BINDING BY DELTAMETHRIN AND RO5-4864 IN TROUT BRAIN MEMBRANES. A.J. Eshleman* and T.F. Murray (SPON: R.A. Dodson). Toxicology Program & Coll. of Pharmacy, Oregon State Univ., Corvallis, Or. 97330.

The GABA-gated Cl^- channels are the main inhibitory neurotransmitter-regulated system in the CNS of many vertebrates. [^{35}S]t-Butylbicyclophosphorothionate ([^{35}S]TBPS) is a useful probe to investigate direct and allosteric interactions of compounds with this channel. Rainbow trout (Salmo gairdneri) are exquisitely sensitive to the neurotoxic activity of pyrethroid insecticides, thus this species is a relevant model to explore the interactions of this class of pesticides with these proteins. Ro5-4864 (4'-chlorodiazepam) is a convulsant benzodiazepine that may share with pyrethroids a common site of action in mammalian brain.

Washed P_2 fractions were prepared from rainbow trout brain in a high salt buffer with 5 uM GABA present in the final incubation mixture. Equilibrium saturation assays of [^{35}S]TBPS binding indicated a density of 424±47 fmol/mg protein and a K_d of 115±22 nM. In competition experiments deltamethrin inhibited [^{35}S]TBPS binding by 95% with an IC_{50} of 660±170 nM. The peripheral-type benzodiazepine receptor (PTBR)ligand, Ro5-4864, for which there are no high affinity sites in this species, enhanced [^{35}S]TBPS binding by 80-90%, with an EC_{50} of 1 uM. The maximal enhancement was inversely dependent on [GABA]; with no exogenous GABA it was 130% while with 25 uM GABA it decreased to 37%. Thus both the PTBR ligand and pyrethroid interact with the oligomeric $GABA_A$ receptor, modulating [^{35}S]TBPS binding in opposite directions. (Supported by a grant from Oregon State University Marine / Freshwater Biomedical Center).

LEARNING AND MEMORY: PHYSIOLOGY I

72.1

A THEORY OF THE HIPPOCAMPUS BASED ON REINFORCED SYNAPTIC MODIFICATION IN CA1. W. B Levy. Dept. Neurosurgery, University of Virginia, Charlottesville, VA 22908.

Moore & Levy (Neurosci. Abstr. '85) show a reinforced version of associative synaptic modification in CA1. There the entorhinal cortex (EC) to CA1 input is shown to act as permissive stimulus for potentiation of an active CA3 to CA1 input. This interaction is unidirectional (CA3 activity is not permissive for modification of EC synapses). The temporal window of associative interaction is as in Levy & Steward (Neurosci. '83), i.e. ordered with the permissive input allowed to follow the other input by 10-20ms. Such observations are consistent with CA3-CA1 synaptic modification in which the strength of a synapse stays proportional to the expected value of its CA3 afferent axon activity given a threshold of EC induced postsynaptic activity is reached in a timely manner in the relevant postsynaptic cell. With a suitable transformation of CA3 activity a CA1 cell could then "compute" the probability of experiencing a threshold EC event in the next 10-20ms. One such transformation is the maximum entropy optimal multiplicative scheme implemented by adding logarithms. Unfortunately such predictions occur only slightly before the predicted EC induced CA1 activity. Zipser suggests a fixed time shifting process, tapped delay lines. Alternately positive feedback loop(s), e.g. CA3-CA3, EC-CA-EC, etc, can act as variable shifting processes. The advantage of feedback over tapped delay lines is that delay lines, while suitable for shifting events into the time window of associative modification, maintain the same delay when generating predictions so that predictions occur just 10-20ms before the predicted event. Feedback, however, can produce earlier predictions by causing successive activity patterns of CA3 to vary only a little from immediately preceding CA3 activity patterns.

Supported by NS15488 and NIMH RSDA MH00622.

72.2

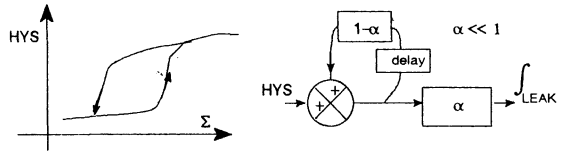
HAUSDORFF NUMBERS CALCULATED FROM POTENTIALS ON THE SURFACE OF THE RABBIT OLFACTORY BULB ARE SPATIALLY UNIFORM AND EVENT-RELATED. Skinner, JE, Martin*, JL, Landisman*, CE, Mommer*, MM, Fulton*, K, Mitra*, M, Mood*, T, Burton*, WD and Saltzberg*, B.(SPON: JE Szilagi). Neurophysiology Section, Neuroscience Program, Baylor College of Medicine, and Neuroscience Signal Analysis Laboratory, University of Texas Medical School, Houston, Texas 77030.

After familiarization with the recording chamber, each of 3 rabbits was presented with a novel odor while recording from an 8 x 8 electrode-array on the surface of the olfactory bulb (bandpass: 20-200 Hz, 3db). The Hausdorff number (HN) is a mathematical descriptor of the number of independent dimensions that appear to generate an observed dynamical process; it can be fractional. Pre-odor epochs (500 msecs each) had HNs that were the SAME, both spatially (mean-HN = 5.04 + 0.02) and temporally (mean HN = 5.04 + 0.02SD). After the odor, the HNs briefly INCREASED ($P<.01$) to new values (6.03 + 0.02), lasting for some seconds, before returning to control. Following only 2 trials of habituation, there was a DECREASE in the odor-evoked HNs. All HNs observed were fractional, with statistical significance. All electrodes showed the same HNs at all times. All HNs had clearly saturated correlation dimensions (15 embedding dimensions); convergence was clear with only 320 digitized data points in the epoch. These results suggest that the electric activities throughout the bulb may be characterized spatially by a single global chaotic attractor, the dimensionality of which is event-related.

72.3

HYSTERESIS, MEMORY & ATTENTION IN ELECTRONIC NEURAL NETWORKS. **J.D. Daniels, Laura Sewall* & C. Brennan***. Div. of Engineering. & Dept. of Psyc., Brown Univ., Prov., RI. 02912.

Connectionist neural network models can be faulted for low memory capacity. Two ways to add biologically plausible memory are with hysteresis non-linearities and with integrators.



In a one-layer neural network, simulated on a computer, we added sigmoid-shaped hysteresis functions connected to leaky integrators at the outputs of the network's Σ amplifiers.

When properly adjusted, the resulting system exhibits some features of attention: An output representing a particular input feature can lock in its response in spite of subtractive and additive noise; and reaction times to cued inputs are faster than un-cued.

If the hysteresis is too wide, modifiable connections (synapses) cannot change enough for the system to learn; if the integration is too slow with respect to input frequency, learning is also impaired.

72.4

A NEURAL NETWORK MODEL SHOWING THE ADAPTABILITY OF CONDITIONING. Alex X. D. Yang. (SPON: Charles J. Smith). Department of Physiology, School of Medicine, SUNY, Buffalo, NY 14214.

The adaptability of conditioning was studied with a neural network model. The network consists of neurons interconnected by Hebbian synapses, and works according to complementary principle, a way to store and organize associative memory into a network. Generalization, differentiation and extinction of conditioning were observed, suggesting the complementary principle a possible mechanism by which information is stored in neural tissue.

72.5

PRESENCE OF EYEBLINK CONDITIONING IN THE DECEREBRATE DECEREBELLATE RABBIT. T.M. Kelly, J.D. McAlduff* and J.R. Bloedel. Division of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85016.

Lesioning studies have suggested that specific regions of the cerebellar cortex and nuclei are essential for classical conditioning of the nictitating membrane response (NMR) and eyeblink reflex. Although the impairment of the conditioned response (CR) with the ablation of these areas has been widely attributed to the destruction of sites necessary for memory trace formation, data from other experiments imply that the motor aspects of this behavior may be more directly affected. We report here that lesions of the cerebellum including all of the cerebellar nuclei and the overlying cortex do not prevent conditioning of the eyeblink reflex in decerebrate rabbits.

Naive rabbits were decerebrated at a precollicular level, the cerebellar nuclei were aspirated bilaterally, and the remaining cortex ipsilateral to the stimulated eye was removed except for the tip of the hemisphere. A 4 kHz tone was used as the conditioned stimulus (CS) and either electric shock to the periorbital area or corneal air puff was used as the unconditioned stimulus (US). During training, the CS began 350 ms prior to the US and the two were coterminus.

Cerebellar lesions did not prevent conditioning. Rather, CR's were evoked 100-150 ms prior to the US during paired trials and persisted during unpaired trials until extinction was complete. When conditioning was repeated with an interstimulus interval of 500 ms, the same temporal relationship between CR and US was maintained. These results indicate that the cerebellum is not necessary for conditioning of the NMR and eyeblink response in the decerebrate rabbit. Supported by NIH grant# NS21958.

72.6

ADAPTIVE GAIN CONTROL OF THE VESTIBULO-OCULAR REFLEX IN GOLDFISH. I. HEMI-CEREBELLECTOMY. R. Baker, M. Weiser*, J.G. McElligott. Dept. Physiol. & Biophys., NYU Med. Ctr., New York, N.Y. 10016 and Dept. Pharmacol., Temple Univ. Sch. of Med., Philadelphia, PA 19140.

Cerebellectomy in either naive goldfish or those with prior VOR training immediately resulted in VOR gains larger than 1.0 (Michnovicz & Bennett, Exp. Brain Res. 66: 287-294, 1987). Those findings were extended by studying adaptive gain control up to several months following various cerebellar lesions. Sinusoidal vestibular and visual stimuli were employed (0.032-2.0 Hz) at 8-40°/sec. Horizontal eye movement gain (eye velocity/head velocity) was tested after training for 4-7 hours. Immediately following hemi-cerebellectomy the adaptive gain modification was partially retained for the eye on the intact side. Combined visual-vestibular interaction produced nearly symmetrical control of velocity in both eyes. One month later adaptive gain training produced nearly normal gain modifications for the eye on the intact side. Gain changes in the contralateral eye occurred to a lesser, more varied extent. In only one case was adaptive gain conjugate. We conclude that normally the cerebellum is a major, if not essential, pathway for adaptive gain control of the velocity of the ipsilateral eye. These data indicate that symmetrical eye velocities are attained through brainstem pathways which, as the next abstract (Weiser et al.) demonstrates, are also modifiable. Supported by EY02007 and NS13742.

72.7

ADAPTIVE GAIN CONTROL OF THE VESTIBULO-OCULAR REFLEX IN GOLDFISH. II. TOTAL CEREBELLECTOMY. M. Weiser*, J.G. McElligott, R. Baker. Dept. Pharmacol., Temple Univ. Sch. of Med., Philadelphia, Pa. 19140 and Dept. Physiol. & Biophys., NYU Med. Ctr., New York, N.Y. 10016

Our accompanying abstract demonstrated retention of adaptive gain control of the VOR following hemi-cerebellectomy. In this study we observed, in 7 of 9 goldfish, the presence of an adaptive gain modification of the VOR in the complete absence of the cerebellum. After acute cerebellectomy that excluded the valvula, VOR gain, typically at lower frequencies, was slightly higher than 1.0. Within the first post-operative month VOR gains stabilized at 1.0 independent of the extent of adaptive gain control that eventually returned. In all experiments, the response to optokinetic and vestibular stimuli alone as well as their interaction remained remarkably unaltered, except for: 1, a significant increase in eye velocity bias; 2, an increasing inability to suppress eye velocity; 3, an asymmetrical adaptive gain control of individual eye velocity and 4, a less robust amplitude of adaptive gain regulation. We conclude that a functional reorganization, unique for each animal, is present in the absence of the cerebellum allowing for continuous VOR recalibration. These observations indicate that neither the cerebellar cortex nor nuclei are necessary for producing the type of adaptive gain control which has been characterized as motor learning. Supported by EY02007 and NS13742.

72.8

CONDITIONING-INDUCED ACTIVATION OF PROTEIN KINASE C IN CEREBELLAR LOBULE HVI. B.Bank*, A.M.Scharenberg*, and D.L.Alkon (SPON: W. Yonekawa). Lab. Mol. Cell. Neurobiol., NIH-NINCDS, Park-5, Rm.435, Bethesda, MD 20892.

Based on previous work in our laboratory we were interested in determining whether PKC redistribution occurs in cerebellar lobule HVI as a result of NMR conditioning. This structure has been shown to be crucial for acquisition and retention of the NMR. PKC was quantified by [^{3}H] phorbol dibutyrate binding to cytosolic and membrane fractions from HVI in a triton X-100/phosphatidylserine mixed micellar assay. The right and left HVI from conditioned, pseudoconditioned (explicitly unpaired CS & US) and naive animals were compared 24h after conditioning. The right eye (and therefore right HVI) was conditioned so that the left HVI could serve as a within animal control. There was a significant redistribution of PKC from cytosol to membrane in HVI of conditioned and pseudoconditioned animals on the right side only. Further comparison of right HVI from conditioned and pseudoconditioned animals revealed a greater decrease in cytosolic PKC and a decrease in total PKC (membrane + cytosol) in the conditioned group. This indicated a greater degree of PKC activation in conditioned HVI resulting in limited proteolysis of PKC, a known consequence of extensive PKC activation. As predicted by the observed PKC redistribution in the right HVI of pseudoconditioned animals, there was a corresponding change in this groups' behavior in the form of increased NMR amplitudes in response to the US over three days of training. These findings suggest that long-term modification of PKC distribution may accompany acquisition and retention of classically conditioned reflexes as well as contribute to pseudoconditioning-induced modification of pre-existing reflexes.

72.9

CONDITIONING-SPECIFIC CHANGES IN HIPPOCAMPAL ^{3}H-PHORBOL-12, 13-DIBUTYRATE AS DEMONSTRATED BY QUANTITATIVE AUTORADIOGRAPHY. J. L. Olds, M. L. Anderson*, L. D. Staten*, D. L. Alkon
Laboratory of Mol. Cell. Neurobiol./NINCDS/NIH Bethesda Md. 20892.

The effect of classical conditioning on the hippocampal distribution of protein kinase C was measured as the changes in specific binding of 3H-PDBU using a modification of the autoradiographic method of Worley et al., (J. Neurosci, 1986). Rabbits were conditioned by paring a 400 msec 1 kHz tone (CS) with a coterminating 150 msec periorbital shock (UCS) for 3 days. Some controls received explicitly unpaired stimuli while others were unmanipulated. Mean bound ^{3}H-PDBU was determined for each animal by computerized averaging of activity along transept lines in autoradiograms of coronal sections of the dorsal hippocampal CA1 region with an MCID Imaging System (Imaging Research inc.). Conditioned animals had significantly higher binding (33 nCi/g±5 vs. 21 nCi/g±3) than pooled pseudoconditioned and naive rabbits (p<.01 Student t-test). These results confirm and extend the earlier findings of Bank et al. (PNAS 1988) and demonstrate that the quantitative autoradiographic method can be used to measure the distribution of learning-dependent changes in protein kinase C within the hippocampus.

72.10

INCREASED CALCIUM-DEPENDENT PHOSPHORYLATION IN RABBIT HIPPOCAMPUS AFTER NM/EYELID CONDITIONING. J.J. Lo Turco, B. Bank*, K.A. Tennes-Rees*, R.J. Delorenzo, and D.L. Alkon. NIH-NINCDS, Park-5, Rm 435, Bethesda, MD 20892. Dept. Neurol., Med. Col. VA, Richmond, VA 23298.

The effect of NM/eyelid conditioning on *in vitro* Ca^{2+}- dependent protein phosphorylation in rabbit hippocampus was studied. 24 h after animals were well trained, hippocampal slices were prepared, and the CA1 and dentate gyrus regions were dissected out. Phosphoproteins were labelled with [γ^{32}P] ATP in the presence of high [Ca^{2+}], and visualized by SDS PAGE. Samples from conditioned, pseudoconditioned and naive rabbits were compared. ^{32}P incorporation was quantified by optical density scanning. There was a 56% increase in the phosporylation of the 50 KD subunit of CaM kinase II (and a corresponsding increase in the 60 KD subunit) relative to pseudoconditioned and naive controls (conditioned: 266.0 ± 12.0, controls: 170.8 ± 11.4, t=5.5, p<.001). There was also a dramatic increase in the phosporylation of a 47 KD phosphoprotein in all conditioned animals. This band was barely detectable in controls. This protein may be F1 (B-50), a substrate for protein kinase C. This is consistent with previous work demonstrating long-term activation of protein kinase C in hippocampal CA1 cells after NM conditioning. Conditioning-specific changes in phosphorylation were not apparent in the dentate gyrus. These results, and those of Bank et al. (1988) indicate that the Ca^{2+}- dependent kinases, CaM kinase II and protein kinase C are persistently activated in rabbit hippocampal CA1 cells by classical conditioning.

72.11

SYNAPTIC PLASTICITY IN THE PARALLEL FIBER-PURKINJE CELL JUNCTION IN ISOLATED TURTLE CEREBELLUM C.Y. Chan, A. Lee* and S.M. Wong* Dept. of Physiology, CUNY Med. School, CCNY, New York, NY 10031.

Synaptic plasticity at the parallel fiber (PF)-Purkinje cell (PC) junction was studied using intracellular recording. PFs were stimulated, without conjoint climbing fiber activation, by identical pairs or trains of current pulses delivered through bipolar electrodes placed on the pial surface. Components were isolated from postsynaptic potentials: Pure EPSPs were selected from on-beam responses. Pure IPSPs were recorded off-beam and their reversal potentials checked. Typically, EPSPs were sensitized by double stimulations. We distinguished 2 types of IPSPs by amplitude and reversal potential. At present, we have tested the type that reversed around 70mV. Double stimulation caused IPSP sensitization that was interval-dependent, peaking around 150 ms; but at longer intervals, IPSP depression occurred, peaking around 300 ms. With a stimulus train, use-dependence was characterized by sensitization of the first few IPSPs and depression of later ones. IPSP depression was reduced by increased stimulus strength. These changes in synaptic potentials were unlikely to result from changes in PF spike amplitude, as our field potential recording showed that the amplitude of PF population spikes did not vary proportionately with postsynpatic potentials. These apparently homosynaptic plastic properties may play a role in nonassociative learning. (Supported by PSC/CUNY 667228 and PHS 2-S07-RR07132).

72.12

ELECTROMYOGRAPHIC RECORD OF EYE WITHDRAWAL CONDITIONING IN THE RESTRAINED OR FREELY MOVING EYE OF THE GREEN CRAB. R. D. Feinman, C. I. Abramson* and R. R. Forman. SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

Electromyograms (EMGs) were recorded from the main eye retractor muscle (19a) of the green crab, *Carcinus maenas*, during classical conditioning of eye retraction. In the conditioning procedure, a mild vibration on the carapace, which has no effect on naive animals, was used as a conditioned stimulus (CS). Pairing of the CS with a brief puff of air to the eye as an unconditioned stimulus (US) rapidly led to acquisition of eye retraction during CS presentation. The EMG record was consistent with reports in the literature. Air puff causes retraction of the eye into the carapace and this correlates with a rapid spiking pattern due to the main retractor neuron followed by tonic firing (as long as the eye remains retracted) due to a slow retractor. This reflex is known to be independent of proprioceptive feedback and the EMG pattern in response to air-puff is not changed if the eye is immobilized. We found that the acquired EMG response to the CS was qualitatively the same as the response to the US but was frequently more robust. Using the EMG record as an indicator, the time course of conditioning was found to be similar in restrained eyes and freely moving eyes. Animals that were conditioned with eyes immobilized showed normal patterns of behavior in extinction and re-acquisition when tested after the eyes were freed. The results indicate that the absence of proprioceptive feedback observed in the eye withdrawal reflex of the crab is preserved in classical conditioning. The results suggest that attenuation of the aversive stimulus is not a major factor in acquisition and limit the possible mechanisms of learning that can account for this type of conditioning. (Supported in part by funds from James C. Marlas and the Research Foundation of the State University of New York).

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS II

73.1

UNIFORMITY IN THE DISTRIBUTION OF THE MAJOR NEUROTRANSMITTER RECEPTORS TYPIFIES THE PRIMARY MOTOR CORTEX OF ADULT RHESUS MONKEY. M.S. Lidow, P. Rakic, D.W. Gallager, and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale University, School of Medicine, New Haven, CT 06510

Primary motor cortex (Brodmann's area 4) is cytoarchitectonically and functionally different from the rest of the neocortex. 2Here we show that this cortical area is also unusual in the distribution of neurotransmitter receptors. We used the *in vitro* autoradiographic techniques and quantitative densitometry to compare the distribution of norepinephrine: α-1, α-2, β-1 and β-2; acetylcholine: M-1 and M-2; serotonin: S-1 and S-2; dopamine: D-1 and D-2; GABA-A and benzodiazepine receptors in primary motor cortex with the distribution of these receptor subtypes in somato-sensory, prefrontal and occipital cortex of four adult rhesus monkeys. Our results show that in the primary motor cortex all neurotransmitter receptor subtypes have identical laminar distributions with the highest concentrations in layers I and II and the upper strata of layer III. The density of these receptors in the deep strata of layer III and in layers V and VI were significantly lower. The similar distribution of the different neurotransmitter receptors in primary motor cortex stands in contrast to the distribution of neurotransmitter receptors in all other cortical areas examined, where each receptor subtype had an individual and often very distinct pattern of localization. The high concentration of neurotransmitter receptors in the upper strata of primary motor cortex may be associated with the paucity of thalamic input and the virtual absence of granular cells in the middle and deep layers of this cortical area. Supported by U.S. Public Health Service Grants NS22807 and NS07224.

73.2

PHOTOAFFINITY LABELING OF BENZODIAZEPINE RECEPTORS IN SLIDE-MOUNTED SECTIONS AND β-ADRENERGIC RECEPTORS IN INTACT CELLS. *P. Ernsberger, V. Arango, L. Iacovitti, and D.J. Reis* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Photoaffinity labeling is often used to identify receptors in membrane fractions, but has never been applied to tissue sections or intact cultured cells. We sought to characterize photoaffinity labeling of (a) the GABA-benzodiazepine receptor complex in brain sections and (b) β-adrenergic receptors in cultured glial cells. Slide-mounted brain sections were incubated with 1 nM ^{3}H-flunitrazepam (^{3}H-FLU) in 170 mM Tris-HCl (pH 7.7) containing 10 μM GABA for 40 min at 4°C. Nonspecific binding was defined by 10 μM diazepam. Slides were irradiated with UV for 5 min, then either washed 2 min in buffer to remove nonspecific binding, or stripped of all but covalent binding by a 30 min wash in buffer containing 10 μM diazepam followed by dehydration in alcohol. In rat forebrain, total ^{3}H-FLU binding (2 min wash) was 77 ± 7 dpm/mm^2, of which 37 ± 3 dpm/mm^2 was nonspecific (N = 4 experiments, 6 sections each). Covalent ^{3}H-FLU binding was 31 ± 2 dpm/mm^2, of which 8 ± 1 dpm/mm^2 was nonspecific (N = 9). Sections not irradiated with UV exhibited virtually no covalent labeling (0.3 ± 0.03 dpm/mm^2). Slides were dipped in emulsion, exposed 4 wk, and developed for high-resolution autoradiographic mapping. Neonatal rat glia were cultured on chambered slides and incubated with 0.1 nM ^{125}I-iodocyanopindolol diazirine (^{125}I-ICYPD) in culture medium at 37°C. Nonspecific binding was defined by 10 μM (-)-propranolol. In the dark, binding was rapid ($t_{1/2}$ = 9 min) and reached plateau by 40 min. A 10 min wash at 4°C selectively reduced nonspecific binding. Glia were irradiated with UV for 4 min, fixed in paraformaldehyde, extensively washed, and stained with antibodies to GFAP, a specific glial marker. Covalent ^{125}I-ICYPD binding was 24 ± 2 dpm/mm^2, of which 7 ± 2 dpm/mm^2 represented nonspecific binding to the plastic slide and 7 ± 1 dpm/mm^2 was nonspecifically bound to the cells (N = 3). Autoradiographic grains were selectively localized over positively stained glia relative to adjacent background. We conclude that cellular localization of receptors is made possible by photoaffinity labeling of receptors in tissue sections and in intact cells.

73.3

QUANTITATIVE AUTORADIOGRAPHY DEMONSTRATES INCREASED TOTAL AND HIGH-AFFINITY β-ADRENERGIC RECEPTOR BINDING IN CEREBRAL CORTEX OF SUICIDE VICTIMS. *V. Arango, P. Ernsberger, P. Marzuk*, M. Stanley, D.J. Reis and J.J. Mann,* Div. of Neurobiology and Lab. of Psychopharmacology, Cornell Univ. Med. Coll., New York, NY 10021

We have reported an increase in 5-HT_2 receptors in the prefrontal cortex (PFC) of suicide victims by both membrane saturation assays and quantitative receptor autoradiography (Arango et al., *Soc Neurosci Abst* 13:216, '87) and an increase in β-adrenergic receptors in PFC membranes (Mann et al., *Arch Gen Psychiatry* 43:954-9, '86). Using quantitative autoradiography we sought to determine: a) the normal cortical distribution of β-adrenergic receptors; b) whether the increased receptor density observed in brains of suicide victims is localized to a specific cortical layer and region; and c) whether this increase involved high-affinity receptors coupled to adenylate cyclase. Suicide victims and controls were paired for assays by matching for postmortem delay, age and gender (PFC = 8 pairs; temporal cortex (TC) = 9 pairs). Toxicology was negative in all cases for centrally active drugs. Slide-mounted sections (15 μm) were incubated in Tris-sucrose (pH 7.4) containing 2 mM $MgCl_2$ and 60 pM ^{125}I-iodopindolol. Total specific and high-affinity sites were selectively blocked by incubations with 100 μM and 17 nM (-)-isoproterenol (ISO), respectively (Arango et al., *Fed Proc* 46:390, '87). Sections were exposed to Ultrofilm (40-60 h). Digitized images representing non-specific (with 100 μM ISO) or low-affinity plus non-specific binding (with 17 nM ISO) were subtracted from images representing total binding and calibrated against standards. In both groups β-adrenergic receptors were evenly distributed across cortical layers in PFC and TC. Total specific binding was increased 36 ± 6% ($p < 0.001$) in PFC and 16 ± 6% ($p < 0.05$) in TC of suicide victims *vs.* controls. High-affinity sites were increased 56 ± 20% ($p < 0.05$) in TC of suicide victims and 40 ± 24% in PFC ($p > 0.05$). We conclude that: a) β-adrenergic receptors are evenly distributed across cortical layers; b) there are more binding sites in TC than in PFC; c) β-adrenergic receptors are increased in PFC and TC of suicide victims; and d) at least in TC this increase involves functionally coupled sites. (Supported by PHS grant MH40210)

73.4

DUAL EFFECTS OF BM-5 IN NEUROPHARMACOLOGICAL INVESTIGATIONS. V.H.Sethy, T.P.Boyle*, J.W.Francis* and R.J.Collins*, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The effects of BM-5 vary from tissue to tissue due to differences in muscarinic receptor reserve (Ringdahl et al., JPET 242:464, 1987). We have further investigated BM-5 in neuropharmacological tests, and the results have been compared to those obtained with oxotremorine (OX) and atropine (AT). Both OX and BM-5 produced salivation and lacrimation, whereas only OX elicited tremors (ED50: 0.1 mg/kg) in mice. Atropine blocked muscarinic agonist effects of both OX and BM-5. Furthermore, BM-5 also blocked OX-induced tremors. The ratio of the Ki's of OX, BM-5, and AT in (3H)-QNB and (3H)-OX-M binding was 240, 29, and 0.63, respectively. OX or AT dose-dependently increased or decreased acetylcholine (Ach) concentration in the striatum, hippocampus, and cerebral cortex of rats, respectively. BM-5 significantly ($p<0.01$) reduced striatal Ach content, and this effect was dose-independent. The hippocampal and cortical Ach content was not altered by BM-5. Like OX, BM-5 significantly ($p<0.01$) decreased (3H)-Ach release from hippocampus slices in the presence of HC-3. However, like AT, BM-5 significantly ($p<0.01$) increased release of (3H)-Ach in the presence of eserine. Both AT and BM-5 blocked OX-M-induced phosphoinositide (PI) hydrolysis in the cerebral cortex. However, BM-5, like OX, stimulated PI hydrolysis in the parotid gland. Thus, the in vitro and in vivo studies indicate that BM-5 is a partial agonist of muscarinic receptors and, as a result, has the ability to elicit both agonist and antagonist effects in central and peripheral tissues.

73.5

CHARACTERIZATION OF [^{3}H]ALPRAZOLAM BINDING TO CENTRAL BENZODIAZEPINE RECEPTORS IN THE RAT BRAIN. J.P. Yezuita*1, R. T. McCabe[1], D.R. Mahan*2, R.B. Smith*2, and J.K. Wamsley[1]. (SPON: B. Grosser). [1]Dept of Psychiatry, U of UT, Sch of Med, SLC, UT 84132, and [2]Upjohn Co., Kalamazoo, MI 49001.

The triazolobenzodiazepine alprazolam (ALP) is a selective central benzodiazepine (BZ) receptor agonist and used for treatment of panic disorder and anxiety associated with depression. The availability of [^{3}H]ALP has allowed the analysis of binding characteristics to membranes and tissue sections of rat brain. The optimal binding conditions for defining BZ receptors with [^{3}H]ALP were obtained from association, saturation, and displacement studies. Tissues were incubated in 50 mM Tris-HCl (4°C) with [^{3}H]-ALP and equilibrium was reached after 45 min. Saturation analysis of [^{3}H]ALP revealed a K_D of approx. 3 nM. Displacement of [^{3}H]ALP was examined by including related and unrelated ligands. The order of potency for competitive inhibition from BZ sites is triazolam>clonazepam>alprazolam>Ro15 1788>BCCE>quazepam. Both GABA and pentobarbital enhanced [^{3}H]ALP binding. Autoradiographic localization of [^{3}H]ALP binding sites showed a distribution consistent with both BZ receptor subpopulations. These results demonstrate that [^{3}H]ALP binding is saturable, specific, and of high affinity and support previous reports that [^{3}H]ALP has properties related to other central BZ agonists.

73.6

Characterization and Localization of Transferrin Receptors in the Rat Brain. J. Pablo*, S. M. Efange*, D.D. Flynn, W. J. Weiner, and D. C. Mash. Departments of Neurology and Pharmacology, University of Miami School of Medicine, Miami, Fl, 33101.

Transferrin, an iron binding transport protein, and its receptor may regulate the intracellular delivery and subsequent sequestration of iron in the central nervous system. Recent studies have demonstrated that iron uptake by neuron and glial cultures is transferrin-mediated (Swaiman and Machen, Neurochem. Res. 9:1241-1248,1986). Using [^{125}I]-ferrotransferrin, we have characterized the binding site for transferrin on membrane fractions prepared from the rodent forebrain. The distribution of transferrin receptors in the rat brain was investigated by in vitro autoradiography. For binding assays, forebrain samples were homogenized with a Brinkmann Polytron in 10 mM phosphate buffer (pH 7.4) containing 150 mM NaCl. The homogenate was centrifuged at 48,000 g for 10 minutes with an intermediate wash in fresh buffer. Membranes were incubated in phosphate buffer containing bacitracin (80 ug/ml), PMSF (0.5 mM), 0.2% bovine serum albumin and increasing concentrations (0.1 - 50 nM) ^{125}I-labelled and unlabelled transferrin. With 20 - 30 mg of membranes, the amount of specific transferrin binding was 70%. Saturation binding analysis revealed a apparent single class of sites with a dissociation constant of 1.8 nM and a binding site density of 15 pmol/g wet weight original tissue. Estimates of the kinetically determined Kd for forebrain membranes were within the 2 - 5 nM range, in agreement with the equilibrium measurements. Apotransferrin and ferrotransferrin competitively displaced the binding of [^{125}I]-transferrin, while ferritin, cytochrome-C and ceruloplasmin failed to compete for the binding site. Autoradiographic localization studies demonstrate that the distribution of transferrin receptors in the CNS is heterogeneous. The distribution of [^{125}I]-transferrin binding sites is more widespread than the regional content of iron determined with iron histochemistry.

Supported by the National Parkinson Foundation

73.7

AUTORADIOGRAPHIC VISUALIZATION OF GLUTAMATERGIC NERVE TERMINALS IN RAT BRAIN UTILIZING ATP-DEPENDENT VESICULAR UPTAKE. P. E. Kish* and T. Ueda (SPON: M. Gnegy) Mental Health Research Institute, University of Michigan, Ann Arbor MI 48109.

ATP-dependent glutamate uptake has previously been demonstrated in purified vesicle preparations. Because of the unique properties of the vesicular uptake system, it has been proposed that this plays a crucial role in selecting glutamate for neurotransmission. In this study we have utilized the specificity of of the vesicular uptake system for glutamate to visualize brain areas having glutamatergic presynaptic terminals. Frozen sections of brain were incubated in a mixture containing ATP, ^{3}H-glutamate, the membrane permeabilizing agent digitonin, and high concentrations of aspartate. Aspartate is a competitive inhibitor for glutamate at the high-affinity plasma reuptake system and the postsynaptic receptor, effectively blocking non-vesicular glutamate uptake and binding. Special washing and fixation techniques are required to immobilize the ATP-dependent accumulation of glutamate. We demonstrate that the properties of the glutamate uptake system in frozen sections are highly similar to those observed in the intact synaptic vesicles, namely ATP-dependency, stimulation by chloride, sensitivity to electrochemical proton gradient dissipaters, and specificity for L-glutamate. Extensive areas of the brain are believed to utilize glutamate as a neurotransmitter. Autoradiography of the ATP-dependent glutamate uptake shows regions within the cortex, striatum, thalamus, and layers of the hippocampus and cerebellum with high levels of uptake. This represents the first autoradiographic demonstration of glutamatergic areas in the brain utilizing a purely presynaptic process for detection. (Supported by NSF Grant BNS 8509679.)

74.1

FUNCTIONAL ORGANIZATION OF THE UTRICULAR MACULA IN THE CHINCHILLA. J. M. Goldberg, G. Desmadryl*, R. A. Baird and C. Fernández*. Neurobiology Laboratories, University of Chicago, Chicago, IL, USA.

Three kinds of endings are labeled in the utricular macula after extracellular injection of horseradish peroxidase (HRP) into the vestibular nerve. Calyx units supply one or a few type I hair cells in the striola. Bouton units contact several type II receptors in the extrastriola. Dimorphic units supply all parts of the crista and innervate both types of sensory cells. More than 90% of the afferents are dimorphic units, less than 10% are calyx units, and less than 1% are bouton units. Intra-axonal HRP techniques were used to label 52 afferents that had been characterized in terms of their excitatory tilt directions, discharge regularity, and responses to externally applied galvanic currents and to sinusoidal linear head accelerations. The excitatory tilt directions of labeled afferents were consistent with the presumed morphological polarization of the hair cells they innervate. Calyx and striolar dimorphic units are irregularly discharging, phasic afferents. Extrastriolar dimorphic units are regularly discharging and tonic. Galvanic sensitivity (ß) was related to discharge regularity and not to terminal morphology as such. Acceleration gains in irregular units show a 10-20x increase as one proceeds from dc to 2Hz, whereas those of regular units are more nearly constant. Because of these differences in response dynamics, it is best to compare units in terms of their dc gains. Calyx units have dc gains (g_A) that are 3x smaller and galvanic sensitivities (ß) that are 3x larger than the corresponding values for dimorphic units. We can estimate the dc synaptic input (g_S) by the formula, $g_S = g_A/ß$. The values of g_S are 10x lower in calyx, as compared to dimorphic, units. The relatively low g_S of calyx units may reflect the small number of hair cells they contact and the low gain of calyx synapses. The latter may be related to the small number of release sites observed in type I hair cells.

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74.2

PHYSIOLOGICAL IDENTIFICATION OF CALYX, DIMORPHIC AND BOUTON AFFERENTS IN THE VESTIBULAR NERVE OF THE SQUIRREL MONKEY. A. Lysakowski, L. B. Minor, C. Fernández* and J.M. Goldberg. Neurobiology Laboratories, The University of Chicago, Chicago, IL, 60637.

Intra-axonal HRP techniques have been used in the chinchilla to relate the peripheral innervation of semicircular-canal afferents with their discharge properties (Soc. Neurosci. Abstr. 12:773). Units can be divided into two classes on the basis of their discharge regularity and rotational gains. The first class, consisting of dimorphic (D) and bouton (B) units, include low-gain, regular and high-gain, irregular units. The second class are calyx (C) units; they are irregular and have low rotational gains. Only a few B fibers were labeled. The present study had two aims: 1) to determine if the same groups exist in the squirrel monkey; 2) to use conduction velocities to identify a group of thin, presumably B fibers. Rather than label axons, we relied on the physiological properties of the afferents. Antidromic conduction velocities were obtained by placing stimulating electrodes in the central part of the vestibular nerve. Discharge regularity was expressed as a normalized coefficient of variation (cv*). Rotational gains and phases (response dynamics) were measured to 2 Hz, sinusoidal head rotations.

In the monkey, as in the chinchilla, there are two groups of afferents. For the first group, there is a positive, nearly linear relation between cv* and rotational gain. The second group consists of irregular units with low rotational gains. Conduction velocities are slightly higher in low-gain (C), than in high-gain (D) irregular units. Presumptive B units were identified by their low conduction velocities; they were regular units whose discharge properties were indistinguishable from those of other regularly discharging, presumably D units. Sensitivity to perilymphatic galvanic currents and rotational response dynamics were related to cv* and not to the afferent type (C, D or B) as such.

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74.3

THE RELATIONSHIP BETWEEN DISCHARGE REGULARITY AND FREQUENCY OF PRIMARY AFFERENTS OF THE POSTERIOR SEMICIRCULAR CANAL OF THE TOADFISH, OPSANUS TAU. T.Tricas and S.M.Highstein. Depts. of Otolaryngology, Anatomy and Neurobiology, Washington University School of Medicine, St. Louis MO 63110.

Caloric induced modulation of the resting discharge rate of primary afferents of the posterior semicircular canal of the toadfish, Opsanus tau was used to study the relationship of discharge regularity (measured as the coefficient of variation = standard deviation of the interspike interval/mean interval, CV=SD-ISI/mean ISI) to the frequency of discharge. Mean, resting rate for 98 fibers in 6 fish was 36.4 spikes/sec ± 25.2; range = 2 - 98). Caloric stimulation significantly changed the resting discharge rate in 75% of fibers tested; firing rate increased during application of heat and decreased when cooled. Ninety-one % of responsive fibers showed a positive and significant curvilinear relationship between mean ISI and CV. Fibers with high rates of resting discharge tended to have better loglinear changes in CV with firing rate than those with lowers resting rates. Normalization of data should lead to the formulation of a parameter called CV*, unique to each fiber, and independent of resting discharge frequency. We will then investigate the relationship of CV* to the response dynamics of individual primary afferents.

74.4

SEMICIRCULAR CANAL AFFERENT RESPONSE DYNAMICS TO ROTATIONAL AND MECHANICAL STIMULATION. J.D. Dickman[1] and M.J. Correia[2]. Depts. of Otolaryngology[12]. and Physiology & Biophysics[2], Univ. of Texas Medical Branch, Galveston, Texas, 77550.

Recently, we developed a method for discrete and reproducible mechanical stimulation of the semicircular canals that allows the animal to remain stationary (Dickman, Reder, & Correia, J. Neuroscience Meth., 1988, in press). The mechanical technique provides a broad frequency range of stimulation and was shown to elicit responses that are directly comparable to those produced by natural rotational stimulation. In the present study, we recorded extracellular single fiber responses from horizontal semicircular canal afferents (HCA) in the pigeon (Columba livia) elicited by both rotational and mechanical stimulation. Sinusoidal frequencies ranged from 0.01 to 15 Hz. Gain and phase values were determined for each response using a fitted sine wave to the binned average of several stimulus cycles. To date, complete protocols have been recorded in 63 HCA units to mechanical stimulation, with complete rotational data also being obtained in 11 of these fibers. For mechanical stimulation, intensity functions of displacement magnitude indicated that for most HCA fibers, a linear range of response occurred between ±0.5 and ±5 μ. Stimulus displacements from ±5.0 to ±10 μ typically elicited responses that showed evidence of rectification and saturation. Bode diagrams of HCA response dynamics indicated that the S-shaped gain and U-shaped phase plots for mechanical and rotational stimulation were analogous when plotted re: mechanical displacement position and re: rotational velocity, respectively. In addition, HCA fiber responses produced by rotational stimulation with a canal fistula introduced (exposed canal duct for concurrent mechanical stimulation) were similar to those reported by Anastasio et al. (J. Neurophys., 1985, 54, 335-347). For example, with the present results, at 0.1 Hz (10 deg/sec max velocity) HCA irregularly firing (CV > 0.2) fibers (N=4) had a mean gain (±SEM) of 1.43 (0.39) $s \cdot s^{-1}/d \cdot s^{-1}$ and a mean phase lead of 31.8 (11.5) degrees. Similarly, at 10 Hz, HCA fibers had a mean gain of 4.17 (0.94) $s \cdot s^{-1}/d \cdot s^{-1}$ and a mean phase lead of 41.6 (7.5) degrees.

Supported in part by NASA grant NAGW-70 and NSF grant BNS 8709303.

74.5

CHANGES IN VISUAL AND VESTIBULAR FUNCTION AFTER CANAL PLUGGING IN THE MONKEY. B.Cohen, D.Helwig,* T.Raphan, J.I.Suzuki,* K.Kaga* and A.Eden. Depts. of Neurology, Physiology, Otolaryngology and Computer Science, Mt. Sinai School of Medicine, New York 10029, Brooklyn College and Teikyo University, Tokyo, Japan.

The semicircular canals were plugged bilaterally in rhesus and cynomolgus monkeys to study effects on OKN, OKAN, the VOR and nystagmus induced by off-vertical axis rotation (OVAR). The lateral canal nerves were also cut bilaterally in several animals. Lesions were verified histologically. The initial jump of horizontal OKN and steady state OKN velocities were enhanced after lateral canal plugging, and the time constant of OKAN became shorter. Thus, direct visual pathways had a higher gain after canal lesions, although velocity storage had habituated. Horizontal but not vertical OKAN was abolished by lateral canal nerve section, but a horizontal component of vestibular nystagmus or OKAN reappeared as animals were tilted upward re the axis of rotation, as previously found by Boehmer and Henn (1985). This demonstrates that the vertical canals can elicit a horizontal component of eye movement and velocity storage, dependent on head position re gravity, even after all input from the lateral canals is lost. Nystagmus was induced by OVAR after all six canals were plugged, as noted by Correia and Money, (1970). The gain decreased by about half, the position dependent velocity modulations increased and the time constant of the velocity storage integrator was shortened. Results were similar after the lateral canals were plugged. There was no effect of anterior and posterior canal plugging on OVAR. This suggests that the lateral canals play the major role in determining the dynamics and steady state properties of the horizontal component of slow phase velocity generated by the velocity integrator. Supported by NS00294, EY04148, EY01867 and a grant from the Young Men's Philanthropic League.

74.6

SIGNAL PROCESSING IN THE VESTIBULAR NUCLEI DURING OFF-VERTICAL AXIS ROTATION (OVAR). H. Reisine, T. Raphan, B. Cohen, E. Katz* Depts. of Neurology, Physiology, Mt. Sinai School of Medicine, NY, NY, 10029 and Dept. of CIS, Brooklyn College, Brooklyn, 11210.

Extracellular unit activity was recorded in the vestibular nuclei to determine the role of different classes of units in generating compensatory eye velocity in three dimensions during OVAR. Units related to lateral semicircular canal activation had firing rates proportional to the bias level of the horizontal component of eye velocity during constant rotations (0 to 120 deg/sec) and showed evidence of being related to velocity storage (Raphan et al, 1979). One subclass of these neurons showed little oscillation about the bias level. Another subclass, that paused during saccades, showed a marked oscillation whose phase was time-locked to head position. These neurons were also activated during horizontal OKN and OKAN. Units activated by the vertical semicircular canals also showed evidence of processing a velocity storage component of eye velocity during OVAR. Firing rates were proportional to the bias level of horizontal eye velocity. Although the activity of these neurons was not generally related to static head orientation, it was strongly modulated during OVAR. The amplitude of the modulation increased with head velocity. Otolith related neurons showed no evidence of velocity storage and had activity modulated in accordance with head position during OVAR. Lateral and vertical canal related neurons in the vestibular nuclei appear to encode the structure of velocity storage. The oscillations in the activity of vertical canal related neurons are probably due to oscillations in the three dimensional velocity estimator (Schnabolk & Raphan, 1988) and to cross coupling within the three dimensional velocity storage integrator (Raphan & Cohen, 1988; Sturm & Raphan, 1988). The otolith related neurons probably also contribute to the eye position changes that occur during OVAR. Thus, neuronal classes in the vestibular nuclei contain the requisite signals to generate eye velocity during OVAR. Supported by EY04148, NS00294, EY01867, NAG 2-336.

74.7

THE SLOW COMPONENT OF THE VOR (VELOCITY STORAGE) DEPENDS ON COMMISSURAL CONNECTIONS CAUDAL TO THE ABDUCENS NUCLEUS. E.Katz*, J.M.B.V.deJong*, B.Cohen, J.Buettner* (SPON: H. Krieger). Depts. of Neurology & Physiology, Mt. Sinai School of Medicine, City University of New York 10029 & Universities of Amsterdam & Munich

Midline section in the medulla shortens the time constant of the VOR (Blair and Gavin, 1981) and abolishes OKAN (de Jong et al. 1980). In this study we cut commissural connections in the midline of the medulla in cynomolgus monkeys to determine effects on gains of nystagmus induced by rotation of the animal about a vertical axis in darkness, on vertical and horizontal OKN and OKAN, on the nystagmus induced by off-vertical axis rotation (OVAR) and on cross coupling of horizontal to vertical or roll OKAN (Raphan and Cohen, 1987). Lesions extended from 1 to 4 mm below the surface from the posterior border of the abducens nuclei to the obex. After operation quick phases were of normal peak velocity. Although the eyes drifted toward the midposition from lateral positions in darkness, they were held stable in light. The gains of the initial jump in slow phase velocity at the onset of rotation and of OKN were unaffected. However, the time constant of the response to constant velocity rotation fell to approximately 5 sec, the time constant of activity elicited in the semicircular canals, and the slow rise in slow phase velocity during OKN and horizontal and vertical OKAN were abolished. Despite modulations in eye position during OVAR there was no steady state velocity, and cross coupling of OKAN was lost. Each of the lost functions depends on the integrity of the velocity storage mechanism in the vestibular system. The data are compatible with the hypothesis that velocity storage depends on bilateral communication of the vestibular nuclei and/or prepositus complex through the commissural connections caudal to the abducens nuclei. Supported by NS00294 and EY01867.

74.8

MODELLING THREE DIMENSIONAL VELOCITY ESTIMATION DURING OFF-VERTICAL AXIS ROTATION (OVAR). C. Schnabolk and T. Raphan. Dept. of CIS, Brooklyn College, Brooklyn, New York, 11210.

Studies of nystagmus during OVAR suggest that gravity induced moving patterns of otolith activation are responsible for the bias component of slow phase eye velocity (Raphan & Schnabolk, 1988). The purpose of this study was to analyze the characteristics of an extension of the estimator to three dimensions. It was assumed that the estimation of head velocity was done in semicircular canal coordinates. When the head velocity was parallel to a canal axis an accurate estimate of the component of the velocity along the axis was obtained. However, while the other components of head velocity were zero, the estmated values were nonzero. Their instantaneous values oscillated and at times were 60% of the magnitude of the head velocity. Their relatve phase was 90 deg, so that the magnitude of the three-dimensional estimate was approximately constant. Each of these estimates approached zero as the angle between the head velocity and gravity increased toward 90 deg. The improvement was attributed to the standing wave form approached by the associated otolith activation pattern. In other simulations the component of the estimate of head velocity along a canal axis improved as the angle between the head velocity and the axis went to zero. In summary, this study indicates that the three dimensional estimator essentially estimates the component of head velocity perpendicular to gravity. This estimate is then normalized with regard to body orientation. Thus, the internal coordinate basis for normalization is important for determining the planes of optimal head velocity estimation during OVAR. Supported by EY04148, NS00294, EY01867, and PSC-CUNY 668285.

74.9

NEURAL NETWORK MODELLING OF VELOCITY GENERATION DURING OFF-VERTICAL AXIS ROTATION (OVAR). R. Fanelli*[1], C. Schnabolk[2], and T. Raphan[2]. Depts. of Physics [1] and Computer and Information Science[2], Brooklyn College of CUNY, Brooklyn, New York, 11210.

Modelling of slow phase velocity during OVAR has suggested that continuous compensation during OVAR arises from phase detection of otolith activation patterns (Raphan & Schnabolk, 1988). However, it is not clear how these units should be connected to obtain robust estimation over a wide range of tilt angles. The purpose of this study was to construct a neural network model to address this problem and predict the kinds of unit activity that would be necessary to implement such an estimator. A feed-forward network of 25 units trained with back propagation was used. The input units were assumed to be otolith cells with different polarization vectors and cells with delayed versions of the signals (Raphan & Schnabolk, 1988). There were two hidden units associated with each cell and its nearest neighbor. There was also one output cell coding the estimated head velocity. The network was trained with varying velocities and tilt angles over a range of ±50 deg/sec. Within the training range, the neural network model produced velocity estimates that are within 2.8 deg/sec of the input velocity. Outside the training range, velocity estimates declined consistent with the decline in steady state velocity vs. stimulus velocity in monkey (Raphan et al, 1981). Simulation of the activity in the hidden units showed that their nonlinear characteristics were mainly responsible for the robust performance of the network. It suggests that the saturation and cutoff characteristics of neurons in the central nervous system that encode velocity estimation are important for the robust performance of the system. Supported by EY04148, NS00294, EY01867

74.10

BIOMECHANICS OF THE HEAD-NECK ARTICULATIONS AND HEAD MOVEMENT STRATEGIES IN MONKEY. W. Graf, P.P. Vidal, C. deWaele* and L.C. Evinger. The Rockefeller University, New York, N.Y. 10021, Lab. Physiol. Neurosens. C.N.R.S.,Paris,France, and SUNY, Stony Brook, NY 11794 (Sponsor: E.E. Brink).

We extended our analysis of the head-neck movement system in vertebrates to the monkey by studying the range of motion of the joints of the head-neck ensemble from X-ray photographs of anesthetized animals, and movement strategies from cineradiographic images of unrestrained behaving animals. The range of motion of the head-neck joints in the monkey is restricted in comparison to other vertebrates studied (e.g. of the atlanto-occipital joint to ca. 35 deg versus 90-110 deg in cat and rabbit). X-ray fluoroscopy revealed an extensive utilization of the cervico-thoracic junction for head movement control. In contrast to other vertebrates, e.g. the rabbit, which stabilizes the head largely by engaging the atlanto-occipital articulation, the monkey, when raising or lowering the head, almost exclusively utilizes the cervico-thoracic junction and thus the orientation of the entire cervical vertebral column for head movements. Strategies employed by guinea pigs and cats are in-between these two extremes.

Our collective data establish a relative changing in involvement of major articulations for head movement control comparing rabbit, guinea pig, cat and monkey, indicating increased utilization of the cervico-thoracic junction and a decreasing involvement of the atlanto-occipital articulation. Supported by INSERM and NEI-04613.

74.11

DEVELOPMENT OF CERVICO-OCULAR REFLEX IN UNILATERALLY LABYRINTHECTOMIZED SQUIRREL MONKEYS. J. Goldberg and M. Marsh*. Department of Otorhinolaryngology and Communicative Science, Baylor College of Medicine, Houston, TX 77030.

Cervico-ocular reflex (COR) generates eye movements in response to neck rotation. Primates develop a compensatory COR after a total loss of vestibular function. To determine whether such COR also develops as a result of a partial vestibular deficit, COR was measured in squirrel monkeys before and after a unilateral labyrinthectomy.

Eye movements were recorded in 3 dimensions via search coils. The head was fixed in space while the trunk was rotated sinusoidally at 0.01-5.0 Hz, 25° amplitude. This neck rotation did not produce a consistent COR in intact animals. The right labyrinth was destroyed under gas anesthesia. Recording was resumed after the operation and continued for three months.

Alert labyrinthectomized monkeys exhibited nystagmus during neck rotation in the horizontal plane. The horizontal component of the nystagmus was modulated by the rotation. COR *gain*, computed as the amplitude ratio of horizontal slow-phase eye velocity modulation to stimulus velocity, increased with post-operative time. Gain decreased steeply with increasing stimulus frequency, while phase remained near compensatory, except for a small lag at higher frequencies. Thus, at 0.05 Hz, maximum COR gains recorded over the three-month period in three animals ranged from 0.19-0.29, while at 2.5 Hz, the corresponding gains ranged from 0.01-0.04.

These preliminary results indicate that a unilateral loss of vestibular function is sufficient to trigger the development of a compensatory COR.

Supported by USPHS grant NS10940

74.12

EFFECT OF CHANGES IN GRAVITY ON OCULAR COUNTERROLLING IN UPRIGHT AND TILT POSITIONS. S.G. Diamond and C.H. Markham, Dept. Neurology, UCLA Sch. Medicine, Los Angeles, CA 90024.

Parabolic flight on the NASA KC-135 aircraft results in approximately 30s of 0G at the top and 30s of 1.8G at the bottom of each of 40 parabolas typically flown. Four healthy subjects were each tested in 20 parabolas, in the upright and at 30° right ear down (RED) and 30° left ear down (LED) positions, to test the hypothesis that inherent asymmetries of the otolith system, possibly well compensated in the 1G environment on earth, may be unmasked in conditions of hypo and hypergravity.

Three subjects showed no eye torsion while upright, regardless of gravity condition; nor did they have any ocular counterrolling (OCR) while tilted in 0G. In 1.8G at the tilt positions, they had considerably more OCR than at 1G. None of these three subjects became motion sick.

The fourth subject showed eye torsion to the left when at 0G, whether upright or tilted. This leftward bias could also be seen at 1.8G when he was tilted LED, the side that induces rightward OCR. At that position he showed less OCR than he had at 1G. This subject became motion sick.

All subjects had normal OCR in ground-based testing.

These results support the hypothesis that asymmetry of the otolith system may be revealed in unaccustomed gravitational situations, and may suggest that exposure of an asymmetric system to unadapted G conditions could be a factor in the unique motion sickness occurring in space.

75.1

ASCENDING PAIN INHIBITION FROM STIMULATION OF THE PERIAQUEDUCTAL GRAY MATTER OF THE RAT. M. M. Morgan, J.-H. Sohn, and J. C. Liebeskind. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

Stimulation-produced analgesia (SPA) from the PAG is known to inhibit nociception at the spinal level. Several studies have suggested that pain modulation may also occur via ascending projections from the PAG (Camarata & Yaksh, 1985; Elberdink et al., 1985). The following study was undertaken to determine if ascending projections from the PAG can inhibit nociception. Rats were anesthetized and chronically implanted with a bipolar electrode in the PAG. Immediately prior to electrode implantation, one group of rats had a wire knife lowered 9 mm into the brain just caudal to the PAG in order to disrupt descending output. A control group received no lesion. After one week, both groups were tested on a pain test organized spinally (tail-flick) and one organized supraspinally (hot-plate). Lesioned rats had significantly shorter baseline tail-flick latencies compared to control rats (mean = 3.9 vs 4.3 s), indictating removal of descending inhibition from the PAG. Inhibition of the tail-flick occurred in only 3 of 22 lesioned rats. In contrast, 13 of these 22 rats showed analgesia on the hot-plate test. The threshold for SPA on the hot-plate test was significantly lower than the current producing behavioral effects (e.g. aversive reactions) (mean = 73 and 228 μA, respectively). Non-lesioned rats displayed SPA on both tests at similar currents. These data demonstrate that analgesia can be produced in rats despite a lack of inhibition at the spinal level. Although nociceptive input can be modulated at the spinal level (as demonstrated by inhibition of spinal reflexes), the ability of PAG stimulation to inhibit supraspinally-organized nociceptive responses in the absence of spinal inhibition suggests ascending modulation of nociception. (NIH grant NS07628)

75.2

AXONAL TRAJECTORIES AND TERMINATIONS OF PHYSIOLOGICALLY CHARACTERIZED RAPHE MAGNUS CELLS IN THE CAT. P. Mason and H. L. Fields, Depts of Neurology and Physiology, UCSF, San Francisco, CA 94143.

Physiologically defined populations of pontomedullary raphe magnus (RM) cells have been described that either decrease (off-cells) or increase (on-cells) their discharge rate during the heat evoked rat tail flick reflex. There is evidence that these cells participate in the modulation of both reflexes and secondary sensory cell responses elicited by noxious stimuli.

RM neurons were recorded intracellularly during the paw withdrawal reflex evoked by noxious pinch or heat in the cat. As in the rat, RM cells either increased or decreased their discharge rate during the nociceptive reflex and responded similarly to noxious stimulation applied anywhere on the head or body surface. These cells were termed on- and off-cells respectively. Many of these neurons were also excited by periaqueductal gray stimulation. In order to determine the axonal projections of on- and off-cells, physiologically characterized cells were subsequently injected with horseradish peroxidase (HRP) and the cats were maintained for 24hrs under deep anesthesia. HRP labeled axons had their somata within the RM and sometimes collateralized within the dendritic arbor of the parent cell. Axons typically traveled caudally, although some axons also terminated in brainstem regions rostral to the cell body. The axons of on- and off-cells collateralized extensively throughout the brainstem raphé and medial reticular formation, reaching regions that were both ipsilateral and contralateral to the parent soma. Collaterals were also seen in the lateral pontomedullary reticular formation, the nucleus tractus solitarius and the sensory trigeminal complex. Axonal varicosities and terminal swellings were observed almost exclusively within regions of axonal collateralization. The HRP label in most parent axons diminished 4-6mm from the injection site as the axons traveled caudally over the lateral inferior olive in the caudal medulla.

75.3

DOSE-RESPONSE RELATIONSHIP FOR HYPERALGESIA FOLLOWING NALOXONE PRECIPITATED WITHDRAWAL FROM MORPHINE D.H. Kim*, N.M. Barbaro*, and H.L. Fields (SPON: P. Weinstein) Depts of Neurosurgery and Neurology, UCSF, San Francisco, CA 94143.

Hyperalgesia occurs during withdrawal in opiate-dependent animals and humans. Previous work from this laboratory has demonstrated facilitation of spinal nociceptive reflexes in rats given naloxone following an analgesic dose of systemically administered morphine.

In the present study, a dose-response relationship for hyperalgesia was established between intravenously administered morphine and naloxone. Rats were maintained in a lightly anaesthetized state with methohexital, and baseline tail flick latencies (TFL) and paw withdrawal thresholds (PWT) were established. Then the rats were given either saline (control) or morphine (0.5, 1.0, or 2.0 mg/kg), followed by naloxone (0.1, 1.0, or 10.0 mg/kg). There were four rats in each of 12 dosage groups. Saline followed by naloxone, at any dose, produced no change in either TFL of PWT. However, when naloxone administration followed morphine, subsequent TFL and PWT values were significantly ($p<.05$) below baseline levels (hyperalgesia). Increasing doses of morphine (followed by the same dose of naloxone) produced progressively lower TFL and PWT values . In contrast, increasing the dose of naloxone at a given dose of morphine did not increase hyperalgesia.

We conclude that dependence to opiates, as measured by hyperalgesia, develops following a single analgesic dose of morphine. Furthermore, this hyperalgesia is a function of the amount of opiate to which the central nervous system is exposed prior to naloxone precipitated withdrawal.

75.4

ROLE OF NUCLEUS RAPHE MAGNUS (NRM) IN MODULATION OF RENAL AFFERENT INPUT. I.L. Holt*, P.R. Coulombe* and M.M. Knuepfer (SPON: M.A. Walz). Dept. of Pharmacology, St. Louis Univ. School of Medicine, St. Louis, MO 63104.

The supraspinal structures that tonically inhibit somatic and visceral inputs to the spinal cord are, as yet, unclear. NRM stimulation generally enhances this inhibition. We sought to define the extent and source of tonic descending inhibition of renal afferent input in chloralose-anesthetized rats. Activity was recorded in the lower thoracic spinal gray from single neurons responding to renal nerve stimulation (RNS). Neurons inhibited by RNS (8/68) were located deeper than excited neurons and had higher spontaneous discharge rates (SDR). Reversible cold block of the cervical spinal cord (CB) caused an increase in SDR (disinhibition) in 73% of neurons excited by RNS and a decrease (disfacilitation) in 17%. Neurons disinhibited by CB had lower control responses to RNS than disfacilitated neurons. We sought to determine whether the source of tonic descending inhibition arose from the NRM by microinjecting a GABA agonist, muscimol (M), into the NRM (1nmol/1μl over 2 min). In 10/11 neurons, a disinhibition of SDR of similar magnitude was observed both with CB and M (R=0.93). M or CB also caused equivalent changes in RNS-evoked responses. We propose that tonic descending modulation of renal afferent input is widespread and predominantly inhibitory and that the NRM may be the source of the tonic inhibition. (Supported by HL37224 and HL38299 and AHA, MO Affiliate.)

74.5

ANTINOCICEPTION AND BLOOD PRESSURE CHANGES INDUCED BY ELECTRICAL STIMULATION OF THE DORSOMEDIAL MEDULLA. Sue A. Aicher and A. Randich. Department of Psychology Univ. of Iowa, Iowa City, IA 52242.

This study examined regions of the dorsomedial medulla for stimulation-induced inhibition of the nociceptive tail-flick reflex (TF) in lightly-anesthetized (pentobarbital sodium) rats (n=60). Sixteen tracking sites on the left side of the medulla were examined, extending in 0.5 mm steps from 0.5 mm caudal to 1.0 mm rostral to obex; from midline to 1.5 mm lateral; and from 0.5 mm to 2.5 mm ventral to the dura. At each site the minimum current necessary to inhibit the TF to a 10 sec cut-off was determined (baseline TF 2.5-3.5 sec). If stimulation produced either motor responses or failed to inhibit the TF with less than 200 uA, the trial was terminated. Sites with the lowest current thresholds for TF inhibition were located 1.0 and 1.5 mm lateral to obex, corresponding to placements in the nucleus tractus solitarius (NTS) and the nucleus reticularis ventralis (NRV). Sites in the NRV had lower thresholds to inhibit the TF than sites in NTS. Midline and 0.5 mm lateral sites had higher thresholds and often produced motor responses to stimulation. Threshold current for inhibition of the TF usually produced an increase in mean arterial blood pressure, for sites in either NTS or NRV. These results support the hypothesis that neural substrates involved in cardiovascular regulation interact with antinociceptive systems.

74.6

VAGAL AFFERENT MODULATION OF THE JAW-OPENING REFLEX ELICITED BY PERIPHERAL AND CENTRAL STIMULATION. W. Maixner, E.A. Whitsel*, J. Nestor*, J. Zuniga* and S. Madison*. Dental Research Center, University of North Carolina, Chapel Hill, N.C. 27514.

The outcomes of several studies show that stimulation of cervical vagal afferents (CVA) diminishes putative nociceptive somatomotor reflexes. In the present study we: 1) identified the CVA conditioning parameters that modulate the jaw-opening reflex (JOR) elicited by stimulating oral and perioral test sites (TS's) and 2) examined the ability of CVA conditioning to alter JOR evoked by electrical stimulation of the trigeminal motor nucleus (TMN).

Cats were anesthetized with α-chloralose and TS stimulating electrodes were positioned in the right maxillary canine, gingival tissue, maxillary skin and the TMN. The JOR was recorded from the right digastric muscle in response to TS stimulation at 1.5-2X threshold. Conditioning stimuli (20 msec trains, 333 Hz, 0.1-1.0 msec, 0-10 mamp) were applied to the central end of the right cervical vagus at various conditioning-test intervals (CTI; 0-1000 msec).

For all peripheral TS's, facilitation of the JOR was observed at CTI's less than 20 msec and inhibition of the JOR was observed at longer CTI's (maximum inhibition at CTI = 200 msec). In general, the inhibitory responses were dependent upon conditioning pulse width and intensity. Vagal afferent conditioning failed to alter the JOR evoked by TMN stimulation.

These data support the view that CVA stimulation modulates nociceptive reflexes and that the observed antinociceptive effects do not result from motor inhibition at sites distal to and perhaps including the TMN. Supported by NIDR grant DE0750901.

75.7

PROPRIOSPINAL MODULATION OF NOCICEPTIVE RESPONSES OF NEURONS IN THE CAT LUMBAR DORSAL HORN.

J.SANDKÜHLER, Q.G.FU*, E.CARSTENS, B.STELZER*, M. ZIMMERMANN
II.Physiologisches Institut, Universität Heidelberg, West-Germany.

The inhibition of nociceptive spinal dorsal horn neuronal responses by segmental or supraspinal systems has been extensively characterized (see Besson & Chaouch, Physiol. Rev., 67(1987)67-186 for a recent review). Little is known, however, about the impact of ascending or descending spinal interneurons on the transmission of nociceptive information in the spinal cord.

In deeply pentobarbital anesthetized cats extracellular recordings were made from multireceptive spinal dorsal horn neurons at the L_4/L_5 segments. All neurons responded to noxious radiant heating of the glabrous skin on the ipsilateral hindpaw. Focal electrical stimulation (25-100μA, 0.1 ms pulses at 100 Hz) or glutamate microinjections (0.5 M, 30-60 nl) was given at identical sites within the ipsi- or contralateral cord at the T_{11} to L_1 or at sacral segments by means of a multibarrel glass pipette (tip diameter $\leq$ 40μm).

Noxious heat-evoked responses of dorsal horn neurons at the L_4/L_5 segments were reduced by electrical stimulation in the lateral or ventral funiculi, the dorsal columns and the dorsal horn at the T_{11} to L_1 and sacral segments. Only few or no effective sites were found in laminae VI to VIII. Glutamate microinjections, which excite cell bodies only, were effective at sites in the dorsal and ventral horn gray matter at T_{11} to L_1 but not at sacral segments. Inhibitions were produced from sites *ipsi-* or *contra*lateral to the neuron under study with the cord left intact or spinalized at T_8 or T_9 segments, indicating that the activation of supraspinal antinociceptive systems is *not* a necessary component of the inhibition observed.

The present study established that some spinal interneurons arising from the dorsal horn at the thoraco-lumbar junction may modulate the nociceptive responses of dorsal horn cells at the L_4/L_5 segments. SUPPORTED BY THE DEUTSCHE FORSCHUNGSGEMEINSCHAFT, THE DAAD AND FULBRIGHT.

75.8

TRANSCRANIAL ELECTROSTIMULATION ENERGY DENSITY DISTRIBUTION IN THE BRAIN AND EFFECTS ON SOMATOSENSORY AND LIMBIC NUCLEI. C.Hymel, P.Jenkins & M.Skolnick* (SPON: R. Marc). Neurophysiology Research Center, University of Texas Health Science Center at Houston, 1343 Moursund, Houston, TX 77030

It has been shown that application of very low amplitude transcranial electrostimulation can elicit profound analgesic effect in rats which is naloxone-reversible. Investigation of the characteristics of the propagation of the stimulation signal through the brain has yielded energy density distribution maps at various points in time during the stimulation pulse. Variations in energy distribution are determined for nuclear regions generally associated with analgesia (i.e. somatosensory and limbic nuclei). These regions are subsequently tested for variations in evoked potentials as a result of electrostimulation alone and in conjunction with the administration of a chemical promotor. The findings include: 1) detection of pulses in the brain from electrostimulation as low as 10 uA, 2) significant attenuation and distortion of the detected waveform as compared to the applied signal and 3) demonstration of asymmetrical in the resultant potential field distribution across the brain.

75.9

THE EFFECT OF LOW CURRENT TRANSCRANIAL ELECTROSTIMULATION ON NEUROTRANSMITTER LEVELS IN RAT CSF AND BLOOD PLASMA
O.B.Wilson, R.Warner*, R.Hamilton*, G.Sood*, C.Johnston*, L.Howard*, M.Skolnick UT Hlth.Sci.Ctr., Houston TX 77030

It has previously been reported that low current transcranial electrostimulation (TCES) applied to the apex of the antihelices in the ears of rats induces analgesia, reduces the severity of opiate withdrawal syndrome, and causes changes in the electrophysiological responses of deep brain neurons to noxious tail pinch stimuli. These effects are slow in onset, persist well after stimulation ceases, and are reversed by the opiate antagonist naloxone. Further work also indicates that p-chlorophenylalanine (pCPA), a serotonin formation inhibitor, reduces the analgesic effect of TCES. The implication is that at least one neurotransmitter system is stimulated by TCES.

Using the TCES prescription previously found to have the greatest analgesic effect on rats (charge balanced rectangular waveform with 2msec pulsewidth, 10Hz repetition rate, and 30 minute duration), we have measured the beta-endorphine content of blood plasma and CSF using radio-immuno assays. Blood was obtained by heart puncture of anesthetized animals, whereas CSF was collected by cannulation of the third ventricle and the cisterna magna. Beta-endorphine levels in stimulated and control animals were compared before stimulation and at times 0, 15, 30, 45 and 60 minutes after treatment ended.

In CSF we have investigated the dynamics of dopamine, serotonin and norepinephrine turnover as affected by TCES. The breakdown products dihydroxyphenylacetic acid (DOPAC), hydroxyindoleacetic acid (HIAA) and homovanillic acid (HVA) were assayed using high performance liquid chromatography. CSF samples were obtained at 15 minute intervals before, during, and after TCES from both stimulated and sham-treated control animals.

75.10

INTRATHECAL BRADYKININ ACTS ON SPINAL NORADRENERGIC TERMINALS TO PRODUCE ANTINOCICEPTION IN THE RAT. O. Laneuville*, T.A. Reader and R. Couture*. (SPON: A. Ferron). Dept. Physiology, Faculty of Medicine, University of Montréal, Montréal, Qué., Canada.

In the awake restrained rat the intrathecal (i.th.) administration of 8.1 pmol-8.1 nmol of bradykinin (BK) and Lys-BK (KD) enhanced reaction time (RT) to a noxious radiant heat stimulus in a dose-related manner, the fragment BK (1-8) was active only at doses higher than 10 nmol. The increases in RT were greatest at 1 (BK) and 6 (KD) min and RT returned to the basal level within 15 min post-administration. The following order of potency was observed in relation to this response: BK > KD >> BK (1-8). The effect of BK (81 pmol) was unaffected by prior i.th. administration of propranolol (β-adrenoceptor antagonist) and naloxone (opioid antagonist) but was significantly blocked ($P < 0.001$) by idazoxan and yohimbine (α_2-adrenoceptor antagonist) and in rats which received an i.th. dose (20 µg) of 6-hydroxydopamine 1 week earlier. The latter treatment reduced by 60% ($P < 0.001$) the noradrenaline (NA) content in the lumbar spinal cord without affecting the endogenous levels of serotonin, dopamine, adrenaline or their main metabolites. There were no significant change in NA in both the brainstem and heart. In rats pretreated with the neurotoxin or with α_2-adrenoceptor antagonists, the effects of neurokinin B (antinociception) and substance P (hyperalgesia) were preserved. We conclude that BK may inhibit spinal nociceptive sensory transmission and produce analgesia by acting presynaptically on terminals of bulbospinal NA-containing fibers.

(Supported by the Medical Research Council of Canada).

75.11

DOSE RESPONSE OF INTRATHECAL CLONIDINE IN THE RAT MECHANICAL VISCERAL PAIN MODEL(m-VPM). R.W. Colburn*, D.W. Coombs*, L.L. Rogers*, K.N. Raye*, and M. Addis* (SPON: S.F. Gonsalves) Anesthesia Research Laboratory, Dartmouth Hitchcock Medical Center, Hanover, NH 03756.

Antinociception of the alpha 2- adrenergic agonist Clonidine against an upper abdominal mechanical visceral stimulus has not been tested. We report here the dose response with intrathecal Clonidine in the rat m-VPM. METHODS: 200-250g Sprague-Dawley Rats were implanted (halothane anesthesia) with duodenal VPM balloon catheters(1) and PE-10 suboccipital intrathecal catheters (2). Rats were infused intrathecally with 10ul of 0.1, 1, 10 ug Clonidine HCl or saline followed by a 10 ul saline flush one half hour prior to VPM testing. Testing protocol consisted of 5 balloon pulses(0.5-0.7ml) followed by one minute held inflation. Writhing response was graded on a 0-3 scale(1). RESULTS: Percent control(n=8) writhing responses are shown below.

Dose Clonidine	% Control Response + SE	n
0.1ug	89 + 20	9
1.0	50 + 17	9
10	13 + 8	8

DISCUSSION: Intrathecal Clonidine yields a linear dose response curve over the range of doses tested. M-VPM shows promise as a bioassay for analgesic activity of spinal alpha-adrenergic agonists against visceral pain. 1.Colburn RW, Coombs, DW, Degnan, CC, Rogers LL: Mechanical Visceral Pain Model. (Behavior and Physiology, In Press 1988).
2.Schmauss C, Yaksh TL. J Pharm Exp Ther 228: 1; 1984. Supported by: Cancer Education Grant CA 19379, Hitchcock Foundation Grant HF103, and Public Health Service Grant CA 33865 .

76.1

A BAG CELL SPECIFIC ANTIGEN COLOCALIZES WITH THE BAG CELL PEPTIDES BUT NOT WITH THE EGG-LAYING HORMONE. W. S. Sossin, T. Kreiner, and R. H. Scheller. Dept. of Biological Sciences, Stanford University, Stanford, CA, 94305

We have recently demonstrated that the amino and carboxy terminal products of the egg-laying hormone (ELH) precursor are packaged into separate dense core vesicles (DCVs) in the bag cells of Aplysia californica (Fisher, J. et al., submitted). The monoclonal antibody (MAb) 4F6 recognizes a bag cell specific protein of 80 kD which is also localized to DCVs. Using double label immunoelectron microscopy, we demonstrate that the antigen recognized by MAb4F6 colocalizes with immunoreactivity to peptides from the amino terminal (e.g bag cell peptides) of the ELH precursor but not with immunoreactivity to the carboxy terminal peptide ELH. Another distinct class of vesicles stains with the MAb 4F6 but not with antibodies directed against peptides from the ELH precursor. The 4F6 specific vesicles are smaller and less dense than the peptide containing vesicles. Thus, at least 5 separate classes of DCVs exist in the bag cells.

76.2

PROGRESSIVE RELEASE OF PEPTIDES FROM BAG CELL NEURONS OF APLYSIA DURING A DISCHARGE. K.J.Loechner, E.Azhderian, R.Dreyer and L.K.Kaczmarek. Dept. of Pharmacology, Yale Univ. Sch. of Med.,New Haven, CT 06510.

Electrical stimulation of bag cell neurons of Aplysia triggers a discharge of action potentials during which several peptides are released. We have examined the time course of release of two of these peptides, α-bag cell peptide (α-BCP) and egg-laying hormone (ELH). Medium (artificial sea water/protease inhibitors) bathing individual abdominal ganglia was exchanged completely at 5 min intervals before, during and after discharges lasting 10-30 min. Peptides in the medium were analyzed by HPLC with post-column derivatization. α-BCP and ELH were identified by retention times, which corresponded to synthetic peptide standards. Pre-discharge samples contained several peaks that did not correspond to known bag cell peptides (n=8). Some of these peaks were diminished during a discharge and recovered after the cells had stopped firing. Samples at times after the onset of a discharge also showed additional peaks, including α-BCP and ELH. A progressive increase in the release of both α-BCP and ELH was seen over the course of the discharge. Once the discharge had stopped, however, the next 5 min sample showed a decrease in both α-BCP and ELH. One hour after a discharge, neither α-BCP nor ELH were detected. Our data show that the release of α-BCP and ELH through the vascularized sheath into the external medium is not tightly coupled to the firing rate of these neurons. The firing rate peaks in the first minute of a discharge and subsequently declines, whereas release increases progressively over the course of a discharge.

76.3

THE NEUROPEPTIDE EGG-LAYING HORMONE OF *APLYSIA* INDUCES PACEMAKER ACTIVITY AND REDUCES ACCOMMODATION OF SPIKING BY MODULATING FOUR IONIC CURRENTS. R.F. Jansen* and E. Mayeri. Department of Physiology, University of California, San Francisco, CA 94143.

Egg-laying hormone (ELH) is a neuropeptide released by the bag cells. It is the hormone that triggers ovulation and it also functions as a neurotransmitter in the abdominal ganglion, where it causes long-lasting excitation of the LB and LC neurons. Using voltage-clamp methods, we previously showed that ELH increases I_R, the inwardly rectifying potassium current and I_X, an inward sodium-sensitive voltage-independent current.

We now report that focal application of ELH (final conc. approx. 10 μ*M*) also suppresses $I_{K(V)}$, a delayed rectifier potassium current and I_A, a transient potassium current, by approx. 20 % each. $I_{K(V)}$ is a slowly activating current that is TEA+-resistant (100 m*M*). It shows slow voltage-dependent inactivation, and reverses close to E_K in a 120 m*M* K^+ medium. I_A is a rapidly activating and inactivating current that can be activated by hyperpolarizing prepulses and is blocked by 5 m*M* 4-AP. ELH modulates all four currents in each LB/LC neuron.

The functional consequences of the actions of ELH are the onset of repetitive spiking and a dramatic reduction in the accommodation of spiking, as measured by a graded series of depolarizing current pulses.

Supported by NIH grants NS16490, NS16033.

76.4

PURIFICATION AND STRUCTURE OF THE DELTA AND EPSILON BAG CELL PEPTIDES OF APLYSIA. G.T. Nagle, M. de Jong-Brink+, S.D. Painter, J.E. Blankenship and A. Kurosky*. Marine Biomedical Institute and *Dept. of Human Biol. Chem. and Genetics, Univ. of Tx. Med. Br., Galveston, TX 77550, and +Dept. of Biology, Free Univ., Amsterdam, The Netherlands.

A factor in the bag cells stimulates calcium uptake into mitochondria of secretory cells in the albumen gland, a female accessory sex gland that deposits a nutritive secretion around oocytes during egg laying. Peptides were extracted from bag cells of 50 animals and fractionated by gel filtration and C18 reversed-phase HPLC. Amino acid sequence analyses demonstrated that the presumed bioactive peptide (delta BCP) was the 39-residue sequence: NH_2-Asp-Gln-Asp-Glu-Gly-Asn-Phe-Arg-Arg-Phe-Pro-Thr-Asn-Ala-Val-Ser-Met-Ser-Ala-Asp-Glu-Asn-Ser-Pro-Phe-Asp-Leu-Ser-Asn-Glu-Asp-Gly-Ala-Val-Tyr-Gln-Arg-Asp-Leu-COOH. This peptide is structurally related to the caudodorsal cell neuropeptide (calfluxin) of Lymnaea, which has equivalent activity in that species. Interestingly, the potential Arg-Arg cleavage site in delta BCP is not utilized in vivo. Amino acid sequence analyses demonstrated that a second, presumably inactive, peptide (epsilon BCP) was: NH_2-Ser-Val-Leu-Thr-Pro-Ser-Leu-Ser-Ser-Leu-Gly-Glu-Ser-Leu-Glu-Ser-Gly-Ile-Ser-COOH. Supported by NSF BNS 17575 and BBS 87 11368, and by NIH NS22079, NS23169 and CA17701.

76.5

PURIFICATION AND STRUCTURE OF HISTIDINE-RICH BASIC PEPTIDE AND PEPTIDE I FROM NEURONS R3-R14 OF APLYSIA. S.D. Painter, G.T. Nagle, J.E. Blankenship and A. Kurosky*. Marine Biomed. Inst. and *Dept. Human Biol. Chem. & Gen., Univ. Tx. Med. Br., Galveston, TX 77550.

The R3-R14 neurons contain a myoactive peptide that excites the heart and enhances gut motility in vitro. The gene expressed in these cells encodes three peptides, only one of which (peptide II) has been isolated and sequenced. We report the purification and sequence analysis of the two remaining peptides, the myoactive histidine-rich basic peptide (HRBP) and peptide I. Peptides were extracted and fractionated by gel filtration and C18 reversed-phase HPLC. Amino acid compositional and sequence analyses demonstrated that the structure of the 43-residue HRBP was: <Glu-Val-Ala-Gln-Met-His-Val-Trp-Arg-Ala-Val-Asn-His-Asp-Arg-Asn-His-Gly-Thr-Gly-Ser-Gly-Arg-His-Gly-Arg-Phe-Leu-Ile-Arg-Asn-Arg-Tyr-Arg-Tyr-Gly-Gly-Gly-His-Leu-Ser-Asp-Ala-COOH. Compositional analysis indicated that peptide I was a 26-residue peptide. Sequence analysis of the first 20 residues yielded the following primary structure: NH_2-Glu-Glu-Val-Phe-Asp-Asp-Thr-Asp-Val-Gly-X-Glu-Leu-Thr-Asn-Ala-Leu-Glu-X-Val. The sequences obtained for the two peptides were identical to those predicted from nucleotide sequence analysis of the HRBP gene expressed in neurons R3-R14. Supported by NIH NS 22079, NS 23169, and CA 17701, and by NSF BNS 85 17575 and BBS 87 11368.

76.6

SEQUENCE OF PEDAL PEPTIDE - A NOVEL NEUROPEPTIDE FROM THE CNS OF APLYSIA. P. E. Lloyd & C. M. Connolly* Dept. of Pharmacol. & Physiol. Sci. & Comm. on Neurobiol., Univ. of Chicago, Chicago IL 60637.

We have identified a novel neuropeptide in Aplysia nervous tissue. The peptide was termed Pedal peptide (Pep) because it was predominantly synthesized in the pedal ganglia. Pep was purified and sequenced from pooled extracts of pedal ganglia and the following sequence proposed:

Pro-Leu-Asp-Ser-Val-Tyr-Gly-Thr-His-Gly-Met-Ser-Gly-Phe-Ala

Enzymatic hydrolysis indicated that Pep had a free carboyxl terminal. A peptide with the proposed sequence was synthesized and compared with the native peptide. Chromatographic properties of the two peptides under 3 different conditions were identical. Native and synthetic Pep produced the same electrophysiological responses in neuron L5 in the abdominal ganglia. The cell body of L5 was covered with a network of Pep-immunoreactive terminals. Both peptides produced net inward currents that were associated with a decrease in membrane conductance. The results from these two procedures confirmed that the proposed Pep sequence was correct. Quantitative measurements of the incorporation of ^{35}S-methionine into Pep suggest that cell bodies which synthesize Pep were present predominantly in the pedal ganglia but should also be found in other central ganglia as well. Pep-like immunoreactive neurons were found predominantly in the pedal ganglia and less frequently in the other ganglia (Pearson & Lloyd, these abstracts). Quantitatively, Pep constitutes one of the predominant peptides in the nervous system of Aplysia. Pep does not appear to be a member of any other previously identified peptide family. Supported by NIH grant 23569 and a Whitehall grant.

76.7

IMMUNOCYTOLOGICAL LOCALIZATION OF PEDAL PEPTIDE IN THE CNS AND PERIPHERY OF APLYSIA. W. L. Pearson* & P. E. Lloyd. (SPON: L. Minor) Comm. on Neurobiol. & Dept. of Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Immunocytology using antisera raised to conjugated pedal peptide (Pep) was used to localize the peptide in the CNS and periphery of Aplysia. A total of over 200 neurons in the CNS exhibited Pep-like immunoreactivity. As expected from results presented elsewhere (Lloyd & Connolly, these abstracts), immunoreactive neurons were heavily concentrated in the pedal ganglia primarily in a broad ribbon on the dorsal surface of each ganglion. This ribbon was comprised of about 60 large contiguous neurons. Several smaller groups of neurons were also found on the ventral side of each pedal ganglion. Smaller and less numerous immunoreactive neurons were found in the cerebral, buccal, and abdominal ganglia. Pleural ganglia contained only 1 or 2 very small positive neurons. A number of neurons primarily located in the abdominal ganglia had dense networks of immunoreactive varicose fibers surrounding their cell bodies. Many immunoreactive axons were observed in peripheral nerves, particularly those nerves leaving the pedal ganglia. Analyses of sections of body wall indicated that Pep-like immunoreactivity was localized to a series of fibers and large varicosities, many of which appeared to be associated with vascular spaces. A significant proportion of the Pep in the body wall appears to come from neurons in the pedal ganglia. Experiments in which newly synthesized Pep in the pedal ganglia was labelled with ^{35}S-methionine indicate that very large amounts of the labelled peptide were transported by fast axonal transport down peripheral nerves towards and into the body wall. These results suggest that Pep is a transmitter-like neuropeptide which is likely to have a number of important physiological actions in Aplysia. Supported by NIH grant 23569 and a Whitehall grant.

76.8

BUCCALIN: DISTRIBUTION OF IMMUNOREACTIVITY IN THE *APLYSIA* NERVOUS SYSTEM AND BIOCHEMICAL LOCALIZATION TO IDENTIFIED MOTOR NEURON B16. I. Kupfermann, E. C. Cropper, M. W. Miller, A. Alevizos, R. Tenenbaum, and K. R. Weiss. Cntr. Neurobiol. & Behav., Coll. Phy. & Surg., Columbia Univ., NYS Psychiatric. Inst., New York, NY 10032.

In order to study modulation of the muscles involved in feeding responses in *Aplysia* we have been investigating peptide cotransmission in the accessory radula closer (ARC) muscle. This muscle, which is involved in the biting response, is innervated by two cholinergic motor neurons B15 and B16. Previous work has indicated that contractions of the ARC can be potentiated by the peptides SCP_A and SCP_B, which are present in neuron B15, and the peptide myomodulin, which is present in neuron B16. It has also been shown that in addition to the SCPs B15 contains a third peptide, buccalin, which decreases rather than increases the size of muscle contractions. Since buccalin appears to act presynaptically, yet modulates muscle contractions produced by stimulation of either B15 or B16 we hypothesized that buccalin was present in B16 as well as B15.

As previously shown for B15, physiologically identified B16 neurons marked with Lucifer Yellow exhibited buccalin-like immunoreactivity in whole mount preparations. Other identifiable cell bodies and richly staining neuropilar regions were present in each of the ganglia. Immunoreactive varicosities, indicative of buccalinergic innervation were present in tissues of the genital system (hermaphroditic ducts, penis retractor muscles) and feeding system (esophagus and ARC muscle). Double-labeling of the ARC muscle revealed two populations of varicosities, one immunoreactive to both $SCP_B$and buccalin (presumably from B15), and a second population exhibiting only buccalin-like immunoreactivity (presumably from B16).

The presence of authentic buccalin in B16 was demonstrated by radiolabeling B16 neurons with ^{35}S methionine and showing coelution of synthetic buccalin and B16 radioactivity through three different sequential RP-HPLC gradients. Furthermore, gas phase sequence analysis of B16 radioactivity yielded counts in cycle two, the only position where methionine is present in buccalin. Therefore, neuron B16 as well as neuron B15 contains buccalin. These data indicate that the two motor neurons for the ARC muscle contain unique as well as overlapping peptides that can provide types of modulation common to both neurons as well as specific to one or the other.

76.9

ACTION AND STRUCTURE OF PARABUCCALIN - A NOVEL NEUROPEPTIDE LOCALIZED TO CHOLINERGIC BUCCAL MOTONEURONS B15 AND B16 OF *APLYSIA*. K.R.Weiss, E.C.Cropper, R.Tenenbaum*, F.S.Vilim and I.Kupfermann. Cntr. Neurobiol. & Behav., Columbia U., and NYS Psychiatr. Inst., N.Y., N.Y. 10032.

Contractions of the ARC muscle are modulated by several peptides that are present in the cholinergic motoneurons B15 and B16. In an attempt to define the full complement of modulatory neuropeptides in the ARC, we fractionated an extract of 1000 muscles using HPLC, and bioassayed the fractions for modulatory actions on ARC contractions elicited by motoneuron stimulation. This uncovered the presence of several substances that were able to either potentiate (SCPa, SCPb and myomodulin) or depress (FMRFa and buccalin) ARC contractions. We have now purified and sequenced another inhibitory peak. A synthetic carboxy-terminally amidated replica of the established sequence had the same retention time and bioactivity as the native peptide. Furthermore, the amidated peptide comigrated through four stages of HPLC with ^{3}H-Leu labeled peptides synthesized by B15 and B16. This confirmed that the structure of the peptide is Gly-Leu-Asp-Arg-Tyr-Gly-Phe-Val-Gly-Gly-Leu-a and localized it to B15 and B16. This peptide, which we have named parabuccalin, shares 7 of 11 residues with buccalin, a peptide we previously characterized. The structural similarity of these two peptides is reflected in the similarity of their actions on the ARC. Both depress motoneuron, but not ACh elicited contractions and depress the EJPs. Thus both peptides appear to exert their action via presynaptic inhibition of ACh release from motoneurons and may function in combination with other peptides to permit selective control of the amplitude and relaxation of muscle contractions. Thus, ARC motoneurons contain different families of structurally related peptides, some of which such as the SCPs potentiate contractions, and others like the buccalins, depress contractions. Possible subtle differences in the actions of these related peptides remain to be explored.

76.10

FUNCTIONAL CHARACTERIZATION OF THE PEPTIDERGIC ARC MOTOR NEURONS B15 AND B16 OF *APLYSIA*: ACTIVITY PATTERNS DURING FEEDING IN FREE MOVING ANIMALS. E. C. Cropper, I. Kupfermann, and K. R. Weiss. Cntr. Neurobiol. & Behav., Columbia Univ. P & S, & NYS Psych. Inst., NY, NY 10032.

The two cholinergic ARC motor neurons B15 and B16 contain multiple bioactive peptides which, when exogenously applied, modulate parameters of muscle contraction. While there is considerable data on the biophysical and biochemical characteristics of these neurons, their role in behavior and the precise function of the peptides they contain is poorly understood. This is, in part, due to the fact that practically nothing is known about their physiological firing patterns. For this reason electrodes were implanted in the ARC muscle and synaptic currents were recorded during the different feeding-related behaviors of *Aplysia*; biting without ingestion of food, biting followed by swallowing, i.e., ingestion of a strip of food, and rejection. Synaptic currents from B15 were distinguished from those of B16 by sacrificing animals and comparing responses evoked by intracellular motor neuron stimulation to those recorded during feeding. Correlations between synaptic events and behavior were made with the aid of video recording and a transducer which recorded tension developed between the radula halves during ingestion of a strip of seaweed.

These experiments demonstrate that B15 and B16 do indeed fire during the consummatory feeding response, during closure of the radula, and that they fire at frequencies which in reduced preparations produce significant muscle contractions. The data also show that one motor neuron is not simply a functional duplicate of the other. While both neurons fire during biting (with or without ingestion of food), during rejection only B16 fires. Furthermore, during biting, the firing patterns of the two neurons are markedly different; B16 always fires alone for about one second and then the two neurons fire together for a period of time that is dependent on whether or not food is ingested. If food is ingested, the motor neurons can fire for as long as 6 seconds, whereas food is not present they typically fire for 3 seconds or less.

In conclusion, we have characterized the physiological patterns of activity of the two ARC motor neurons B15 and B16. These patterns can now be simulated in reduced preparations where it will be possible to test in a behaviorally relevent context hypotheses concerning the role of peptide cotransmission in the ARC neuromuscular system.

76.11

CELLULAR DYNAMICS OF PEPTIDES CONTROLLING EGG-LAYING BEHAVIOR AND GROWTH IN THE SNAIL *LYMNAEA STAGNALIS*. E.W. Roubos*, W.R.A. van Heumen*, E.D. Schmidt* and J. Slootstra* (SPON: ENA). Dept. of Biology, Vrije Universiteit, P.O. Box 7161, 1007 MC Amsterdam, The Netherlands.

The Caudodorsal Cells (CDC) of the snail *Lymnaea stagnalis* control egg-laying behavior by releasing *ca* 11 peptides. Two genes encoding for these peptides have been characterized. Peptide dynamics have been studied with tannic acid and immunoelectron microscopy. The peptides are partly colocalized in secretory granules. Release occurs from neurohaemal terminals into the haemolymph, and from collaterals into the intercellular space of the CNS. Neurohaemal release is calcium-dependent and occurs during a 1 h of electrical activity ("discharge"). Most likely, first the αCDCP-peptide and then other peptides are released. The discharge is initiated by membrane depolarization and is mediated by cAMP. Release from the collaterals occurs at morphologically unspecialized release sites ('nonsynaptic communication'). It is stimulated by depolarization and is calcium-dependent, but unlike neurohaemal release, it is minimal during the discharge. This suggests that neurohaemal and collateral release are controlled independently. Apparently, the CDC control peripheral targets (gonad, accessory sex glands) by neurohaemal secretion, whereas collateral release regulates CNS targets inter- and motoneurons). CNS receptors for one of the CDC-peptides, calfluxin, have been localized.

The Light Green Cells (LGC) stimulate body growth by releasing an (molluscan) insulin-related peptide (MIP) from neurohaemal axon terminals into the haemolymph. The MIP-gene has been characterized. The LGC are under control of two types of dopamine-receptor and an adenylate-cAMP system. MIP seems to belong to a large family of insulin-(related) peptides that control growth throughout the animal kingdom.

It is suggested that complex behavior is controlled in a multipeptidergic way, whereas body growth is controlled in a more simple (conservative?) fashion, by one peptide.

77.1

CUES FOR THE ADJUSTMENT OF SYNAPTIC STRENGTH IN THE CRICKET. A. Chiba* and R.K. Murphey (SPON: M. Hirsch). Department of Biology, SUNY Albany, NY 12222.

Synapses made by cercal sensory receptors change their strengths during postembryonic development (Chiba et al., Science in press 1988). We focused on three identified receptors which had different synaptic strengths in control animals, and examined the role of age and receptor hair size in adjusting synaptic strength. First, we changed the age of the presynaptic structure by cutting the cercal nerve in the 7th or 8th instar, and allowing the sensory axons to regenerate. The strengths of three identified synapses on the medial giant interneuron were the same as in control adults. This result eliminated the age of the presynaptic structure as a cue for adjusting synaptic strength. Next, we changed the age of the entire receptor, including the sensory neuron soma, by amputating the cercus in the 6th or 7th instar, and allowing cercal regeneration to occur. The three receptors of interest could usually be identified on the regenerate cercus. Their synapses had strengths which were better correlated with receptor size than with their absolute age. The result seems to eliminate age of the sensory neuron soma as a controlling variable. We are testing the hypothesis that hair size is coupled to synaptic strength through its effect on sensory neuron activity. Supported by an NSF grant to RKM.

77.2

THEME AND VARIATIONS ON NEURONAL EVOLUTION S. R. Shaw, Life Sciences Ctr., Dalhousie Univ., Halifax, NS, Canada.

Evolutionary considerations have found relatively little place in modern neuroscience, to the extent that it is unclear which features of neurones change during evolution. From Golgi/EM neuroanatomy of the insect visual system, I now confirm our earlier inference based on axon counts, that all the cell types present in the lamina neuropil of recent Diptera have recognizable homologs in Stratiomyidae, a family of ancient lineage that probably diverged >200 Myr ago. Additionally, the relative position occupied by each neurone within the lamina cartridge has been identified, and has remained practically invariant.

By contrast, the relative sizes of the neurones and the positions of some monopolar cell bodies vary markedly between the distant families. Of more functional significance, the branching patterns and synaptic terminations of neurones have altered, some radically, some less so, implying that other synaptic connections must have changed beyond the one already documented (Shaw and Meinertzhagen, PNAS 85: 7961, 1986). Some of the implied connectivity changes have been confirmed directly by EM. A picture emerges of extreme developmental constraints on the basic architecture of the cartridge, contrasting with plasticity of the synaptic connections. Of the possibilities that can be detected with neuroanatomical approaches, synaptic promiscuity emerges as the most likely vehicle for evolutionary progression. [Supported by NSERC Canada, A9593]

77.3

MODIFICATION OF TRANSMITTER RECEPTOR PROPERTIES MEDIATED BY CELL CONTACT PRIOR TO SYNAPSE FORMATION. S. Sanchez-Armass* and P. Drapeau, McGill University Centre for Neuroscience, 1650 Cedar Ave., Montreal, Canada H3G 1A4.

Identified leech neurons reform specific synapses in culture. Serotonergic Retzius cells can reinnervate pressure sensitive (P) neurons and activate only one of the two 5-HT receptors in the target P cells: the Cl (gCl_S) but not the monovalent cation receptor channels ($gCation_S$), as observed in vivo. We have examined the mechanism of receptor selection.

$gCation_S$, the non-synaptic response, was reduced 5-fold both in innervated P cells and in P cells contacted but not yet innervated by Retzius cells. Depleting the Retzius cells of 5-HT by incubation in reserpine did not prevent the reduction of $gCation_S$ whereas incubating single P cells with 5-HT did not affect $gCation_S$. Both receptors were observed in the Retzius cell but $gCation_S$ was unaffected by synapse formation. These results suggest that the reduction of $gCation_S$ occurs upon cell contact prior to synapse formation and is specific for the target neuron. The loss of counter-effective postsynaptic receptors appears to be a prelude to synapse formation. We are testing the effects of modifying the Retzius cell surface in order to obtain direct evidence for a contact-mediated selection of receptors. Supported by the MRC of Canada and the FRSQ and FCAR of Quebec.

77.4

COMPETITION BETWEEN CONVERGENT SYNAPSES IN DEVELOPMENT: ELECTRICAL ACTIVITY MODULATES EFFICACY AND NUMBER OF INPUTS. R.D. Fields, C. Yu, E.A. Neale, and P.G. Nelson. Lab. of Develop. Neurobiology, NIH, NICHD, Bethesda, MD 20892.

Afferents of (DRG) neurons converging on spinal cord neurons of mice in vitro form more and stronger synapses when exposed to a phasic pattern of electrical stimulation during development. Synaptic efficacy was diminished in competing afferents which were not provided with electrical stimulation, but the number of axonal inputs was not reduced. Dissociated neurons were cultured in a three-compartmental chamber such that axons from DRG neurons in the two side compartments formed synapses on spinal cord neurons in the central compartment. A phasic pattern of electrical stimulation was applied to the DRG axons extending from one of the side compartments, and the amplitude of EPSPs was compared after 3-5 days of stimulation. The median EPSP amplitude increased from 5.5 mv in unstimulated controls to 10.0 mv in stimulated afferents, and decreased to 3.9 mv in non-stimulated convergent inputs ($p < 0.03$). The number of inputs, determined by graded stimulation, increased from a mean of 1/side to 1.6 after stimulation ($p < 0.001$), but non-stimulated afferents also increased to 1.4, because of an increased number of these inputs to proximal neurons. Activity-dependent synaptogenesis and modifications of synaptic boutons may not progress to elimination of weak convergent or proximal afferents.

77.5

DEVELOPMENT OF THE RETINOTECTAL PROJECTION IN ZEBRAFISH EMBRYOS UNDER TTX-INDUCED BLOCKADE OF NEURONAL ACTIVITY

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To determine whether an impairment of neural activity would affect the pathfinding of growing retinal axons and/or the precision of the developing retinotectal map zebrafish embryos of 28-34 h of age were injected with 9-19 nl, 0.12 mmol TTX prior to axonal outgrowth. The embryos were paralyzed for several days. Axons from either dorsal (D) and ventral (V) or nasal (N) and temporal (T) retina were stained with DiI and DiO in embryos at 35, 48 and 56 h of age and viewed at 70 to 86 h.

Similar to axons of normal embryos, the TTX-blocked axons from D and V retina traveled through separate aspects of the optic nerve and tract and terminated at retinotopically correct sites in tectum. T and N axons were segregated in the nerve but mixed in the tract and initially coextensive over the rostral tectal half. Then, N axons progressed into and settled in the caudal tectum whereas T axons ended in rostral tectum as in normal embryos. Individual TTX-blocked axons were of normal morphology. They were either unbranched or carried a few filopodial sprouts. At retinotopic sites, they gave rise to terminal arbors ranging in size between 8 x 14 to 14 x 33 μm at 70-75 h, and between 11 x 18 to 42 x 62 μm at 78-83 h (mean arbor size: 17 x 27 μm). Thus, the arbors in TTX-blocked embryos were no larger than normal arbors. The tectal area covered by the mean of the arbors was 1,3 %.

These findings suggest that TTX-induced impulse blockade does not interfere with the formation of orderly pathways nor with the establishment of a precisely ordered retinotopic map in zebrafish embryos.

77.6

AXON-TARGET INTERACTIONS ARE ARRESTED AND ABNORMAL IN WEAVER CEREBELLUM

C.A. Mason, Dept. of Pathology, College of Physicians and Surgeons of Columbia University, New York, N.Y. 10032.

In the weaver mouse cerebellum, granule neurons fail to migrate along Bergmann glial processes and die in ectopic positions. To understand how afferent axons respond to the loss of one target cell population, we examined axon morphology and cellular relations by labeling axons with HRP in cerebellar slices, or with rhodamine via their brainstem sources in intact animals, and correlative electron microscopy. The majority of afferents in mature weaver cerebellum have large oval en passant terminals that follow the contours of the misoriented dendrites and the soma of Purkinje cells. Rhodamine injections into the inferior olives identify these axons as climbing fibers, and show that exuberant arbors innervate multiple Purkinje cells at the perikaryal level, despite the loss of perisomatic processes. Compared to the wild type, a reduced number of mossy fibers are labeled via their pontine source, and mossy terminals have immature shapes. Terminals of both afferents contact Purkinje cells and the few surviving granule neurons.

This view of afferent morphology in the mutant cerebellum supports previous physiological and ultrastructural data, and shows a wider range of effects from the loss of the granule neuron, implicated as a primary site of action of the weaver gene. The maintenance of multiple innervation and immature terminals suggests an auxilliary model, that molecules mediating interactions among a number of neuron and glial cell populations are defective in weaver, yielding both reduced specificity and arrested development. Supported by NIH grant NS 16951.

77.7

TARGET-DEPENDENCY OF CATECHOLAMINERGIC AFFERENTS IN AN ANIMAL MODEL OF MICROENCEPHALY INDUCED BY PRENATAL METHYLAZOXYMETHANOL ADMINISTRATION. MP Abbracchio*, M Cimino*, M Di Luca*, L Mennuni*, P Zaratin* and F Cattabeni, Inst. Pharmacol. Sci., Via Balzaretti 9, 20133 Milan, Italy.

The administration of the antimitotic agent methylazoxy methanol (MAM) to pregnant rats at day 15 of gestation results in a microencephaly in the offspring characterized by a reduced cortical field. Noradrenergic afferents to the cortex seem to develop normally, leading to a consistent hyperinnervation. We have studied whether this hyperinnervation found in 2-month-old offspring could undergo regressive events, in view of the known target-dependency of synapse survival. Noradrenergic cortical markers were measured in 1, 2 and 4-month-old offspring of pregnant rats treated with graded doses of MAM. A dose-dependent increase of these markers was observed in 1 and 2-month-old animals, whereas at 4 months these markers were significantly lower in treated animals. This pattern was observed also in n. striatum for the dopaminergic afferents. We can therefore conclude that also the catecholaminergic system is target-dependent although regression seem to occur over a much longer period of time when compared to other systems, as recently reported by Ashwell (Dev. Brain Res. 35, 199, 1987) in the same animal model.

77.8

CRITICAL PERIOD FOR ANDROGENIC BLOCK OF NEUROMUSCULAR SYNAPSE ELIMINATION. C.L. Jordan, M.S. Letinsky and A.P. Arnold. Departments of Psychology and Physiology, UCLA, Los Angeles, CA 90024.

In the androgen-sensitive levator ani (LA) muscle of the rat, the normal developmental transition from multiple to single innervation of muscle fibers (mfs) occurs largely between postnatal days (P) 14-28 and androgen treatment during this time blocks this transition. Adult LA muscles retain their neonatal pattern of multiple innervation after juvenile (P7-34) androgen treatment (Jordan, Letinsky and Arnold, Soc. Neurosci. Abstr. 12: 1213). Juvenile androgen treatment also increases the diameter of adult LA mfs. The present experiment was done to determine when in development androgen is most effective in preventing synapse elimination and altering the size of adult mfs.

Male rats were castrated on P7, 21 or 35, then given daily injections of either testosterone propionate (TP) or the oil vehicle during the 2 weeks following castration. All animals were sacrificed at 9 weeks, and their LA muscles removed and stained with tetranitroblue tetrazolium, which stains motor axons and their terminals. TP during the first treatment period (P7-20) increased the percentage of multiply innervated mfs in 9 week LA muscles (54% in TP-treated vs. 12% in oil-treated) without increasing the size of LA mfs (26.8 μm vs. 27.0 μm). TP during the two subsequent periods had little or no effect on the amount of multiple innervation (P20-34: 24% in TP vs. 19% in oil; P35-48: 25% vs. 24%), but did increase mf size (31 μm in TP vs. 25 μm in oil; 36 μm vs. 28 μm). These data suggest that there are different critical periods for androgen effects on synapse elimination and on muscle fiber size, and that androgen can prevent synapse elimination independent of its effect on muscle fiber size. Supported by NIH grants HD15021 and HD7228.

77.9

SELECTIVE ELIMINATION OF CROSS-COMPARTMENTAL INPUTS DURING SYNAPSE ELIMINATION. S.P. Donahue* and A.W. English. Dept. Anatomy. Emory Univ. Atl, GA 30322.

Neuromuscular compartments are the territories of muscle innervated by primary (first order) muscle nerve branches. We used intracellular recording to examine the distribution of nerve terminals from axons coursing in different primary nerve branches to the medial head of the rat lateral gastrocnemius (LGm). We exposed and cut the LGm primary nerve branch, thus denervating all fibers innervated exclusively by axons coursing in the cut branch. During the first postnatal week of life, only about 5% of cells in LGm received innervation from axons which crossed into the LGm head from an adjacent compartment. The synapses responsible for this innervation were less able to evoke muscle cell action potentials. They were also restricted to an area of LGm not having a tendinous boundary to separate LGm from adjacent LG compartments. The loss of these inputs occurred at a rate different than that of compartmental synapse elimination: no cross-compartmental innervation could be detected at a time when 50% of muscle cells remained polyinnervated (P7). Furthermore, tenotomy delayed compartmental synapse elimination but had no effect on the loss of cross-compartmental innervation. We conclude that a very small number of axon branches initially cross compartment boundaries and that these branches are lost selectively during synapse elimination. Supported by USPHS Grant 20545.

77.10

SYNAPSE ELIMINATION IS SLOWED BY PARTIAL POSTSYNAPTIC ACTIVITY BLOCKADE IN THE NEONATAL RABBIT SOLEUS MUSCLE. E. M. Callaway and D. C. Van Essen, Division of Biology, Caltech, Pasadena, CA 91125.

To examine the role of postsynaptic activity in regulating the rate of neuromuscular synapse elimination, we continuously superfused α-bungarotoxin (α-BGT) over the surface of neonatal rabbit soleus muscles. Superfusion was begun at 6 days postnatal and continued for a variable duration (2 to 5 days) before muscles were analyzed. *In vitro* tension measurements indicated that suprathreshold synaptic transmission was blocked in only a small minority of the soleus muscle fibers. The percentage of polyinnervated fibers was assessed both physiologically and anatomically for toxin-treated muscles, contralateral muscles from the same animals, control (saline-superfused) muscles, and normal muscles of the same age.

Within muscles exposed to α-BGT for 5 days, an average of 55% of endplates remained polyinnervated based on either assay. This value was significantly greater than for any of the control groups, in which average polyinnervation ranged from 7% to 18%. Differences between toxin-treated and control groups were smaller at intermediate ages (8-10 days), but were statistically significant after just 2-3 days of the treatment. The anatomical assay further revealed that the retention of polyinnervation in α-BGT treated muscles was most pronounced near the muscle's surface, where the activity blockade was most extensive. However, endplates at the center were also affected, even though the activity blockade appeared to be minimal in this region.

These observations indicate that the rate of synapse elimination depends upon the levels of functional acetylcholine receptors. Since the percentage of endplates at which synapse elimination was delayed was greater than the percentage whose activity was completely blocked, we infer that synapse loss was slowed even in muscle fibers retaining some postsynaptic activity. Hence, the rate of synapse elimination may be regulated independently at individual endplates in response to graded changes in postsynaptic activity.

Supported by NSF grant BNS-8408213 and NIH training grant T-32 GM07737 to E.M.C.

77.11

A COMPUTER MODEL OF NEUROMUSCULAR SYNAPSE ELIMINATION. J. M. Soha, E. M. Callaway, and D. C. Van Essen, Division of Biol., Caltech, Pasadena, CA 91125.

Synapse elimination is a complex process likely to involve a variety of distinct mechanisms and cellular interactions. It is often difficult to predict reliably how a hypothesized component of this process might affect the sequence of developmental events, and thus contribute to experimentally observed outcomes. We have developed a computer model of neuromuscular synapse elimination that provides an objective framework for analysing the roles which certain proposed mechanisms might play during synaptic maturation.

The current version assumes that presynaptic terminals compete for limited available space at each endplate. The model dynamically simulates the growth or retraction of individual terminals at each iteration. Actual size changes at each terminal are determined stochastically, based upon the momentary bias toward growth or retraction. This growth-retraction bias is calculatedly analytically, based upon a set of parameters whose values depend on the mechanism(s) being simulated.

To date, three general mechanisms have been studied: 1) an extracellular synaptic stabilization molecule ("scaffolding"); 2) a muscle-derived trophic factor that is taken up presynaptically and optionally can be transported to the cell body; and 3) intrinsic restrictions on metabolic capacity that reduce the competitive abilities of nerve terminals in proportion to motor unit size. Each hypothetical mechanism was able to simulate most, but not all, of 9 selected experimental observations that are descriptive of both normal and experimentally perturbed development.

The performance of both the scaffolding and trophic factor models is critically dependent on how the size of a presynpatic terminal affects its tendency to grow. Unless larger terminals tend to be at an advantage over smaller ones, synapse elimination proceeds very slowly in these models. We also examined several potential roles for presynaptic and postsynaptic activity. We found that an advantage to less active terminals could simulate development of the orderly relationship between recruitment order and motor unit size known to exist in adult muscles. Supported by NSF grant BNS-8408213.

78.1

INTERNEURONS INTERPOSED IN OLIGOSYNAPTIC PATHWAYS BETWEEN THE SUPERFICIAL PERONEAL NERVE AND THE FLEXOR DIGITORUM LONGUS MOTONEURONS IN THE CAT. A. K. Moschovakis, M. Solodkin and R. E. Burke. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20892.

Previous work in this laboratory has demonstrated the existence of a disynaptic, low threshold pathway between the superficial peroneal (SP) nerve and the flexor digitorum longus (FDL) motoneurons (Fleshman et al., *Exp. Brain Res.* 54:133, 1984). In an attempt to locate interneurons in this pathway, we employed two approaches: 1) retrograde transynaptic transport of lectin-conjugated horseradish peroxidase (WGA-HRP; see Harrison et al., *Neurosci. Lettr.* 45:15, 1984) to label candidate interneurons; and 2) spike triggered averagIng of ventral root potentials to investigate the location, response properties and synaptic actions of individual segmental interneurons that were activated by low threshold SP afferents. In the anatomical experiments, WGA-HRP was injected bilaterally into the FDL muscle nerves under anesthesia and aseptic conditions. Two days later, the cat was re-anesthetized and maintained under halothane anesthesia for up to 54 hours. The SP of the left leg was continuously stimulated through cuff electrodes with repetitive trains (4 shocks, 200 Hz, 3 trains per sec, 2X threshold) while monitoring the compound action potential through a separate cuff electrode. Transneuronally labelled interneurons were plotted from serial sections after standard tissue fixation and TMB histochemistry. Labeled neurons were scattered bilaterally in laminae V to VII of spinal segments S1 to L4, with greatest concentration in L7 and L6. There were about twice as many labeled neurons on the left (stimulated) side.

Maximum field potentials after stimulation of SP afferents (2xT) were found within a more restricted region in the central and lateral/dorsal horn. This region overlapped the location of some HRP-labeled interneurons. Extra- and intracellularly recorded interneurons (n=26) that responded monosynaptically to SP stimulation (central latency: 0.4-1.2 msec) were found in the same region. Averaged records of ventral root potentials recorded with the sucrose gap technique and triggered from interneuron spikes revealed that some of these cells produce postsynaptic potentials in motoneurons.

78.2

CHEMICAL MODULATION OF MEDULLARY OUTPUT NEURONS WHICH CONTROL EXCITABILITY OF HINDLIMB ALPHA-MOTONEURONS IN CATS. K. Takakusaki*, T. Sakamoto* and S. Mori. Dept. of Physiology, Asahikawa Med. Col., Asahikawa 078 Japan

Pontomedullary reticular formation contributes to the excitability level setting of alpha-motoneurons (MNs). We tried to elucidate pontine contribution to the medullary output neurons in acute, decerebrate cats. A group of reticulospinal cells in the nucleus reticularis gigantocellularis (NRGc) were tonically activated by microinjection of carbachol (100 mM, 0.1-0.25 µl) into the nucleus reticularis pontis oralis (NRPo), while intrapontine injection of noradrenaline (100 mM, 0.1-0.25 µl) resulted in suppression of the NRGc cells. A train pulse microstimulation (20-40 µA, 3 pulses with 1 ms interval) to the NRGc evoked mixed postsynaptic potentials (PSPs) with short (5-20 ms) and long latencies (30-60 ms) in alpha-MNs. With microinjections of carbachol, noradrenaline and atropine sulfate into the NRPo, the stimulus effects of the NRGc upon alpha-MNs changed. With carbachol injection, IPSP components were markedly augmented with suppression of EPSP components, while IPSP components were suppressed with augmentation of EPSP components by noradrenaline and atropine injections. These results indicate that selective activation of acetylcholine and noradrenaline receptive cells in the NRPo modulates the effects of the medullary output neurons upon hindlimb alpha-MNs in a reciprocal manner.

78.3

SYNAPTIC RESPONSES EVOKED BY VENTRAL ROOT STIMULATION IN NEONATAL RAT MOTONEURONS. Z. G. Jiang*, M. Y. Wang* and N. J. Dun. Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153

Intracellular recordings were made from antidromically identified motoneurons (MNs) in transverse (500 µm) spinal cord slices removed from neonatal (< 20 days) rats. Ventral root stimulation evoked a hyperpolarizing potential (IPSP) in about 10% of MNs. When recorded at the resting membrane potential between -55 and -75 mV, the mean amplitude and duration of IPSPs was 2.8 mV and 12 ms, respectively. The IPSP was associated with a decrease of input resistance; it was reversed between -70 and -80 mV. Strychnine (0.1-2 µM) as well as d-Tc (10 µM) reversibly blocked the IPSP, indicating that the latter is probably di-synaptic similarly to the Renshaw cell circuitry established for the cat MNs. In addition to the IPSP, ventral root stimulation evoked an excitatory potential (EPSP) in about 30% of MNs. A biphasic response consisting of an IPSP followed by an EPSP was observed in a few MNs. The EPSP exhibited a long latency (1-5 ms); the conduction velocity was estimated to be less than 1 m/s, which is slower than that of the motor axons (8-50 m/s). In the majority of MNs, the EPSP was associated with a decrease of input resistance. As the EPSP was reversibly depressed by low Ca solution and by excitatory amino acid antagonist kynurenic acid (0.2-1 mM), it is probably mediated by an excitatory amino acid. The results suggest that MNs receive excitatory synaptic inputs from fibers coursing in the ventral roots. (Supported by NS24226)

78.4

PHARMACOLOGICAL ANALYSIS OF IPSPS GENERATED BY SINGLE INTERNEURONS MEDIATING NON-RECIPROCAL INHIBITION IN THE CAT SPINAL CORD. I. Jiménez*, J. Quevedo*, M. Solodkin* and P. Rudomin. CINVESTAV, México, D.F. 07000

Previous work (J. Neurophysiol. 57: 1288, 1987) has disclosed the existence of several types of intermediate nucleus interneurons with inhibitory synaptic connections on motoneurons. We now investigated the sensitivity to systemic injections of strychnine (STR) and picrotoxin (PTX) of IPSPs elicited by interneurons mediating non-reciprocal postsynaptic inhibition with axon collaterals to the Clarke's column nuclei (CC). In barbiturate anesthetized cats with the dorsal columns sectioned at L1 level, CC stimulation produced monosynaptic IPSPs in the ventral roots (recorded with the sucrose-gap technique) that were abolished by STR (0.1 mg/kg) but not by PTX (1 mg/kg; n=5). STR also abolished the monosynaptic IPSPs elicited by CC stimulation in single spinal motoneurons (n=4). Spike-triggered averaging of root potentials following activity of single interneurons responding to group I and to low-threshold cutaneous inputs that were antidromically activated from the CC, revealed the existence of time-locked population IPSPs in the ventral roots that dissapeared after i.v. STR and were substituted by common excitation potentials (ACEPs; n=5). Computer analysis indicated that after STR no population IPSPs were present in record samples in which ACEPs were smallest. The population IPSPs were not abolished by i.a. PTX that reduced the DRPs produced by stimulation of group I flexor fibers up to 50% of control (n=2). It is concluded that monosynaptic IPSPs generated in motoneurons by interneurons mediating Ib-non reciprocal inhibition are mediated by glycinergic synapses. Partly supported by grants NIH NS-09196 and CONACyT 041739.

78.5

ELECTROPHYSIOLOGICAL PROPERTIES OF FACIAL MOTONEURONS (FMNs) IN BRAINSTEM SLICES FROM GUINEA PIG. Y. Nishimura*, P.C. Schwindt and W.E. Crill Dept. of Physiology & Biophysics, Univ. of Washington Sch. of Med., Seattle WA 98195.

Electrical properties of guinea pig FMNs were studied in *in vitro* brainstem slices at 30-35°C using constant current injection and voltage clamp. FMNs were identified histologically and by antidromic activation. The response to a subthreshold depolarizing current pulse was larger than to a hyperpolarizing pulse. Subthreshold depolarizing voltage clamp commands from resting potential evoked a persistent, tetrodotoxin (TTX)-sensitive inward current. Injection of suprathreshold depolarizing current pulses evoked repetitive firing characterized by an initial period of rapid adaptation and subsequent maintained firing. The relation between firing rate and current (f-I relation) was bilinear upwards during adaptation, but became shallower and linear during sustained firing. Single evoked action potentials were followed by a fast afterhyperpolarization (fAHP) and a subsequent medium duration AHP (mAHP). Replacement of Ca^{2+} with Co^{2+} abolished the mAHP and corresponding ionic currents, but spike duration was unaffected. The f-I relation became steeper and fast adaptation was reduced. Addition of tetraethylammonium (TEA: 10 mM) abolished the fAHP, prolonged the spike, enhanced the mAHP and reduced firing rate. We conclude that FMNs possess both a fast, TEA-sensitive K^+ conductance involved in spike repolarization and a slower, Ca^{2+}-mediated K^+ conductance that, together with a persistent Na^+ conductance, largely controls the repetitive response. Supported by NIH grants NS16972 and NS22410.

78.6

ELECTROPHYSIOLOGICAL PROPERTIES OF ADULT GUINEA PIG HYPOGLOSSAL MOTONEURONS STUDIED IN VITRO. F. Viana and A.J. Berger. Dept. of Physiology & Biophysics, Univ. of Washington School of Medicine, Seattle, WA 98195.

We have recently examined the basic firing behavior of hypoglossal motoneurons recorded intracellularly from transverse brainstem slices of guinea pigs (200-350 g) (Faseb J. 2:A513, 1988). In the present study we used pharmacological agents known to block specific ionic conductances and ionic substitution in the bath solution to characterize the ionic mechanisms underlying motoneuron firing behavior. Action potentials were evoked antidromically or with brief intracellular current pulses. Addition of 10-mM TEA to the perfusate caused a moderate increase in spike duration, suppression of the fast AHP (afterhyperpolarization), and an increase in the medium-duration AHP. Substitution of $Ca2^+$ with $Co2^+$ reduced the medium AHP with no effect on spike repolarization. Under these conditions the steady-state firing frequency was greatly enhanced. Intracellular Cs^+ markedly increased the spike duration, with additional spikes generated on the falling shoulder of the first spike. In the presence of extracellular 10-mM TEA, or in Cs^+-loaded cells after extracellular addition of TTX, a brief intracellular current pulse produced a sustained depolarizing plateau with superimposed spikes. Both the plateau and the spikes were completely eliminated when $Ca2^+$ was substituted with $Co2^+$. We conclude that the firing behavior of hypoglossal motoneurons is complex and is governed by a subtle balance between inward and outward currents. (Supported by NS 14857)

78.7

DIRECT EXCITATORY INTERACTIONS BETWEEN RAT PENILE MOTONEURONS. W.F. Collins, III and J.T. Erichsen, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794

In male rats, copulatory behavior is accompanied by rapid penile reflexes involving striated perineal muscles innervated by motoneurons (MNs) in the lumbar spinal cord. Using intracellular recording and staining techniques, we have examined a subset of these MNs (those located in the dorsal medial nucleus (DM or SNB) which innervate the bulbospongiosus (BS) and anal sphincter (AS) muscles) for evidence of electrical coupling. In gonadally intact male rats (Sprague-Dawley; 250-400 g), anesthetized with chloral hydrate or ketamine + xylazine and artificially ventilated, spinal cord segments L_1-S_3 were exposed and all lumbar and sacral dorsal roots cut bilaterally. Intracellular recordings were obtained from antidromically identified DM MNs using KCl (3M) or Lucifer Yellow (LY; 4% in 0.1M LiCl) filled glass micropipettes. Ipsilateral (ipsi) muscle nerves (BS or AS) or the contralateral (contra) PMN were stimulated individually (0.3-0.5 Hz) and the resulting MN response averaged. Short latency (300-700 us; from onset of antidromic field potential), graded EPSPs were observed. Peak amplitudes varied from 30-1200 uV (500-1200 uV, ipsi homonymous nerve stimulation (liminal); 100-400 uV, ipsi heteronymous nerve stimulation; 30-200 uV, contra PMN stimulation). In two experiments (of 8) intracellular LY injection resulted in dye coupling between ipsi DM MNs. These results suggest that rat penile MNs are electrically coupled. Supported by RO1-NS24206, RO1-EY04587, RO1-NS16996 and PO1-NS14899.

78.8

COMPUTER MODELING STUDIES OF THE EFFECT OF DENDRITIC LOCATION ON ANALYSIS OF SOMATIC EPSP AMPLITUDE FLUCTUATIONS. M. Solodkin, A.K. Moschovakis, J.D. Clements and R.E. Burke, Lab. of Neural Control, NINCDS and Lab. of Develop. Neurobiol., NICHD. , NIH, BETHEDSA, MD 20892.

A dynamic compartmental model based on the actual morphology of an α-MN was used to evaluate the effect of electrotonic distortion on the amplitude of somatic EPSPs generated by synaptic boutons distributed within a branched dendritic tree to mimic group Ia synapses. We assumed that the probability of activation of each bouton was independent and binomially distributed. Amplitudes of EPSPs resulting from the different combinations of activation probabilities were measured at and around the EPSP peak. Amplitude fluctuation analysis was done under one of two assumptions: 1) all boutons produced identical conductance changes; or 2) all boutons produced identical amplitude EPSPs at the soma (see Jack et al., *J. Physiol.* 321:65, 1981). Using assumption (1), the number of peaks in the EPSP amplitude distribution equals the number of boutons only when all boutons are at the same electrotonic distance from the soma. In addition, because of non-linear interactions between synapses, the increments between peaks are equal only when all these boutons are also located in different dendritic compartments. Under all other conditions, the number of peaks in the noise-free amplitude histograms exceed the number of boutons and are separated by unequal voltage increments. These problems were mitigated only partially when conductances were increased so as to generate EPSPs with equal amplitudes at the soma irrespective of electrotonic distance (assumption 2). The interpretation of quantal EPSP amplitudes educed by deconvolution analysis of synaptic events in the presence of noise is greatly complicated when applied to group Ia EPSPs, which are generated by synaptic boutons that are widely distributed in motoneuron dendrites.

(M.S. was supported by an International Fogarty Fellowship).

78.9

VOLTAGE ATTENTUATION IN CABLE MODELS OF VAGAL MOTONEURONS. R. Nitzan*, Y. Yarom* and I. Segev* (SPON: O. Abramsky). Dept. of Neurobiology, Hebrew University, Jerusalem, Israel.

Cable models of morphologically and physiologically characterized vagal motoneurons from guinea pig brainstem slices were constructed. Four different types of steady-state voltage attenuation factors along the dendritic trees of these models were calculated based on Rall's cable theory: 1) $AF_{T\rightarrow S}$, the attenuation factor calculated iteratively from each dendritic terminal to the soma, averaged; 2) $AF_{S\rightarrow T}$, the attenuation factor from the soma to each of the dendritic terminals, averaged; 3) AF_{av}, or cosh (L_{av}), where L_{av} is the average cable length of the paths from the soma to dendritic terminals; and 4) AF_{peel}, or cosh (L_{peel}), where L_{peel} is the cable length calculated by "peeling" exponentials from voltage transients in the model, produced by a brief current injection to the soma. A new parameter, $\Upsilon = AF_{T\rightarrow S}/AF_{S\rightarrow T}$, is defined to characterize the asymmetry of voltage attenuation in the dendritic tree in the centripetal as compared to the centrifugal direction. In these cells, $AF_{T\rightarrow S} = 7.3$, $AF_{S\rightarrow T} = 1.4$ and $\Upsilon = 5.1$. Comparison between AF_{av} (1.2) and $AF_{S\rightarrow T}$ (1.4) shows that AF_{av} is a good estimate for the average voltage attenuation from the soma to the dendritic terminals. On the other hand, AF_{peel} (1.7) was found to be significantly larger than AF_{av} (1.2). This discrepancy is the result of trees whose dendrites have unequal electrotonic lengths, when the recording site (soma) is not in the midpoint of the cable. In this case, L_{peel} may reflect up to twice the average cable length from the soma to dendritic terminals when the dendritic to soma conductance ratio, ρ, is large and even somewhat more when ρ is small. The latter result indicates that, for a correct estimation of cable length of neurons, both electrophysiological and morphological data are essential.

78.10

ENCODING OF INFORMATION BY POPULATIONS OF DSCT NEURONS IN THE CAT. R.E. Poppele, Lab. of Neurophysiol., Univ. of Minnesota, Minneapolis, MN and C.E. Osborn, Kresge Hearing Res. Inst., Ann Arbor, MI.

Neurons of the DSCT respond to various stimuli of the hindlimb with long lasting changes in post-stimulus firing probability. Changes produced by small passive rotations of the foot follow stereotyped patterns of excitation and inhibition. These responses were characterized by principal component analysis of their waveform, and response types grouped by cluster analysis.

We investigated the responses of a randomly selected population of 115 neurons to passive flexion and extension of the foot and found that over 95% responded to this stimulus (7 rotations lasting about 100 ms). About half of the neurons in each response group gave the same response to both ankle flexion and extension. The remaining units in each group responded differently according to movement direction but all the responses within a group were generally changed in the same way. For example, units inhibited by flexion were either inhibited the same way by extension or else excited by extension. There were also different combinations of early (20-30 ms) and late (50 ms) responses. Thus, even though nearly all the cells of the DSCT respond to passive rotations of the foot, only a fraction of them encode the direction of the movement.

Supported by NIH grant NS 21143.

78.11

INPUT FROM THE SPINAL CORD TO THE VL NUCLEUS OF THE CAT THALAMUS. R. Mackel and T. Noda*. The Rockefeller University, 1230 York Avenue, New York, N.Y. 10021.

Intracellular recording techniques were used to study, in chloralose anesthetized cats, synaptic responses in cerebellocerebral relay neurons to dorsal column (DC) and spinothalamic tract (ST) stimulation. All neurons (n=88) were monosynaptically excited from the contralateral deep cerebellar nuclei and 25/88 were identified as thalamocortical projection neurons by their antidromic activation from the motor cortex. The recording sites in VL were anatomically localized by depositing a dye mark(pontamine sky blue) through the micropipette(filled with K-acetate). 74/88 neurons (84%), including 22/25 projection neurons, responded with short or long latency excitatory or inhibitory postsynaptic potentials to single or double shock stimulation of the spinal cord. Six neurons were tested for spatial facilitatory interactions between the DC and ST pathways. They did not respond to DC or ST stimulation in isolation, but were excited(4/6) or inhibited(2/6) when bothpathways were stimulated together. The spinal input to VL is primarely mediated polysynaptically, probably via brain stem structures. The findings extend recent work (1) and show that VL can no longer be regarded as a simple relay between the cerebellum and the motor cortex. Instead it appears to be a site of considerable sensorimotor integration.

(1) Mackel.R., Noda.T.: Brain Res. (1988),440,348-351.

78.12

COULD SPINOTHALAMIC TRACT (STT) TRANSMIT MUSCLE AFFERENT INPUT TO PRIMARY MOTOR CORTEX (MI) IN MONKEYS? S.F. Hobbs, D.C. Bolser, M.J. Chandler and R.D. Foreman, Dept. Physiol., Univ. of Okla. HSC, Okla. City, OK 73190.

Group II muscle afferent input from hindlimb excites MI (latency 15 ms). Cells in thalamic nucleus ventroposterolateralis oralis (VPLo) project to MI. Dorsal column nuclei and STT project to VPLo. Dorsal columns provide input to MI areas controlling distal limb muscles. Pathways contributing input to MI areas controlling proximal limb and axial muscles are undefined, but anatomical data suggest that STT may contribute. If this is true, STT cells that project to VPLo should 1) receive input from proximal or axial muscles, 2) conduct afferent input from muscle to thalamus in <15 ms, and 3) receive group II afferent input. STT cells in the L_2 to S_3 spinal segments in 9 anesthetized monkeys (Macaca fascicularis) were identified by antidromic activation from VPLo. Mechanical stimulation of thigh, tail, or abdominal muscles excited 25 (86%) of 29 STT cells. Electrical stimulation of muscle afferents was used to determine latency of muscle afferent input in 7 STT cells. Latencies averaged 6.1 ± 0.5 ms from hamstring or tail muscles to STT cells and 5.9 ± 0.6 ms from thalamus to cell for a total latency for muscle to VPLo via STT of 12.0 ± 0.8 ms. Calculated conduction velocity of muscle afferent input ranged between 34 and 58 m/s (mean 46 ± 3 m/s), for the 7 STT cells. These data support the hypothesis that primary motor cortex controlling proximal and axial muscles receive muscle group II input via STT. (NIH grants HL22732, HL07930, NS08150, and HL07304)

78.13

PARTIAL SPIKES ARE GENERATED IN THE DENDRITES OF MOTONEURONS. E. Sernagor*, Y. Yarom* and R. Werman* (SPON: H.Parnas). Dept. of Neurobiology, Hebrew University, Jerusalem, Israel.

We have investigated the sites of generation of partial spikes which develop in axotomized cat L_7 motoneurons. Partial spikes appear together with synaptic potentials, usually close to the maximum voltage of excitatory postsynaptic potentials. Partial spikes of different shapes and amplitudes may appear in the same cell as a result of synaptic stimuli from one or more sources. Rarely, partial spikes may appear during inhibitory postsynaptic potentials. The distribution of partial spike amplitudes appeared to be bimodal-like in 35.3% of the cells and monomodal in the others. Partial spike incidence and amplitude are reduced by hyperpolarization while they are increased by depolarization and may even, rarely, be evoked by somatic depolarization alone. We have recorded spontaneous partial spikes which, when averaged, appeared to be elicited by tiny excitatory postsynaptic potentials which could not be clearly discerned in single traces. Indeed, partial spike incidence and amplitude are increased by extracellular iontophoresis of excitatory amino acids without any significant change in the resting potential recorded in the soma, suggesting either that partial spikes are generated in restricted areas characterized by very low tresholds or that they have a more distal origin. Moreover, these findings also indicate that partial spikes might be generated in the vicinity of more-or-less remote excitatory synapses. Shape indices, however, indicate that almost all partial spikes discerned in somatic recordings are generated in the proximal five compartments of a ten compartment dendritic model of electrotonic length, L=1, as found experimentally.

SPINAL CORD AND BRAINSTEM: MOTOR OUTPUT

79.1

ELECTRICALLY AND CHEMICALLY INDUCED LOCOMOTION FOLLOWING ACTIVATION OF THE MEDIOVENTRAL MEDULLA IN THE RAT. N. Kinjo*, Y. Atsuta*, R.D. Skinner, M. Ort Webber* and E. Garcia-Rill, Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Previous reports from our labs have described the presence of an area in the cat medioventral medulla (MED) which, 1) can be electrically and chemically activated to induce locomotion, and 2) receives input from the mesencephalic locomotor region (MLR) (Garcia-Rill and Skinner, Brain Res. 411, 1987). The present study employed the precollicular transected adult rat preparation to determine the characteristics of this area in the rodent brain. Electrical stimulation of an area 1mm dorsal to the pyramids, posterior to the trapezoid body and anterior to the inferior olive was found to induce controlled locomotion on a treadmill at low thresholds (20-60 μA, 20-50 Hz pulses of 0.5-1.0 ms duration). Injections (0.5 μl) into the same area of the cholinergic agonist, methacholine (0.5 mM), and the GABAergic antagonist, picrotoxin (2-5 mM), were found to induce short episodes of locomotion. Injections of GABA (0.5-1M) into the MED were found to block MLR-induced locomotion. Combined with previous anatomical results showing projections from the MLR to the MED in the rat (Garcia-Rill *et al*, Brain Res. Bull. 17, 1986), these findings suggest that, in the rat, 1) the MED may provide reticulospinal elements which can control spinal pattern generators, 2) this area is under cholinergic and GABAergic influence, and 3) the main descending output of the MLR may be mediated by the MED in the rodent (as in the feline) brain. Supported by USPHS grants NS 21983 and NS 20246.

79.2

ROLE OF THE TELENCEPHALON IN LOCOMOTOR-RESPIRATORY COUPLING DURING WALKING IN GEESE. G.D.Funk, W.K.Milsom & J.D.Steeves. Dept. Zoology, U.B.C., Vancouver, Canada.

The role of the telencephalon in synchronizing locomotor and respiratory rhythms was examined in geese by comparing intact birds (n=4) during treadmill walking (8min) with decerebrate geese (n=9) that were induced to walk (10min) by focal electrical stimulation (25-100uA, 60Hz) of: 1) the nucleus and tract of the Trigeminal nucleus (TTD), and 2) the caudal reticular formation. Oxygen consumption, minute ventilation, tidal volume, breathing frequency (fv) and stride frequency (fs) were monitored throughout each trial. The ratio of fs/fv was calculated over each 10 sec interval and percent entrainment (E) was defined as the portion of time fs/fv occurred as an integer or 1/2 integer value (±0.05). The degree of locomotor-respiratory coupling in the two groups was virtually identical (dec=28.9±3.3%; int=28.3±3.9%) and greater than that predicted by random chance. These data suggest that interactions within the brainstem and/or spinal cord are sufficient for the synchronization of locomotor and respiratory rhythms. (supported by NSERC of Canada)

79.3

'FICTIVE' LOCOMOTION ELICITED BY ELECTRICAL OR CHEMICAL STIMULATION OF THE AVIAN MID- AND HINDBRAIN. G.N. Sholomenko and J.D. Steeves. Dept. of Zoology, U.B.C. Vancouver, Canada, V6T 2A9.

Electrical or neurochemical stimulation of sites in the mid- and hindbrain has been shown to evoke locomotion in the acute decerebrate Canada goose or Pekin duck (Sholomenko and Steeves, SN Abs,1987). Our present studies demonstrate that all modes of locomotion (as monitored from activity in peripheral nerves [ENGs]) present in the unparalyzed animal (eg. walking, running, transition from running to flying and flying alone) can also be produced in the curarized decerebrate preparation. The intensity of electrical current necessary to evoke locomotion was slightly higher in the paralyzed condition. Direct intracerebral infusion (chemical stimulation) of carbachol (1μl/27mM in PBS) or NMDA (0.4μl/30mM in PBS) was equally efficatious in both the unparalyzed and subsequently paralyzed trials. These results demonstrate that phasic afferent input is not essential to the expression of a diverse number of avian locomotor patterns at different levels along the spinal axis. (supported by NSERC of Canada)

79.4

INTERNEURONS INVOLVED IN STINGRAY LOCOMOTOR PATHWAYS. N.A. Bernau and R.B. Leonard. Marine Biomed. Inst., Univ. of Tex. Med. Branch Galveston, Tx 77550.

Vertebrate spinal cord neurons are capable of generating motor patterns during locomotion. We are using the Atlantic Stingray, Dasyatis sabina, to study the input to these neurons. Stingrays have a simple limb with pairs of segmentally organized and innervated muscle bundles. Extracellular recordings in paralyzed animals were used to study neurons associated with circuits generating locomotion. Stimulating electrodes were placed in the brain stem to evoke locomotion, which was recorded as alternating activity in nerves innervating one pair of elevator and depressor muscles. We recorded from neurons within the spinal cord whose activity was altered during locomotion. Then we tested to see if they receive input from the brain stem or the sensory nerve. Thirty-nine cells were found whose activity was altered during locomotion; 7 cells were tonically excited, 9 were inhibited, and 21 discharged phasically. One of the tonically excited and 6 of the phasic cells received input from the brain stem. One of the tonically excited and 3 of the phasic cells received sensory input. One of the tonic and 1 of the phasic cells received input from both the sensory nerve and the brain stem. These cells may be involved in integrating descending and sensory inputs with pattern generation. This work is supported by NS 11255 and NS 07185.

79.5

PHRENIC NERVE AND DORSAL RESPIRATORY GROUP NEURON RESPONSES TO NORMOCAPNIC HYPOXIA IN KITTENS. A.L. Sica, M.R Gandhi* and A.M. Steele*. Research Center, Schneider Children's Hosp, SUNY Stony Brook, New Hyde Park, New York 11042.

To obtain information about age-related responses of respiratory neuron discharges during normocapnic hypoxia (10% or 15% O2 in N2), dorsal respiratory group (DRG) neuron activities were recorded extracellularly in 24-120 d kittens. Experiments were carried out in anesthetized, paralyzed, artificially ventilated (100% O2), vagotomized and thoracotomized animals. The discharges of many neurons occurred during central inspiratory (I) phases; some, however, discharged during early expiration (E), i.e., early-E neurons. In animals <30 d, hypoxic stimulation (5-6 min) elicited an initial period (1-2 min) of facilitation of phrenic (PHR) and neuronal discharges, followed by a decrease in discharges toward or below control levels, and the occurrence of apnea, i.e, 'biphasic' response of the neonate. Such changes of discharges were typical of both I and early-E neurons. In animals >30 d, PHR discharges were usually facilitated during hypoxia; however, neuronal responses were quite variable: some were facilitated, whereas others were depressed. This latter result is similar to that reported for adult cats, supporting the concept of an uneven distribution of peripheral chemoreceptor afferents among DRG neurons. On the other hand, the decrease and subsequent absence of neuronal discharges in neonatal kittens suggest that such afferents exert a depressant effect upon DRG neurons.

79.6

MICROINJECTIONS OF EXCITATORY AMINO ACIDS INTO THE REGION OF THE KÖLLIKER-FUSE NUCLEUS ALTER PHRENIC MOTONEURONAL ACTIVITY IN THE RAT. M. Fournier & J.L. Feldman. Systems Neurobiology Lab., Dept. of Kinesiology, UCLA, Los Angeles, CA 90024.

The functional organization of the region of the Kölliker-Fuse nucleus (K-F) involved in respiratory control was studied by injecting picomole quantities of excitatory amino acids into discrete areas. Experiments were performed in anesthetized, spontaneously breathing rats. Phrenic (Phr) motoneuronal activity was monitored by recording diaphragm electromyographic activity. Multibarrel pipettes were used for pressure injection of drugs and iontophoresis of a marker. Barrels contained 10 mM solutions of L-glutamate (L-GLU), DL-homocysteic acid (DLH) and kynurenic acid (KYN) prepared in 165 mM NaCl (pH 7.4). A saline barrel was used for control injection. Volumes ejected were directly measured by monitoring the displacement of the meniscus in a barrel. The pipette was positioned in the K-F region using stereotaxic coordinates and was advanced in 50 µm steps at which small volumes (< 20 nL) of drugs were injected. Responses to injection of L-GLU or DLH were markedly site-specific and repeatable with transient increases or decreases of Phr discharge without arterial pressure change. Most potent effects were obtained with DLH injections; volumes < 1 nL could increase Phr discharges for periods > 10 sec. At some K-F sites DLH injection totally abolished Phr discharge for a few sec. Injection of equivalent volumes of saline produced no change in Phr discharge. These effects were attenuated or blocked by concurrent or prior injection of antagonist KYN. These results indicate the heterogeneous organization of the K-F and that receptors for excitatory amino acids are involved in the modulation or transmission of respiratory-related activity. Supported by NIH Grant HL-37941.

79.7

Extracellular Recordings from the Turtle Red Nucleus in an *In Vitro* Brainstem-Cerebellum-Spinal Cord Preparation.
J. Keifer and J.C. Houk. Northwestern University Medical School, Dept. Physiology, Chicago, IL, 60611.

The cerebellorubrospinal circuit has been postulated to function in the generation of motor patterns that may be adaptively tuned. This pathway is comprised of recurrent connections among the red nucleus (RN), the lateral reticular nucleus and the cerebellar nuclei, and is thought to form an excitatory feedback loop. Hence, bursts of activity recorded in the RN may be generated through this recurrent circuit and adaptively modulated by inhibitory inputs from cerebellar Purkinje cells. As an initial stage in examining this circuitry in detail, we report extracellular recordings from the RN in an *in vitro* brainstem-cerebellum-spinal cord preparation from the turtle *(Chrysemys picta)*.

The cerebellorubrospinal circuit was activated by stimulation of the contralateral spinal cord while activity in this circuit was sampled with extracellular recordings from RN neurons (n=73). Single units responded to stimulation with latencies clustering into three groups: antidromic responses (3-4msec), short latency synaptic (5-8msec), and long latency synaptic responses (11-14msec). Antidromic responses are not blocked in low Ca-high Mg bathing solution, while units activated at a latency of 5msec and longer are reversibly blocked. Recordings from single units in the RN of this preparation also reveal bursts of activity to single pulse stimulation of the contralateral spinal cord. Preliminary evidence shows that bursting activity is blocked in low Ca-high Mg, suggesting that it is driven by a synaptic network. Furthermore, removal of Purkinje cell inhibition from the cerebellum by cutting the contralateral cerebellar peduncle greatly enhances bursting discharges and spontaneous activity recorded in the RN.

These results show that the cerebellorubrospinal circuitry can be examined in detail in an *in vitro* preparation from the turtle. Furthermore, this preparation makes feasible the examination of the role of this circuitry in the generation of adjustable motor patterns.

79.8

CONVERGENCE FROM CUTANEOUS AND DEEP INPUTS ONTO THE NEURONS OF THE CAT MAIN CUNEATE NUCLEUS(MCN) PROJECTING TO CEREBELLUM AND TO OLIVE. L.Domich and F.J.Rubia*.Dept.of Physiol. U.of Alicante.Alicante;Dept.of Physiol.U.Complutense.Spain.

Convergence between cutaneous and propioceptive afferents onto the MCN neurons is considered key in the sensory-motor integration and kinesthesis. Nevertheless,the data shows a clear discrepancy.For some authors a meagre convergence(2%) is observed(Golovchinsky,1980;Dykes,1983)in marked contrast with others high proportion(69%)reported(Millar,1979). All data belongs to cuneothalamic neurons,less is known about the convergence onto the other cells in the MCN.In previous results(Domich et al,1987) we have reported specific and complex responses on these cells to parameters of movement.

In anesthetized cats,we have analized the responses evoked by a passive movement at wrist level on the cuneocerebellar and cuneolivar projecting cells,identified antidromically. The responses consist in a phasic,rapidly adapting,component or one tonic,slowly adapting,the mostly were the mixed responses.The partial or complete denervation associated or not to cut off of tendons have allowed us to eliminate almost selectively these components.The phasic responses appears to belong to footpad receptors and the two tonic one correspond to joint or muscles inputs.The mixed responses,phasic-tonic, were striking more frequent on cerebellar cells than the olivary projecting cells in which predominate the phasic responses.These data suggest that the convergence exist on the cuneocerebellar and olivary cells projecting and being crucial in the sensory-motor integration processes.

79.9

MEMORY SUBSTRATES IN PRIMATE SPINAL CORD PRODUCED BY OPERANT CONDITIONING OF H-REFLEX ARE STILL APPARENT AFTER TETANIC POTENTIATION. J.G. Calaitges*, J.S. Carp, C.L. Lee, and J.R. Wolpaw (SPON: L.D. Reid). Wadsworth Labs, NYS Dpt Hlth, Albany, NY 12201.

Monkeys can gradually increase or decrease the wholly spinal, largely monosynaptic triceps surae (TS) H-reflex if reward depends on reflex amplitude (J. Neurophysiol. 57:443-458, 1987). Conditioning causes changes in lumbosacral cord that persist after thoracic cord transection (Wolpaw & Lee, this vol). We evaluated the effect of tetanic potentiation on these memory substrates.

In 16 animals (Macaca nemestrina), the TS H-reflex in one leg was increased (8 HR↑ animals) or decreased (8 HR↓ animals) by conditioning. Animals were then deeply anesthetized, and monosynaptic reflexes to L6-S1 dorsal root stimulation were recorded from TS nerve in both legs before and after tetanization for 3 days after thoracic cord transection. Animals remained anesthetized throughout and were sacrificed by overdose.

Reflexes were much larger 20 s after tetanization. Nevertheless, the task-appropriate reflex asymmetries visible in the awake animal and in the unpotentiated reflexes of anesthetized transected animals were still apparent. In HR↑ animals, potentiated reflexes were larger in HR↑ legs than in control legs. In HR↓ animals, potentiated reflexes were smaller in HR↓ legs than in control legs. These asymmetries remained through the 3 days of post-transection study.

A possible source of the persistent conditioned reflex asymmetries is plasticity at the Ia afferent-alpha motoneuron synapse. The finding that tetanic potentiation, which also acts at the Ia synapse, does not eliminate these asymmetries suggests that they may reflect a different and more fundamental synaptic modification. (Supported by NIH NS22189 and United Cerebral Palsy.)

79.10

RETICULAR FORMATION DISCHARGES AND BEHAVIORAL CHANGES IN THE RAT. O.J. Andy, D.F. Peeler, and M. Andrews*. Depts. of Neurosurgery and Preventive Medicine, U. of Mississippi Med. Ctr., Jackson, Ms. 39216

Reticular discharge-induced behaviors are usually related to the alerting response. Specific sensory and motor control systems located in the brainstem reticular formation were investigated in the adult rat. Bipolar electrodes were placed in the brainstem reticular formation. Electrical stimulation and recordings were made through sockets attached to an acrylic cap screwed to the skull. After 2-3 days of daily stimulations, EEG discharges and behavioral changes occurred. Discharges consisted of fast and slow frequencies with periodic buildups of high voltages. Behaviors consisted of extension of the neck, lowering the head, grooming the face and the tail, deep sleep, and states of varied excitability. Aggression was reduced. Normal behavior followed 1-3 weeks of stimulation.

79.11

RETICULO-RETICULAR AND RETICULO-SPINAL CONNECTIONS AFFECTING EMG ACTIVITY IN RAT BACK MUSCLES. A. Robbins*, S. Schwartz-Giblin and D.W. Pfaff. (SPON: M.S. Herness) Lab. of Neurobiology and Behavior, Rockefeller University, N.Y., N.Y. 10021.

Electrical stimulation in medullary reticular formation (MRF) produces EMG activity in rat back muscles, and effective MRF stimulation sites receive a strong projection from contralateral MRF (MRFc) (Robbins et al., Neurosci. Abs. 13: 21.3,1987). To determine if the MRFc cells also project to the lumbar spinal cord, rhodamine microspheres were injected at the effective MRF site and a fluorogold (FG) pellet was implanted in the lumbar spinal cord. The lack of double labeled cells in MRFc indicated that separate cell populations project to the effective MRF site and to lumbar cord. The typical effective MRF site appeared to be dorsal to retrogradely labeled reticulospinal cells. This relationship was tested more precisely by tracking the threshold for EMG activation in the MRF of 2 rats in which a FG pellet had been implanted in the lumbar cord. Highly effective sites were dorsal to the FG labeled reticulospinal cells, and more caudal MRF effective sites were ventral, among FG labeled cells. Dorsal effective MRF sites that do not project to the lumbar cord may powerfully augment direct reticulospinal outputs by virtue of their commissural connections within the MRF, and thus amplify EMG responses in back muscles.

79.12

RESPONSE CODING IN POPULATIONS OF PRIMATE CORTICOMOTONEURONAL AND RUBROMOTONEURONAL CELLS AND FORELIMB MOTOR UNITS. E.E. Fetz[1], P.D. Cheney[2], K. Mewes[3] & S. Palmer[4], Depts. of Physiology, Univ. of Wash.[1,4], Seattle, WA, and Univ. of Kansas[2,3], Kansas City, KS.

The post-spike effects of corticomotoneuronal (CM) and rubromotoneuronal (RM) cells on forelimb muscles (12 total) were documented in behaving monkeys by spike-triggered averages of EMG activity. RM cells facilitated more muscles per cell (mean: 3.0 of 6 synergist muscles) than CM cells (2.4/6). Both groups had "reciprocal" cells which also suppressed antagonists of their facilitated target muscles. Unlike CM cells, some RM cells co-facilitated flexor and extensor muscles (5.8 of 12 muscles).

During performance of a standard ramp-and-hold force tracking task the firing patterns of CM and RM cells, as well as single motor units (MU), fell into distinct response types. To estimate population activities the response histograms of different cells were summed (with force ramps aligned) in proportion to the relative frequency of each cell type. The population response histogram of CM cells was phasic-tonic, consistent with the predominant response type. The population response of RM cells was also phasic-tonic, but had a less prominent tonic component. The population histogram of MUs of a muscle was proportional to the average of rectified multiunit EMG, and typically exhibited decrementing activity during the static hold. The phasic discharge of the supraspinal premotor cells was larger and earlier than their target muscle activity, and their tonic discharge was more sustained. The difference between responses of forelimb MUs and these descending inputs may reflect intrinsic motoneuron properties and/or additional sources of synaptic input.

Unlike MUs, which were recruited over a range of forces, the CM and RM cells were usually active at the lowest force levels. The increase in tonic firing rate per increase in static torque was less for RM cells (mean: 117 Hz/Nm) than for CM cells (379 Hz/Nm) and MUs (340 Hz/Nm). These results allow quantitative estimates of the contribution of CM and RM cells to MU activity.

79.13

A STRUCTURAL COMPUTER SIMULATION OF THE INFERIOR OLIVARY NUCLEUS. M. Lee* and J. M. Bower, Division of Biology 216-76, Caltech, Pasadena CA 91125.

A computer model of the mammalian inferior olivary nucleus (IO), constrained by anatomical and physiological data, is described. Our objective is to explore the possible cellular and network mechanisms underlying the correlated climbing fiber-mediated activity observed by recording complex spikes from multiple Purkinje cells in rat cerebellum (Bower & Llinás, *Soc. Neurosci. Abs.* 9:607, 1983; Nelson *et al., Soc. Neurosci. Abs.*, this volume). We are also interested in studying the means by which the low-frequency oscillatory behavior seen in the IO might be initiated and modulated by afferent input and recurrent collateral and deep cerebellar feedback. As a basis for the network model, we have developed a model IO neuron incorporating membrane properties inferred electrophysiologically (*cf.* Llinás & Yarom, *J. Physiol.* 315: 549-567, 1981). Upon suprathreshold stimulation, an initial Na^+ spike is followed by an afterdepolarizing potential mediated by a high-threshold Ca^{2+} conductance; a subsequent afterhyperpolarizing potential (AHP), of variable duration, mediated by a Ca-dependent K^+ conductance; and a low-threshold rebound Ca^{2+} conductance. The low-threshold Ca^{2+} conductance, which is normally inactivated at resting potential, can be activated by rapid repolarization at the close of the AHP and drive the cell to spike again, thus providing a mechanism for intrinsic oscillation of the neuron. Networks of these model neurons are constructed according to known microanatomical features (*e.g.* synaptic glomeruli, dendritic thickets) and available biophysical data. We are examining the capabilities of these cellular and network properties for generation and modulation of oscillatory and spatially correlated patterns of activity in cerebellar cortex. (Work supported by NIH grant NS22205, NSF grant EET-8700064, an NSF Graduate Fellowship, and the Lockheed Corporation.)

79.14

CONTROL OF AMPLITUDE IN A CUTANEOUS REFLEX SUBSERVING LORDOSIS: A ROLE FOR A PROGESTERONE METABOLITE. Susan Schwartz-Giblin and Donald W. Pfaff. Rockefeller University, New York City, N.Y. 10021.

During manually-elicited lordosis behavior, EMG activation in deep back muscles occurs concomitant with vertebral dorsiflexion (Exp.Neurol.1984). Consequently, in urethane-anesthetized rats, a brief latency EMG response recorded in lateral longissimus following electrical stimulation of either the ipsilateral, contralateral or bilateral nerves to flank skin must reflect activity in neuronal circuitry for the behavior. Conditioned reflex testing with EMG recording reveals prolonged facilitation following the contralateral-evoked response but a period of inhibition following the ipsilateral-evoked response. The inhibition has a time course that parallels Renshaw feedback inhibition. Consistent with recurrent inhibition is its dependence on axial motoneuron discharge; a weak ipsilateral EMG response does not inhibit subsequent reflex activity. The progesterone metabolite, 3 α -OH-5 α -dihydroprogesterone (250 ug i.v.) antagonizes the delayed inhibition resulting from ipsilateral cutaneous nerve stimulation.

Contralateral cutaneous pressure and contralateral cutaneous nerve stimulation are known to inhibit Renshaw cells (Wilson et al., J.Neurophysiol. 1964). Therefore, bilateral flank palpation and progesterone may act to increase axial muscle gain by reducing Renshaw negative feedback during lordosis behavior.

79.15

PROJECTIONS OF LARGE MUSCLE AFFERENTS TO THE MOTONEURON POOLS OF THIGH MUSCLES IN MAN. Ahmed Bayoumi* and P. Ashby (SPON: R.D.G. Blair) Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, Ont. M5T 2S8

The projections from group 1 muscle afferents to the motoneuron pools of biceps femoris(BI), semitendinosus/semimembranosus (ST), vastus medialis (VM), and vastus lateralis (VL) were examined in man. Muscle afferents were stimulated by electrical stimulation of muscle nerves and by tendon taps. Changes in the firing probability of single motor units were used to derive the afferent connections to individual motoneurons. All muscles examined showed short latency homonymous facilitation. Heteronymous facilitation was easily demonstrated between the two vasti muscles but not from ST to BI nor (unequivocally) from BI to ST. Reciprocal inhibition of ST and BI from low threshold afferents in the femoral nerve was readily demonstrated but inhibition of the vasti was not demonstrable from BI afferents and rarely from ST afferents. These projections differ from those of cats and baboons likely reflecting the differing functions of these muscle in each species.

80.1

TETRAETHYLAMMONIUM ATTENUATES HYPOXIA-INDUCED SPREADING DEPRESSION IN HIPPOCAMPAL SLICES. P.G. Aitken and G.G. Somjen. Dept. of Physiology, Duke Univ. Medical Center, Durham NC 27710.

During ischemia or hypoxia, brain tissue typically undergoes a process that is very similar, if not identical, to spreading depression. This hypoxic SD has been shown to be an important part of the mechanisms by which hypoxia/ischemia causes neuronal damage. In these experiments we asked whether the potassium channel blocker tetraethylammonium (TEA) could attenuate hypoxic SD in the CA1 region of hippocampal tissue slices. Transverse rat hippocampal slices were maintained at 35.5°C in an interface chamber. Each slice was exposed to two brief periods of hypoxia, 35-45 minutes apart, one in control medium and one in 10mM TEA (with the order alternated between experiments). The extracellular potassium concentration ($[K^+]_o$), DC potential, and evoked synaptic responses were monitored in stratum pyramidale of CA1. The average $\Delta[K^+]_o$ response was decreased from 41.3 mM in control medium to 17.5 mM in TEA ($p < 0.01$), and the ΔV response was decreased from 14.4 mV to 9.0 mV ($p < 0.05$). Latency of hypoxic SD was also decreased, although the change was not significant (75.4 vs. 105.2 sec). That only part of the K^+ efflux is blocked by TEA suggests that hypoxia opens K^+ channels that do not open under physiological conditions.

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80.2

SYNAPTIC PHYSIOLOGY OF HIPPOCAMPAL AREA CA1 AFTER TRANSIENT FOREBRAIN ISCHEMIA IN THE GERBIL. L. Urbán*, K. Neill*, B.J. Crain, J.V. Nadler and G.G. Somjen. Depts. Physiology, Pharmacology, Pathology and Anatomy, Duke Univ. Med. Ctr., Durham, NC 27710.

Transient carotid occlusion in the Mongolian gerbil leads to degeneration of CA1 hippocampal pyramidal cells after a delay of 2-3 d. Several lines of evidence suggest that the critical events responsible for delayed neuronal death take place during the first few hours of reflow. The present study tested the hypothesis that pyramidal cell death is preceded by a period of excessive synaptic excitation.

Transverse hippocampal slices were prepared from unoperated and sham-operated control gerbils and from gerbils that had survived from a few minutes to 10 d after occluding both common carotid arteries for 5 min under 2.5% halothane. Synaptic responses were evoked by stimulating the Schaffer collateral-commissural projection to area CA1 or the perforant path and were recorded extracellularly. Effects of ischemia were deduced from analysis of component input-output curves.

Immediately after the occlusion and for the next 4 h the initial slope of the extracellular EPSP in area CA1 was significantly increased with no enhancement of postsynaptic excitability and no change in the amplitude of the antidromic population spike. In area CA1 of slices prepared 10 h after the occlusion both the extracellular EPSP and orthodromic and antidromic population spikes were severely depressed and they continued to decline over the next 2 d. No recovery was detected. In the same slices, responses evoked in the fascia dentata by perforant path or antidromic stimulation were little affected by ischemia. After completion of the recordings, slices were fixed in paraformaldehyde, cut into sections and stained by silver impregnation. Neuronal degeneration in this material was identical to that previously reported in postischemic hippocampal formation perfused in situ.

We conclude that CA1 pyramidal cells lose electrophysiological function well before definite morphological signs of degeneration become visible. Our observation of enhanced excitatory synaptic transmission during the early reflow period supports the idea that delayed ischemic neuronal death results from excessive excitation. (Supported by NIH grant NS 06233.)

80.3

ABSENCE OF ELECTROGRAPHIC SEIZURES AFTER TRANSIENT FOREBRAIN ISCHEMIA IN THE GERBIL. D.R. Armstrong, K. Neill*, B.J. Crain and J.V. Nadler. Depts. Pharmacology, Pathology and Anatomy, Duke Univ. Med. Ctr., Durham, NC 27710.

Certain neuronal populations in the CNS are selectively vulnerable to a brief period of cerebral ischemia. This pattern of neuronal degeneration can be reproduced in the gerbil by occluding both common carotid arteries for 5-10 min. Because many gerbils are prone to develop seizures, it has been suggested that ischemia in these animals initiates seizures and that these seizures are at least partially responsible for the ischemic damage. The present study used depth electrode recordings to determine whether carotid occlusions sufficient to produce extensive brain lesions also evoke electrographic seizures in the damaged regions.

Bipolar EEG recording electrodes were chronically implanted in hippocampal area CA1 and the dorsolateral striatum. Skull screws were implanted in the frontal bone to record neocortical EEG. After a recovery period of 3-14 d, EEG was continuously recorded from freely-moving subjects for 24 h before and 96 h after occluding both common carotid arteries for 5-10 min (N = 16). Five sham-operated gerbils were also studied. At the end of the recording period, the extent of neuronal cell death was determined by silver impregnation.

During the first few hours after the occlusion, the EEG was dominated by delta waves similar to those recorded from human brain after a damaging episode of cerebral ischemia. This period was followed by behavioral hyperactivity associated with a normal-appearing EEG record. Amplitudes of the hippocampal and striatal EEG declined markedly with time during the recording period, presumably as a result of neuronal degeneration. Ictal activity was never recorded, even from animals that suffered extreme damage to the hippocampal formation and striatum. Therefore ischemic cell death in gerbils does not result from seizure activity. (Supported by NIH grant NS 06233.)

80.4

PHARMACOLOGICAL CHARACTERIZATION OF THE PERSISTENT DEPOLARIZATION INDUCED BY EXPERIMENTAL ISCHEMIA. R.K. Rader, G.B. Watson, T.H. Lanthorn. CNS Diseases Research, G.D. Searle, Chesterfield, MO 63198.

We are utilizing a model of experimental ischemia (EI) which involves exposure of in vitro hippocampal slices to both hypoxia and hypoglycemia (2mM D-glucose). Synaptic responses are lost within two minutes from the onset of hypoxia and this is followed by anoxic depolarization(AD). During anoxic depolarization, the membrane potential depolarizes up toward zero. The slice is reoxygenated one minute after AD. During reoxygenation, following AD, synaptic failure persists and the membrane potential remains depolarized.

Intracellular recordings show that competitive (100μM CPP) and non-competitive(10μM MK-801 and 30μM PCP) NMDA antagonists perfused during reoxygenation will allow the membrane potential to repolarize and regain an EPSP with action potential. Cells treated with PCP only partially repolarize. Perfusion of the slice with 0.5mM Ca^{++} and 5.0mM Mg^{++} before, during and after AD also allows for repolarization after EI. These data suggest that a calcium channel plays a major role in whether the cell will recover its membrane potential. Studies looking at variety of calcium channel blockers as well as the timing of the protective effect of low calcium will be shown which may determine which channels are responsible for PSF.

80.5

EFFECT OF GLOBAL ISCHEMIC INSULT ON ELECTROPHYSIOLOGICAL RESPONSES OF RAT HIPPOCAMPAL SLICES. F. El-Sabban*, K. H. Reid, H. L. Edmonds, Jr. and C. B. Shields Laboratory for Cellular Neuroscience, School of Medicine, University of Louisville, Louisville KY 40292.

Male Wistar rats (250-300g) were subjected to an 8 minute total loss of blood pressure (global ischemic insult, Edmonds et al, submitted) and then resuscitated, 2-3 weeks prior to study of electrophysiological responses of hippocampal slices in vitro. Unlike controls, these rats consistently showed sound-induced behavioral seizures post-insult. Population spike responses were recorded from the CA1 region in 3.5 mM K+ artificial CSF, using standard techniques (Reid et al, Brain Res 302:387, 1984). When [K+] was raised to 8.0 mM, spontaneous burst activity developed in both CA3 and CA1. Bursts were classified as "countable" (consistently greater than a preset threshold), "detectable" (visible on the display, but less than threshold amplitude) or "absent" (no detectable spontaneous activity). Rats subjected to global ischemia showed fewer "countable" bursts (in CA1 17/70 vs 65/133 in controls, $p<.001$) and more non-bursting slices (35/70 vs 47/133 in controls, $p<.05$) than controls. Burst frequency in CA3 and CA1 was highly correlated in both ischemic ($r=.95$, df 12) and control ($r=.98$, df 86) groups for all "countable" bursts. The lower ability of hippocampal slices from insulted rats to sustain burst activity is consistent with selective destruction of neurons in this region after ischemic insult (Kirino et al, Prog. Brain Res. 63:39,1985). The site of origin of behavioral seizures in these post-ischemic rats remains undetermined.

80.6

GANGLION CELL VULNERABILITY FOLLOWING AN ACUTE EPISODE OF COMPLETE RETINAL ISCHEMIA IN THE CAT. R. Siliprandi*, R. Canella*, G. Carmignoto* and G. Vantini (SPON: L. Facci). Fidia Research Laboratories, Abano Terme, Italy.

An acute episode of complete retinal ischemia in the cat was produced by increasing for 90 minutes the intraocular pressure above the level of the systolic blood pressure. Retinal functionality during and after the induction of the ischemic insult was assessed by means of electroretinographic recordings (ERG). In particular: the electrical activity of the outer retinal neurons was evaluated by recording the ERG in response to a sinusoidally modulated uniform field (FERG) whereas the electrical activity of the inner retinal neurons (particularly the ganglion cells) was evaluated by recording the ERG in response to a contrast reversing grating (PERG). As soon as the ischemic conditions were established both the FERG and PERG responses were simultaneously abolished. However, the recovery of the two ERG responses was found to be different: the PERG was completely impaired even 30 days after the ischemic insult, whereas the FERG always recovered. Morphological analysis performed on transverse sections and whole-mount preparations 30 days after the ischemic episode revealed a dramatic cell death limited to the ganglion cell layer. These results suggest that only a selected population of retinal neurons (presumably the ganglion cells) is selectively damaged following an acute ischemic insult.

80.7

CALCIUM/CALMODULIN-DEPENDENT *IN VITRO* PROTEIN PHOSPHORYLATION IS REDUCED IN ISCHEMIC TISSUE. A. Kochhar*, T. Saitoh, and J.A. Zivin. Dept. of Neurosciences, School of Medicine, Univ. of California, La Jolla, Ca 92093.

Ischemia initiates a series of biochemical events that can eventually lead to cell death. Energy stores are depleted rapidly and intracellular calcium homeostasis is disrupted. These initial ischemic events could have profound effects on the calcium/calmodulin-dependent protein kinase (PK-B) system. We examined the effects of 1 h of ischemia on PK-B-mediated protein phosphorylation in rabbit spinal cord. This ischemia duration produces irreversible neurological damage. Spinal cords were rapidly excised from control and ischemic animals and frozen. Tissue samples were homogenized and centrifuged. In *in vitro* protein phosphorylation assays, membrane and cytosolic fractions were incubated under phosphorylating conditions, in the absence or presence of PK-B activators, calcium and calmodulin. Proteins were separated by SDS-polyacrylamide gel electrophoresis, stained with Coomassie Blue, and phosphorylated proteins were detected by autoradiography. The PK-B-mediated *in vitro* protein phosphorylation pattern was altered in ischemic tissue. *In vitro* phosphorylation of all substrate proteins, particularly in the membrane fraction, was reduced after 1 h of ischemia. This study shows that the PK-B system is affected by ischemia. Alterations in this critical regulatory system may contribute to irreversible ischemic injury.

80.8

NMDA RECEPTOR BLOCKADE DOES NOT DECREASE 45Ca UPTAKE IN CA1 NEUROPIL DURING "ISCHEMIA". D. Lobner* & P. Lipton. Dept. of Physiol., Univ. of Wisconsin, Madison, WI 53706.

5' exposure to buffer equilibrated with 95%N_2-5%CO_2 and devoid of glucose causes long-term transmission failure (LTF) between the Schaffer collaterals and CA1 pyramidal cells in the rat hippocampal slice. If 1mM Ketamine is included in the ischemic buffer there is no LTF (recovery=101±9%). 100uM PCP partially prevents LTF (recovery=36±19%).

We tested the ability of NMDA antagonists to attenuate the calcium build-up in the neuropil of CA1. Slices were equilibrated with 45Ca for 45' and then exposed to ischemia for 5', still in the presence of 45Ca. 5' ischemia increased neuropil Ca by 25%. The increase in the presence of 1mM Ketamine and 100uM PCP were somewhat larger, 35-40%. Thus, tissue accumulation of calcium during ischemia is not mediated by NMDA receptors. Both Ketamine and PCP reduce 45Ca accumulation in control conditions by about 20%.

The results are consistent with 3 possible mechanisms for NMDA induction of ischemic damage. A) NMDA activation in normoxic conditions produces some Ca entry and so raises resting levels of cytosolic Ca. During ischemia cell Ca levels are thus higher and more damaging. B) NMDA activation elevates ischemic cytosolic Ca by releasing Ca from intracellular stores, perhaps producing a relative decrease in total cell Ca accumulation. C) NMDA activation exacerbates damage in a Ca independent fashion.

80.9

ISCHEMIA-INDUCED INHIBITION OF CAM KINASE II ACTIVITY IN GERBIL BRAIN. K. A. Tennes-Rees,*[1] S. B. Churn,*[1] W. C. Taft,*[1] M. L. Billingsley[2] and R. J. DeLorenzo.[1] [1]Dept. of Neurology, Medical College of Virginia-VCU, Richmond VA 23298, [2]Penn. State Univ., Hershey PA 17033.

The precise molecular mechanisms responsible for ischemia-induced cell death are not known. However, loss of Ca^{2+} homeostasis is one event which has been implicated in the mechanism of ischemic brain damage. CaM Kinase II phosphorylation is involved in mediating some of the effects of Ca^{2+} on neuronal function. To examine the effects of ischemia on CaM Kinase II, this study used the gerbil model of forebrain ischemia, which involves cessation of blood flow to the brain for 5 min by bilateral carotid occlusion (Brain Res.447:159,1988). Ischemia significantly decreased the autophosphorylation of the 50 and 60 kDa subunits of CaM Kinase II and also the phosphorylation of other substrates, including synapsin I. This effect was measured in homogenates of both hippocampus and cortex as early as 10 sec and also at 30 min, 2 hours, 2 days and 7 days post-ischemia. No modification in CaM Kinase II activity occurred in brainstem, which is not ischemic in this model. Autophosphorylation of CaM Kinase II purified from ischemic brain was significantly less than that from control. Protein staining, phosphopeptide maps and biotinylated-calmodulin binding properties of purified ischemic kinase were not significantly different from control. The results indicate that ischemia induces a rapid and long lasting change in CaM Kinase II activity and suggest that modification of this enzyme may be an early event in the development of ischemia-induced cell death.

80.10

CALCIUM ACCUMULATION IN GERBIL THALAMUS FOLLOWING REPEATED ISCHEMIC INSULTS. J. Ikeda*, F. Joo*, J. Lohr*, C.A. Reutzler*, T.S. Nowak, Jr. and I. Klatzo. Lab. of Neuropathol.&Neuroanat. Sci., NINCDS, Bethesda, MD 20892

In a series of experiments we have characterized the effects of repeated ischemic insults on physiological, biochemical and histological parameters in gerbil brain. In this study we examined the consequences of 3 ischemic episodes of 1.5 min duration, repeated at 1 h intervals. Upon recirculation there was a hypoperfusion period similar in magnitude and time course to that observed after single or repeated ischemia of longer duration. There was no significant edema at any recirculation interval, in marked contrast to the severe secondary edema observed after repeated 5 min occlusions, and there was negligible mortality. Histological examination at 7 d revealed frequent loss of hippocampal CA1 neurons, as well as a striking accumulation in thalamus of granules which stained positive for calcium and acid mucopolysaccharides, as well as with periodic acid Schiff reagent. There was no apparent cell loss in thalamus. Autoradiographic studies of Ca-45 uptake revealed selective accumulation of label in thalamus beginning within 24 h recirculation, continuing for at least 3 d. Labeling of CA1 was evident at the longer interval. While thalamic injury has also been noted after single ischemic insults, our results suggest that thalamic involvement is particularly evident after repeated carotid artery occlusions in the gerbil.

80.11

VISUALIZATION OF HYPOXIA-INDUCED CYTOSOLIC CALCIUM ELEVATION IN HIPPOCAMPAL NEURONS. [1]Y. Kudo*, [1]A. Ogura*, [2]S. Sugita* and [2]H. Higashi* (Spon: T. Amano) [1]Dept. Neurosci., Mitsubishi Kasei Inst. Life Sci., Machida, Tokyo 194 and [2]Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830, Japan.

Hippocampal CA1 neurons are vulnerable to hypoxic invasion. In order to analyze the cellular basis of the vulnerability, we exposed the CA1 neurons to a medium deprived of oxygen and monitored cytosolic calcium ion concentration ([Ca]i) by fluoromicroscopic image analysis. When a guinea-pig hippocampal slice loaded with fura-2 was superfused for 10 min with a glucose-free hypoxic medium (gassed with 95% N_2/5% CO_2), a significant elevation in [Ca]i was recognized in a layer of CA1 stratum radiatum. Elevations in [Ca]i in layers of strata pyramidale and oriens were less prominent. A monotonous depolarization of membrane potential indicated an irreversible cell damage. Exposure of a slice to a high K (75 mM)-containing medium for 5 min caused a marked and practically irreversible increase in [Ca]i in s. radiatum. Increases in [Ca]i in s. pyramidale and s. oriens were reversible. A similar tendency was recognized in a dissociated cell culture of rat hippocampal CA1 neurons. When the cultured neuron was exposed to a deoxygenated medium, the rise in [Ca]i was greater in magnitude and longer in duration in cell processes than in cell soma. These results suggest a topographical difference in Ca-mobilizing and Ca-extruding capabilities within single CA1 neurons and a preferential damage of the cell's dendritic region to hypoxic invasion.

80.12

Protein synthesis and ATP in different cell populations of the rat hippocampal slice following 5' ischemia: role of calcium and NMDA receptor activation. K.M. Raley and P. Lipton. Univ. Wisconsin-Madison, Madison, WI 53706.

We previously demonstrated that 5' ischemia (N_2-CO_2-equilibrated buffer lacking glucose) causes a profound and prolonged inhibition of protein synthesis in CA1 pyramidal cells of the rat hippocampal slice. This inhibition is prevented if the ischemic buffer lacks calcium and contains 1 mM ketamine, suggesting that both extracellular calcium and NMDA receptor activation are involved in this effect. The present study documents two additional aspects of the effects of ischemia on protein synthesis in this preparation.

A. Changes in ATP associated with protein synthesis inhibition: effects of NMDA receptor blockade.

ATP levels decrease to <10% of control levels immediately after 5' ischemia in the CA1 stratum pyramidale. 3 hr postischemia, a time when protein synthesis rates are 30% of control rates, ATP levels have recovered to 70% of control levels. 0 Ca^{2+}-1mM ketamine buffer, which completely prevents the protein synthesis inhibition in this cell type, does not attenuate the immediate fall in ATP levels, but does allow a better recovery of ATP levels, to 83% of control levels.

B. Effects of ischemia on protein synthesis in glia and capillary endothelial cells: involvement of calcium and NMDA receptor activation.

In marked contrast to the protein synthesis inhibition in CA1 pyramidal neurons, 5' ischemia produces a dramatic increase in synthesis in glia and capillary endothelial cells (3- and 2-fold, respectively). The stimulation of protein synthesis is not attenuated by exposure to 0 Ca^{2+}-1mM ketamine buffer during the ischemia.

81.1

DEVELOPMENT OF A SUB-SET OF SOMATOSTATIN NEURONS IN RAT VISUAL CORTEX. S.C. Feldman. Dept. of Anatomy, UMDNJ-New Jersey Medical School, Newark, N.J. 07103.

We have previously reported that perinatal visual cortex (VC) contains a population of cells adjacent to the white matter some of which are immunoreactive for somatostatin (SRIF). These cells, which are small and round, may be precursors of SRIF neurons in the adult. Alternatively, they could be glia. To resolve this issue two studies were performed. In the first, birthdates of neurons in VC were determined using ^{3}H-thymidine incorporation into DNA. Autoradiographic analysis demonstrated that the cells in this zone are 'born' between E 15.5-17.5. Autoradiography combined with immunocytochemistry showed that all SRIF containing cells are born by E 17.5. In the second study the identity of the cells in this zone was confirmed by the demonstration that they are immunoreactive for neuron-specific enolase but not for the glial antigens GFAP or S100. Our results suggest that there are two populations of SRIF neurons in VC, an early population born before E15 whose cells reach the surface of the cortical plate before birth and a later set, born after E 15, which synthesize SRIF while in their immature form and before migration is complete. The presence of these cells would also account for the dense plexuses of SRIF-containing fibers in VC during the time of cell migration.

81.2

DISTRIBUTION OF GABA-LIKE IMMUNOREACTIVITY IN PIGEON VISUAL AREAS: POST-HATCHING MODIFICATIONS AND EFFECTS OF UNILATERAL RETINAL ABLATION. G.Fontanesi*, L.Domenici*, P.Bagnoli and P.Streit. Dept.of Physiol.Biochem. Univ., Inst.of Neurophysiol.C.N.R.,Pisa,I-56100 and Brain Res.Inst., Zurich,CH-8029.

The pigeon brain was recently shown to contain numerous GABA-ergic systems which have been identified by means a monoclonal antibody obtained following immunization with GABA-BSA (mAb 3A12; Matute,C. and Streit,P., Histochem.,86:147,1986; Domenici et al.,J.Neurosci.,in press). In the present work we employed mAb 3A12 to investigate: i) the distribution of GABA-like immunoreactivity (GABA-LI) in the pigeon primary visual areas during the first ten days after hatching and, ii) possible changes of GABA-LI distribution in the same areas of adult pigeons which had early unilateral retinal removal. Perfused brains were conventionally cut and processed for immunohistochemistry using the PAP method. GABA-LI distribution in the visual areas changed during the first period after hatching and the adult pattern was reached around the 9th day. From hatching to 9 days, the most primary visual areas showed a progressive decrease in the relative density of labeled cells and an increase of GABA-ir terminal like elements. Adult pigeons with early retinal removal showed an incomplete development of GABA-ergic systems and the GABA-LI distribution was similar to that found at the early posthatching stages. In conclusion, GABA-ergic systems in the pigeon visual areas are incompletely developed at hatching and their maturation seems to depend upon the arrival of retinofugal axons.

81.3

CHARACTERISTICS OF CAT GAP: A GAP43-LIKE PROTEIN IN VISUAL CORTEX. H. McIntosh, D. Parkinson, M.B. Willard, and N.W. Daw, Wash. Univ. Med. School, St. Louis, MO 63110.

We are studying biochemical changes during the critical period of cat visual cortex. Our studies have concentrated on GAP43/F1/B50 because of its association with axonal growth, axonal regeneration and synaptic plasticity.

We have purified a GAP43-like protein (cat GAP) from cat forebrain and identified it in the cat visual cortex. Its isoelectric point is about 4.7 and its apparent molecular weight in SDS gels varies inversely with the acrylamide concentration. The mobility of purified cat GAP in SDS gels is slightly less than that of GAP43. Similarly, the GAP43-like protein in membranes from cat visual cortex, which is recognized on immunoblots, has a lower mobility than GAP43. Cat GAP chromatographs with an apparent MW weight greater than 100 kD on gel filtration. Following cleavage of a blocked N-terminus, an amino acid sequence was obtained (-RRTKQVEKND-) which is identical to amino acids 6-15 of rat GAP43 (Karns et al, Science 236:597-600, 1987). As in visual cortex, the forebrain concentration of cat GAP is decreased after the peak of the critical period.

In summary, we have isolated a GAP43-like protein and demonstrated its presence in cat visual cortex. We have shown that it has the expected properties. Amino acid sequencing data is also consistent with its identity as a GAP43-like protein. In addition, we have shown, both chromatographically and immunologically, that its concentration declines after the peak of the critical period.

81.4

POSTNATAL CHANGES IN GENE EXPRESSION IN KITTEN VISUAL CORTEX AND THE EFFECTS OF DARK-REARING. R.L. Neve and M.F. Bear. The Children's Hospital, Boston, MA 02115, Brown University, Providence, RI 02912.

The kitten striate cortex displays considerable synaptic plasticity during a critical period of postnatal development. We are exploring the possibility that specific patterns of gene expression bestow the cortex with this remarkable capacity to change.

RNA isolated from the visual cortex of kittens at various postnatal ages was examined with Northern blots and quantitative dot blots to determine the developmental course of expression of several genes. Expression of the growth associated protein GAP-43 was 6.6x the adult level at postnatal day 10 (P10, the youngest age examined). GAP-43 RNA dropped steeply to 170% of adult by P36, and decreased gradually thereafter. In contrast, the expression of the α subunit of calcium/calmodulin dependent kinase II (CaM kinase II) was only 14% of adult level at P10 and rose to adult level by P53. Glutamic acid decarboxylase (GAD) RNA levels were somewhat variable and did not show a clear developmental trend.

Visual experience given to kittens that have been reared in complete darkness from birth leads to extremely rapid changes in cortical organization, even after the classically defined critical period. When GAP-43 gene expression was analyzed in P31-43 striate cortex from dark-reared kittens, levels of its RNA were found to be ~150% of those in control kittens of the same age, and was comparable to the increase in GAD expression. CaM kinase II gene expression showed the most striking change: its RNA was increased 2-fold in dark-reared kittens compared to normal. (Supported by NIH grant NS06929)

81.5

POSTNATAL CHANGES IN GLUTAMATE-STIMULATED PHOSPHATIDYL INOSITOL TURNOVER IN NEOCORTICAL SYNAPTONEUROSOMES. S.M. Dudek, W.D. Bowen, and M.F. Bear, Center for Neural Science and Division of Biology and Medicine, Brown University, Providence, RI 02912.

NMDA receptors have recently received a great deal of attention for their role in activity dependent synapse modification at several locations, including the developing visual cortex. A current working hypothesis is that the NMDA-dependent calcium flux acts as an intracellular second messenger specifically to increase synaptic gain. Recent work in other laboratories has suggested that excitatory amino acid (EAA) receptors may also be linked to the metabolism of inositol phospholipids (PI), particularly during early postnatal development. As a first step toward exploring the possible role of this second messenger system in activity-dependent synapse modifications in the neocortex, we have studied the development of glutamate (GLU) stimulated PI turnover in unfiltered rat cortical synaptoneurosomes.

Using the method of Gusovsky and Daly (*Neuropharm.* 27(1) 95-105,1988), GLU-stimulated accumulation of ^{3}H- inositol phosphate was measured in Li-pretreated synaptoneurosomes prepared from rat neocortex at different ages. Maximum stimulation was observed at [GLU] $\geq$ 300 μM, and in adults was ~30% above basal levels. In striking contrast, the maximum stimulation in 1 week rats was 135%, 4.5 times the adult level. At 3 weeks, the maximum stimulation was still 2.7 times adult. However, by 5 weeks of age, the GLU-stimulated PI turnover was reduced to the adult level. To assess the subtype of EAA receptor involved in the stimulation of PI turnover by GLU, synaptoneurosomes from 3 weeks rats were stimulated with either ibotenate (IBO), kainate (KA), or NMDA. IBO concentrations as low as 10 μM produced stimulation of ~45% above basal; the other agonists had negligible effects even at mM concentrations.

These data suggest that a specific subclass of EAA receptor, linked to PI metabolism, is transiently expressed in the neocortex during early postnatal development. Preliminary experiments in kittens indicate that significant GLU-stimulated PI turnover occurs in the striate cortex as late as 50 days of age, suggesting a possible link with the critical period for experience-dependent synapse modification. (Supported by ONR contract N00014-81-K0136 and NIH grant NS06929)

81.6

AUTORADIOGRAPHIC LOCALIZATION AND ONTOGENESIS OF BINDING SITES FOR THE VESICULAR ACETYLCHOLINE TRANSPORT BLOCKER AH5183 (VESAMICOL) IN THE CAT VISUAL CORTEX. G. Prusky and M. Cynader, Department of Ophthalmology, The University of British Columbia, Vancouver, BC, Canada V5Z 3N9.

Vesamicol, a noncompetative inhibitor of high-affinity acetylcholine transport into cholinergic vesicles, was used as a selective marker of cholinergic nerve terminals in the developing cat visual cortex. [^{3}H]Vesamicol binds specifically, saturably and with a heterogeneous distribution to slide-mounted sections of adult cat visual cortex. During the first week of postnatal life, specific [^{3}H]Vesamicol binding sites are homogeneously distributed across all visual cortical layers. The second and third weeks of postnatal life are characterized by the development of a pattern with dense binding localized in the superficial (layers I-III) and deep (layers V-VI) cortical layers with the highest concentration in the superficial layers. The basic laminar distribution remains similar during the next several weeks, but the density of binding sites in the deep layers increases and becomes most prominent. By 10 weeks of age the adult pattern of binding is achieved with the most concentrated labelling in the superficial cortical layers with less pronounced binding the the deep layers. The developmental profile of [^{3}H]Vesamicol binding sites do not closely follow the idiosyncratic M_1 and M_2 muscarinic (Prusky et al, 1987) or nicotinic (Prusky et al, 1988) receptor profiles. It may, however, reflect their combination.

81.7

INSITU HYBRIDIZATION AND IMMUNOCYTOCHEMICAL LABELING OF GABA NEURONS DURING DEVELOPMENT OF MONKEY VISUAL CORTEX. AE Hendrickson, RD Mehra*, BioStructure & Ophth., U. Wash., Seattle 98195 and A Tobin, Biology, UCLA, Los Angeles 90024

We have examined the developmental sequence for the inhibitory neurotransmitter GABA in Macaca visual cortex. Immunocytochemical (imcyt) staining was done on frozen sections of aldehyde-fixed brain using antisera to GABA and to its synthetic enzyme glutamic acid decarboxylase (GAD). Insitu hybridization was done on the same tissue using a S35-labeled riboprobe generated from a cDNA clone for human GAD. Both labels were done at F75, 125, 162 and postnatal 1d, 3d, 9wk, 20wk, and 5+ yrs.

Imcyt labeling for GABA at F75 showed multipolar cells in subplate, marginal zone (MZ) and deep cortical plate (CP), and vertically-oriented cells resembling migrating neurons in upper CP. By F125 GABA+ neurons filled whole of cortex and adjacent white matter, with little change thereafter. GAD labeling in somata was seen first in deep cortex and layer 1 at F90-125 and later in upper layers. GAD label in neuropile was faint until near birth, rapidly increased to more than adult level by P6-9wk, and then decreased to adult levels. Insitu labeling for GAD at F75 showed a few heavily-labeled cells under and in lower CP and many in MZ; lighter labeling occurred in upper CP. By F162 all cortical layers contain many labeled cells with 50% more grains than in adult cortex. These findings indicate that GAD message increase occurs simultaneously or slightly earlier than imcyt-detectable product. (NIH EY01208, EY04536, EY01730)

81.8

ALTERATIONS IN THE $GABA_A$ RECEPTOR COMPLEX IN CAT VISUAL CORTEX FOLLOW EARLY VISUAL DEPRIVATION. C. Shaw* and M. Cynader (SPON: D.E. Mitchell), Dept. of Ophthalmology, University of British Columbia, Vancouver, Canada V5Z 3N9

We have shown that the number of $GABA_A$ receptors in cat visual cortex increases dramatically following early monocular eyelid suture (Shaw and Cynader 1987, 1988). We have now examined another aspect of the $GABA_A$ receptor complex, i.e., the benzodiazepine receptor, and those GABA receptors not associated with this complex ($GABA_B$). Cats of the following groups were studied: normally reared; early monocular eyelid suture (MD); early split chiasm - MD(XCMD); adult MD; adult monocular enucleation (ME); early strabismus; dark reared (DR). Receptors were labelled as follows: $GABA_A$ - [^{3}H]muscimol; benzodiazepine (BZ) - [^{3}H]flunitrazepam; $GABA_B$ - [^{3}H]baclofen. Slide-mounted sections from various animals were incubated appropriately, and apposed to Ultrofilm. Later, the sections were scraped from the slides and the bound radioactivity measured. For $GABA_A$ receptors significant increases in number were obtained following early MD (+93%) and XCMD (+80%) ($P<0.001$). Smaller increases followed adult MD (+40%), adult ME (+31%), or DR (+59%) ($P<0.04$). For BZ receptors, only early MD increased BZ receptor number (+64%). For $GABA_B$ a decrease (-37%) in the cortex contralateral to the open eye was found after early MD. Thus, major changes in the $GABA_A$ receptor complex follow various forms of visual deprivation with greater effects on $GABA_A$ than BZ receptors and opposite smaller effects on $GABA_B$ receptors. These changes argue for an overall alteration in the "chemical circuitry" of the cat visual cortex following abnormal visual experience.

81.9

ORGANIZATION AND PLASTICITY OF GABA NEURONAL SUBPOPULATIONS IN MONKEY AREA 17, IDENTIFIED BY COEXISTENCE OF CALCIUM BINDING PROTEINS K. Omidi*, S.H.C. Hendry, E.G. Jones, and P.C. Emson. (SPON: C.N. Honda) Dept. of Anatomy & Neurobiology, University of California, Irvine, Irvine, CA 92717 and Institute of Animal Physiology, Cambridge, UK.

GABA neurons immunoreactive for either parvalbumin or 28 kD vitamin D-dependent calcium binding protein (calbindin) were examined in area 17 of normal and monocularly deprived Old World monkeys (M. fascicularis). Calbindin immunoreactive neurons make up a large proportion of the GABA cells in layers II & III and as described previously for New World monkeys (Celio et al., 1986; Nature 323:715-717) they surround the patches of cytochrome oxidase (CO) staining. However, in monkeys injected monocularly with tetrodotoxin, the immunostaining around deprived patches is greatly reduced while the staining around the non-deprived patches appears to increase and invade the patches.

Parvalbumin immunostained somata and processes are present in all layers but are densest in layers IVA and IVC. In addition, immunostained processes in layers II & III form patches that coincide with the CO patches. Most of the parvalbumin processes in layers II & III and in layers IVA & IVC do not display GABA immunoreactivity, but arise from large axons that invade the cortex from the underlying white matter. The immunostaining of parvalbumin somata and processes is greatly reduced within layers IVA, IVC and the layer II-III patches of columns dominated by a TTX-injected eye. These findings indicate that calbindin and parvalbumin immunostaining in separate populations of area 17 GABA neurons and in afferent axons, possibly originating in the LGN, varies with synaptic activity. Supported by NEI Grants EY 06432 & EY 07193.

81.10

LONG-TERM SYNAPTIC POTENTIATION AND NMDA RECEPTORS IN THE RAT PUP VISUAL CORTEX F.Kimura* T.Tsumoto, A.Nishigori* T.Shirokawa, Dept. of Neurophysiol., Biomed. Res. Center, Osaka University Medical School, Kitaku, Osaka, 530 Japan

Among the three types of excitatory amino acid receptors, N-methyl-D-aspartate (NMDA)-preferring receptors are suggested to be involved in synaptic plasticity in the developing visual cortex (Tsumoto et al., Nature, 327, 513, 1987). Long-term potentiation (LTP) of synaptic efficacy is a form of synaptic plasticity seen in the hippocampus. In this study, we tested whether LTP could be induced in the visual cortex and if so, whether the induction of LTP could be blocked by a selective NMDA receptor antagonist, 2-amino-5-phosphonovalerate (APV). Visual cortical slices were prepared from rat pups aged between 21 and 40 days. Activities of neurons in layers II/III of the cortex were recorded extracellularly as field potentials or intracellularly as synaptic potentials evoked by electrical stimulation of the underlying white matter. In 17 of the 33 slices tested, LTPs of the field responses were induced by tetanic stimulation (5 Hz for 60 sec) of the white matter. LTPs of excitatory post-synaptic potentials (EPSPs) were also observed in 6 of the 11 cells tested. An application of APV with 25-50 μM prevented the induction of LTP in 11 of the 12 slices in which field responses were measured and in 9 of the 10 cells in which EPSPs were observed. These results suggest that NMDA receptors may play a role in inducing LTPs in the rat pup visual cortex.

81.11

EFFECT OF CORTISOL ON PLASTICITY IN THE CAT VISUAL CORTEX N.W. Daw, H. Sato and K. Fox, Dept. of Cell Biol., Washington Univ. Med. Sch., St. Louis, MO 63110.

Glucocorticoids are known to be low in young rats, and to be inhibitory to the development of their nervous system (Sapolsky & Meaney, Brain Res. Rev. 11,65-76, 1986). We tested whether glucocorticoids affect plasticity in the cat visual cortex, and whether their rise correlates with the decline of the critical period. Kittens were injected with glucocorticoid, starting around 35 days of age, with one eye closed around 45 days and recordings made into the cortex contralateral to the closed eye around 55 days under halothane anesthesia. Three untreated binocular animals were normal controls. Three animals with eyelid suture but no glucocorticoid treatment were monocularly deprived controls. Treated animals were given daily doses of cortisol in sesame oil, two each at 1, 10 and 20 mg/kg/day.

Three numbers were calculated from the ocular dominance histograms: weighted shift, weighted binocularity and percentage of cells in group 7. All three showed dose-dependant changes with cortisol treatment such that 10 mg/kg/day reduced plasticity by about half and 20 mg/kg/day almost eliminated it. Glucocorticoid treatment therefore resulted in a reduction of the ocular dominance shift. Measurement of cortisol levels in normal kittens show that levels are low a few days after birth and rise to adult levels after three months of age. Cortisol levels therefore affect plasticity, and rise along with the decline of the critical period, but are not correlated with the start of the critical period at 2-3 weeks of age.

81.12

COMPARISON OF THE VISUAL CORTICAL SEROTONIN SYSTEM IN NORMAL AND DARK REARED CATS. G.D. Mower and R. Rustad*. Dept. of Neuroscience, The Children's Hospital, Boston, MA 02115.

Serotonergic innervation and receptors in visual cortex (VC) develop during the early postnatal period. The present study asked whether normal development of the serotonin (5-HT) system requires visual experience by comparing normal and dark reared (DR) cats.

In-vitro receptor binding techniques, using (^{3}H) 5-HT as the ligand, were used to compare VC of normal and DR cats. Saturation kinetics and pharmacological specificity indicated that the binding was to a single high affinity (2nM) site with the characteristics of the 5-HT_1 receptor. A consistent increase (20%) in specific binding was found in DR cats in each of 4 matched comparisons. This difference reflected an increase in Bmax and no change in Kd. Autoradiography indicated that 5-HT_1 receptors are distributed rather uniformly across all VC layers and there was no marked difference between normal and DR cats. Analysis of 5-HT_2 receptors is in progress.

Immunohistochemistry (anti-5-HT) showed that 5-HT fibers are densest in superficial layers (I-III), least dense in layer IV, and intermediate in deep layers (V,VI). This distribution was similar in normal and DR cats.

These results indicate that the VC 5-HT system matures to a large extent independently of visual experience. The increase in receptor number due to dark rearing could be related to the elevated VC plasticity of DR cats.

81.13

EXPERIENCE-DEPENDENT SYNAPTIC MODIFICATIONS IN THE VISUAL CORTEX STUDIED USING A NEURAL NETWORK MODEL. Eugene Clothiaux*, Mark F. Bear and Leon N Cooper (SPON: D. Berson). Center for Neural Science and Department of Physics, Brown University, Providence, RI 02912.

Neurons in the mature visual cortex of normal cats are tuned for the orientation of a bar of light and most respond to stimulation of either eye. Both of these properties depend on the visual environment experienced during a critical period of postnatal development. For example, prolonged binocular deprivation eliminates orientation selectivity, and binocular connections are modified after brief periods of monocular deprivation, strabismus, and reverse suture. The question we have asked is whether a single mechanism can account for these varied experience-dependent synaptic modifications.

We have found that a mean field neural network architecture (Cooper and Scofield, *PNAS* 85: 1973, 1988), with some LGN-cortical synaptic modification according to the learning rule devised by Bienenstock, Cooper and Munro (BCM, *J. Neurosci.* 2: 32, 1982), successfully accounts for the experimentally observed results in all cases. A critical feature of the BCM learning rule is a "modification threshold": a critical level of postsynaptic activity at which the sign of the modification changes from negative to positive. Further, the value of the modification threshold is itself a function of the average postsynaptic response. The results of more recent pharmacological experiments in which cortical activity was manipulated by the chronic administration of muscimol (Reiter and Stryker, *Soc. Neurosci. Abs.* 13: 344.2, 1987) and APV (Kleinschmidt et al., *Science* 238: 355, 1987) may be readily understood in terms of changing the relationship of the postsynaptic response to the modification threshold. We have begun exploring the hypothesis that the physiological basis of the modification threshold is a critical level of NMDA receptor-dependent calcium flux. A molecular model based on this assumption is currently under development. Preliminary analysis indicates that the model captures the essential features of the BCM theory and hence is able to account for the various experience-dependent modifications that have been observed in visual cortex. (Supported by ONR contract N000-14-86-K-0041)

81.14

LITHIUM REDUCES EFFECTS OF MONOCULAR DEPRIVATION. T. Ohashi*, K. Imamura and T. Kasamatsu. Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115.

Lithium salts have been effectively used to treat manic-depressive disorders. Lithium at low concentrations decreases release of catecholamines (but not serotonin) from nerve endings in the brain. Lithium at therapeutic concentrations also blocks adrenergic and cholinergic agonist-induced increase in GTP binding in rat neocortex, suggesting that G proteins are the molecular site of lithium's action (Avissar et al., 1988). Recently, contributions of both adrenergic and cholinergic afferents to the regulation of ocular dominance plasticity has been implied in kitten visual cortex (Bear and Singer, 1986). In the present study, we wanted to test directly the involvement of lithium-mediated mechanisms in this matter.

Several-week-old kittens were i.p. injected with 25 mg/kg Li_2CO_3 2-3 times daily for 10 days, preceding monocular lid suture which lasted for a week. The lithium injections were repeated throughout the week of monocular deprivation. Ocular dominance distribution was assessed following Hubel and Wiesel's criteria (1962). In visual cortex of lithium-injected kittens, we found that 1) a shift in ocular dominance to the nondeprived eye was less than usually expected following brief monocular deprivation, and 2) the proportion of binocular cells was higher than that in control. We conclude that ocular dominance plasticity may be in part regulated by lithium-mediated mechanisms. [NIH Publications #85-23, Rev. 1985]

81.15

EXTRAOCULAR PROPRIOCEPTIVE INPUTS TO VISUAL CORTEX : AN ELECTROPHYSIOLOGICAL STUDY DURING DEVELOPMENT IN THE CAT. C. Milleret *, E. Gary-Bobo * and P. Buisseret * (SPON : European Brain and Behavior Society). Lab. de Neurophysiologie, Collège de France, 75231 Paris, FRANCE, Cedex 05.

We have previously shown that unit activity in Area 18 can be modulated by stimulation of the proprioceptors of the extraocular muscles in normal cats (Milleret et al., 1987). In the present study, we examined the development of these projections between 3 weeks of age and adulthood.

Responses of cortical single units to intraorbital stimulation of the afferents from either the Inferior obliquus (electrical stimulation of its branch of the IIIrd nerve, S1) or of the Rectus lateralis (stretch, S2) were recorded in anaesthetized and paralyzed cats and processed as PSTH. Efficiency of the stimuli was monitored by recording field potentials from the superior colliculus where proprioceptive inputs from extraocular muscles have already been described (Donaldson and Long, 1980). Proprioceptive origin of the responses was ascertained by their reversible disappearance after local application of xylocaine onto the stimulated nerve or into the streched muscle.

Our results show that : (1) the percentage of cells activated either by S1 or S2 decreased with age (S1, 85 to 65% ; S2, 65 to 45%) ; (2) the majority of these responsive cells displayed a selectivity for an orientation of the visual stimulus close to the orthogonal of the action plane of the muscle from which they receive proprioceptive inputs ; (3) this relation sharpened during development.

Thus, a specific organization seems to develop in normal rearing conditions between a given extraocular muscle and some visual cortical cells.

81.16

POSTCRITICAL-PERIOD PLASTICITY IN VISUAL CORTEX CELLS OF EARLY MONOCULARLY DEPRIVED AND LATE SPLIT CHIASM CATS. U. Yinon and A. Hammer*. Physiol. Lab., Goldschleger Eye Res. Inst., Tel-Aviv Univ. Faculty of Medicine, Sheba Med. Center, Tel-Hashomer, 52621, Israel.

Whether neuronal plasticity exists after the critical period for monocular deprivation effects, was studied in early monocularly deprived cats following cancellation of the direct geniculocortical contralateral pathways during maturation. 12 cats were monocularly deprived (MD) from birth; in 8 of them the decussating optic nerve fibers were transected in the chiasm during adulthood (OCMD). Unit recording was extracellularly made in visual cortex areas 17 and 18. In the MD cats 86.5% of the cells reacted to the nondeprived eye, 3.2% to the deprived eye and the rest of them binocularly. In the OCMD cats 78.9% of the cells responded to the nondeprived eye, 12.7% to the deprived eye and the rest of them binocularly. In the hemisphere ipsilaterally to the deprived eye of the MD cats 83.2% of the cells were driven by the nondeprived and 4.2% by the deprived eye while in the OCMD cats 32.8% of the cells in this hemisphere had reacted to the nondeprived eye and 40.5% to the deprived eye. Thus, the postnatal occupation of cortical cells by the experienced eye is not final and can be remarkably reduced even after the termination of the plasticity period.

81.17

POSTNATAL DEVELOPMENT OF GABA-ERGIC INHIBITION IN RAT NEOCORTEX. H.J. Luhmann* and D.A. Prince. Stanford University Medical Center, Dept. of Neurology, Stanford, CA 94305.

Maturation of the GABAergic system was studied in layers II/III of rat neocortical slices between P4 and P35. Evoked IPSPs could be first demonstrated at P13 and often consisted only of the bicuculline (BMI)-insensitive, long-latency component. By about P16, nearly all cells also showed a fast IPSP, which could be blocked by BMI. Spontaneous, BMI-sensitive IPSPs were recorded with 2 M KNO_3 electrodes from adult animals and before the maturation of evoked IPSPs in P9-P10 cells. Both $GABA_A$-and $GABA_B$-responses were elicited by extracellular pressure ejection of GABA and baclofen respectively in the first postnatal week. In neurons younger than P10, GABA application at the soma and apical dendrite most often produced a pure depolarizing ($GABA_A$-2) response, but occasionally biphasic hyper- and depolarizing responses were present (see also Mueller, A.L. et al., J. Neurosci., 4: 860, 1984). At this age, somatic $GABA_B$-responses were relatively weak and fragile and could be evoked in only about 40% of the cells. By about P25, the GABAergic system was mature and characterized by multiphasic $GABA_A$- and clear somatic $GABA_B$-responses.

The presence of GABA-receptors before the formation of inhibitory synaptic circuits in immature rat cortex, suggests that GABA might have a non-neurotransmitter function in early postnatal development.

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81.18

CHARACTERIZATION AND ONTOGENESIS OF AN ATROPINE-SENSITIVE EFFECT OF CARBACHOL ON THE *IN-VITRO* VISUAL CORTEX. G. Vaknin AND T.J. Teyler. NE Ohio Univ. College of Medicine, Rootstown, OH 44272.

The basal forebrain is the source of major excitatory acetylcholine (ACh) afferents to the cortex. The action of ACh on the synaptic response of the hippocampus and olfactory cortex *in-vitro* was found to be suppressive and atropine-sensitive. The levels of muscarinic receptors in the cortex of developing rat are reported to peak in adults with a small decline in aged rats. The present study is focused on the characterization and ontogenesis of an atropine-sensitive effect of carbachol (CCH) on the superficial layers (100-250um below the pial surface) of the *in-vitro* rat visual cortex. Stimulation was delivered in the same cortical layer, 300-400um lateral to the recording site using a microbipolar electrode. The Current Source Density analysis revealed sinks in layers II/III with corresponding sources below in layer IV. After establishing a stable baseline, 100uM CCH was added to the perfusate for two min, follwed by a recovery period of 20-30 min, after which 10uM atropine was added and perfused with the CCH for two min. CCH suppressed the synaptic response; atropine eliminated the CCH effect. The ontogenesis of the muscarinic depression was studied in four age groups: 9-10d, 15d, 30-40d and 20 months. The results indicate a significant difference between the infants (10-15% suppression) and the adults (30-40% suppression). The recovery time after CCH administration was shorter in infants (10 min) compared to adults (20-30min). Supported by ONR & EPA.

82.1

CARDIAC SYMPATHOEXCITATORY RESPONSES TO L-GLUTAMATE MICROINJECTIONS INTO THE INTERMEDIOLATERAL COLUMN (IML) OF THE SPINAL CORD OF THE RAT. K. Sundaram*, J. Murugaian* and H.N. Sapru, Section of Neurosurgery, UMDNJ - New Jersey Medical School, Newark, NJ 07103.

Mean arterial pressure (MAP), heart rate (HR), the rate of increase in the left ventricular pressure (dp/dt) and contractility index were monitored in pentobarbital anesthetized, immobilized and artificially ventilated male Wistar rats. On the right side, microinjections (10-20 nl) of L-glutamate (0.9-1.77 nmol in 0.9% saline, pH 7.4) into the IML at T2 level produced marked tachycardic responses with small changes in contractility and no changes in MAP. On the left side, microinjections of glutamate into the IML produced marked increase in dp/dt and contractility index with small increase in HR and no change in MAP. These responses were smaller at T1 and T3 level and absent at C8 and T4 level. N-methyl-D-aspartic acid (NMDA), 1-100 pmol, induced similar responses. The effects of glutamate and NMDA were blocked by microinjections of GDEE and D-AP7, respectively. This model may prove to be useful in studies involving interactions between brain stem cardiovascular areas and the IML.

Support: NIH (HL 24347) and AHA (NJ).

82.2

EFFECT OF SPINAL GAMMA AMINOBUTYRIC ACID RECEPTOR BLOCKADE ON TONIC AUTONOMIC INPUT TO CARDIOVASCULAR EFFECTORS. W. Laskey* (SPON. G. Melvill Jones) Dept. Physiology McGill University, Montreal, P.Q. H3G 1Y6.

Blockade of spinal gamma aminobutyric acid (GABA) receptors increases arterial pressure (AP) and heart rate (HR) of rats (Gordon, F.J. Brain Res. 328:165, 1985). To determine if GABA mediated inhibition has a supraspinal source, effect of spinal GABA receptor blockade was compared in CNS-intact and spinal rats. Male S-D rats were anesthetized, paralyzed and artificially ventilated. Bicuculline methyl iodide (BMI) was administered intrathecally at the caudal edge of the thoracic cord. AP and HR were monitored via a carotid artery. In CNS-intact rats, 5-10 µg BMI increased AP and HR, but 2.5 µg BMI lowered AP by 43.2 ± 2.2 mmHg (n=6, P 0.01) without changing HR. The depressor, but not the pressor response to BMI was abolished by spinalization. Both responses were abolished by ganglionic blockade (20 mg/kg hexamethonium i.v.). The depressor response was abolished by adrenalectomy. These data suggest that GABA mediated tonic inhibition of sympathetic preganglionic neurons (SPN) controlling the heart and the blood vessels has a major propriospinal component, while that exerted on SPN innervating the adrenal gland is dependent on a supraspinal input to these neurons.

82.3

SEROTONIN REGULATION OF SYMPATHETIC OUTPUT AT THE SPINAL LEVEL: INTRATHECAL STUDIES. V.V. Romita,* and J.L. Henry (SPON: S. Lal) Depts. Physiol & Psychiat., McGill Univ., Montreal, H3G 1Y6

Serotonin was administered intrathecally at the T9 or T2 spinal level in male Sprague Dawley rats (300-350 g) anaesthetized with urethane (2.5 g/Kg). Effects on heart rate and arterial pressure were studied. Administration of 100 nmoles at the T9 spinal level had no effect on heart rate or arterial pressure (n=3); 200 nmoles caused an increase in heart rate to a maximum of 22 ± 6.7 bpm at 2 min after administration (n=13). Arterial pressure was unaffected. At the T2 level 200 nmoles of serotonin increased heart rate by a maximum of 45 ± 8.4 bpm at 2 min (n=14). Again arterial pressure was unaffected. Vehicle failed to change heart rate or arterial pressure at either spinal level (n=31). Prior intrathecal administration of 200 nmoles of methysergide, a serotonin antagonist, partially blocked the effects on heart rate elicited by administration of 200 nmoles of serotonin at the T2 spinal level (n=8). Hexamethonium (10 mg/kg i.v.), a blocker of nicotinic transmission in autonomic ganglia, did not block the effects of serotonin on heart rate (n=4). After intrathecal administration of 15 µL of lidocaine, serotonin was without effect on heart rate or arterial pressure (n=10). These results suggest that serotonin regulates sympathetic output by an action at the spinal level, possibly with a non-nicotinic relay in the sympathetic ganglia. (Supported by a grant from the Quebec Heart Foundation to JLH)

82.4

RELEASE OF TONIC INHIBITION OF SYMPATHETIC OUTPUT BY INTRATHECAL ADMINISTRATION OF BICUCULLINE AND STRYCNINE TO THE T2 SPINAL SEGMENT IN THE RAT Hong, Y.,* K. Yashpal* and J.L. Henry (Spon: S.N. Young), Depts. Physiol. & Pschiatry, McGill Univ., Montreal, H3G 1Y6

Sympathetic preganglionic neurones in the intermediolateral nucleus of the spinal cord are depressed by GABA and by glycine when applied directly by iontophoresis (Brain Res. 277:365, 1983). To determine whether GABA and glycine are involved in tonic inhibition of these neurones the respective antagonists were given intrathecally to male Sprague Dawley rats (approx 350g) anaesthetized with urethane (2.5g/kg i.p.) and implanted acutely with a PE-10 intrathecal catheter passed via the atlanto-occipital junction to the T2 level. A PE-60 catheter in the carotid artery was used to measure arterial pressure and heart rate. After 30 min for stabilization, baseline readings were taken over 5 min and bicuculline methiodide (1 µg; n=6) or strychnine SO4 (25 or 100 µg; n=12) were given (in 10 µL of artificial CSF). Bicuculline increased arterial pressure (2-15 min; peak 10 min) with little or no effect on heart rate. Strychnine increased heart rate (2-11 min; peak 4 min) without an effect on arterial pressure. CSF alone had no effect on either parameter (n=8). These results suggest that GABA and glycine express tonic depression of sympathetic output, specifically regulating arterial pressure and heart rate, respectively.

(Supported by a Quebec Heart Foundation grant to JLH; KY is a Fellow of the Canadian Heart Foundation)

82.5

SUBSTANCE P AND TRH: EFFECTS ON ADRENAL AND NON-ADRENAL SYMPATHETIC PREGANGLIONIC NEURONES IN CAT THORACIC INTERMEDIOLATERAL NUCLEUS. S.B. Backman, H. Sequeira-Martinho* & J.L. Henry. Depts. of Physiol.& Psychiat., McGill Univ., Montreal, Quebec H3G 1Y6.

As substance P (SP) and thyrotropin-releasing hormone (TRH) coexist in nerve terminals apposed to adrenal and non-adrenal sympathetic preganglionic neurones (SPNs) (Brain Res.415:137,1987), in the present study the two types of SPN were compared for their responses to SP and to TRH. Cats were anaesthetized (chloralose), paralyzed (pancuronium bromide) and ventilated artificially. Multibarrelled micropipettes were used to record single unit extracellular spikes in the intermediolateral nucleus of spinal segments T8-T10. Barrels for iontophoresis contained SP (1mM in 0.16M NaCl, pH 5.5), TRH (5mM in 0.16M NaCl, pH 5.5) and control solution (0.16M NaCl, pH 5.5). A unit was classified as SPN if it responded antidromically to electrical stimulation of the greater splanchnic nerve or adrenal medulla, with non-adrenal SPNs responding only to stimulation of the nerve. SP and TRH (30-100 nA) increased the firing rate of 4/7 adrenal, 13/24 non-adrenal SPNs, and of 3/12 adrenal, 10/29 non-adrenal SPNs, respectively. Depression was not observed. Excitation was always typically slow in onset (0.5-1 min) and prolonged in after-discharge (0.5-2.5 min). These data suggest that adrenal and non-adrenal SPNs are excited by SP and TRH. (Support: Quebec Heart Fndn. grant to JLH; Can. Heart Fndn. award to SBB & NATO award to HS-M.)

82.6

STIMULATION OF SUPRASPINAL ALHPA$_2$-RECEPTORS BY CLONIDINE INHIBITS THE PRESSOR RESPONSE TO INTRATHECAL INJECTION OF NEOSTIGMINE IN CONSCIOUS RATS. V. Magri and J.J. Buccafusco. Dept. Pharmacology and Toxicology, Med. Col. Ga. and Vet Admin. Med. Ctr., Augusta, Georgia 30912.

Intrathecal (i.t.) injection of neostigmine (NEO) produces a marked increase in blood pressure in freely-moving rats. The pressor response is mediated through local inhibition of cholinesterase and through the release of spinal acetylcholine (Soc. Neurosci. Abs. 13:745). Our earlier studies have also demonstrated that stimulation of central alpha$_2$-receptors with clonidine inhibits the release of acetylcholine. The purpose of this study was to determine whether i.t. injection of (1-10µg) clonidine could block the pressor response to i.t. injection of NEO. 5µg NEO produced a pressor response (41mmHg) which became maximal by 10-15 min and remained elevated for at least 90 min. Pretreatment with i.t. clonidine did not significantly reduce the pressor response to NEO but delayed its onset. In contrast, 100µg s.c. or 10µg injected into the cisterna magna each significantly reduced the pressor response to i.t. NEO. Supraspinal administration of clonidine also inhibited the behavioral changes to i.t. NEO. These results are consistent with the presence of an ascending spinal cholinergic system sensitive to clonidine inhibition which mediates a hypertensive response following i.t. injection of cholinergic agonists. Supported by HL30046 and the Vet. Admin.

82.7

THE PRESSOR RESPONSE TO SPINAL CHOLINERGIC STIMULATION IN SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE (NT) RATS. N.F. Makari, V. Magri and J.J. Buccafusco. Dept. Pharmacology and Toxicology, Med. Col. Ga. and Vet Admin. Med. Ctr., Augusta, GA 30912.

Several laboratories have demonstrated that central cholinergic stimulation in SHR results in an exaggerated pressor response as compared to NT controls. Recent studies in this laboratory have demonstrated a spinal cholinergic pressor system in the NT rat. The purpose of this study was to determine whether the pressor response to spinal cholinergic stimulation is enhanced in SHR. In freely-moving rats previously implanted with intrathecal (i.t.) and intraarterial catheters, i.t. injection of neostigmine or carbachol (1-5µg) produced a dose-related hypertensive response in both strains of rats. While both agonists produced similar maximal increases in blood pressure in NT rats, the pressor responses to both agonists were significantly greater in SHR, although neostigmine was more effective in this regard. These differences were more apparent at the lower doses where the response to 1µg of drug in the SHR was increased by 77% and 109% of the response in NT rats for carbachol and neostigmine, respectively. The facts that both direct and indirect acting agonists produced greater responses in SHR and that spinal depletion of acetylcholine did not reduce blood pressure in SHR suggest that the spinal cholinergic pathways involved ascend to higher centers. Suppt:HL30046 & Vet. Adm.

82.8

NEUROPEPTIDE Y-, SUBSTANCE P-, AND 5-HT-IMMUNOREACTIVE SYNAPSES IN THE INTERMEDIOLATERAL CELL COLUMN OF RABBIT UPPER THORACIC SPINAL CORD. I.J. Llewellyn-Smith*, D.A. Morilak and J.P. Chalmers* (SPON: M. West). Department of Medicine, Flinders University, Bedford Park,South Australia 5042 Australia.

Two groups of neurons in the rostral ventrolateral medulla oblongata (RVLM), which have spinal projections and may have vasopressor functions, are adrenaline/neuropeptide Y (NPY) neurons and substance P (SP)/5-HT neurons.We sought to establish in rabbit upper thoracic spinal cord whether nerve fibers containing NPY, SP or 5-HT formed synapses in the intermediolateral cell column (IML), where vasomotor sympathetic preganglionic neurons are located. Rabbits were perfused with phosphate-buffered 4% formaldehyde, 0.5% glutaraldehyde with or without 0.2% picric acid, and rapidly post-fixed with microwaves. Vibratome sections (80um) of T4-T6 were washed in 50% ethanol, stained to reveal NPY-, SP- and 5-HT-immunoreactive nerve fibers using an avidin-biotin-horseradish peroxidase detection system, and processed for electron microscopy. At the light microscope level, the IML in T4-T6 contained dense plexuses of varicose NPY-, SP- and 5-HT-immunoreactive nerve fibers. At the electron microscope level, all three types of fibers made synapses with clustering of vesicles and membrane densities.

These observations are consistent with a direct synaptic connection between RVLM neurons containing NPY, SP, or 5-HT and sympathetic preganglionic neurons, which could mediate the pressor responses seen upon stimulation of the RVLM.

82.9

ULTRASTRUCTURAL EVIDENCE OF PEPTIDERGIC AND MONOAMINERGIC INPUTS TO SYMPATHETIC PREGANGLIONIC NEURONS (SPNs) IN THE INTERMEDIOLATERAL CELL COLUMN (IML) OF THE RAT AND RABBIT. P.L.Vera,K.E.Miller & V.R.Holets, University of Miami, Dept. of Neurological Surgery, Miami, Fl. 33136.

Peptidergic and monoaminergic processes have been located within IML, closely apposed to retrogradely labeled SPNs (Holets & Elde,1982,Neurosci.,7:1155;Vera et al.;Soc.Neurosci.Abs.,1987,13:734). However, anatomical evidence for actual synaptic contacts between descending transmitters and SPNs can only be obtained at the ultrastructural level. The object of this study was to examine the relationship between different peptidergic and monoaminergic transmitters and SPNs located within IML of the rat and rabbit, in an attempt to identify direct synaptic contacts between the two systems.

In order to identify SPNs in the rat (n=4), the cervical sympathetic trunk was exposed to HRP. In the rabbit (n=4), IML can be clearly identified within the lateral horn at upper thoracic segments, where it is partially separated from the intermediate grey matter by the intermediolateral fasciculus. Animals were perfused with 4% paraformaldehyde and vibratome sections (40-50 um) of segments T2-T4 were taken. In the rat, tissue was processed for HRP histochemistry. Subsequently, Substance P-, enkephalin-, and serotonin-like immunoreactivity was visualized using an immunoperoxidase reaction. The sections were then osmicated and embedded for electron microscopy.

In the rat, labeled terminals for each transmitter were observed in contact with retrogradely labeled processes. In the rabbit, labeled terminals were found on presumptive SPNs within IML forming both somatic and dendritic terminals.

Funded by The Miami Project.

82.10

COEXISTENCE OF NEUROCHEMICALS IN VENTRAL MEDULLARY CELLS THAT PROJECT TO THE INTERMEDIOLATERAL CELL COLUMN (IML) OF THE RAT. C.A. Sasek, M. Galeazza#, M. Wessendorf and C.J. Helke. Uniformed Services Univ. of the Health Sci., Bethesda, MD 20814.

Substance P (SP), serotonin (5HT), thyrotropin releasing hormone (TRH) & enkephalin (ENK) are contained in neurons of the ventral medulla, an area involved in cardiovascular regulation. SP, 5HT & TRH neurons project to the IML. ENK containing neurons also project to the spinal cord but their exact site of termination is unknown. Each of these neurochemicals has been localized in fibers in the IML. It is not known if SP & TRH; TRH, SP & 5HT; or ENK & TRH, SP or 5HT coexist in ventral medullary IML projecting cells. Consequently, we examined the extent of coexistence of SP, 5HT, TRH and ENK in ventral medullary cells that project to the IML.

Rats received 40nl injections of rhodamine labeled microspheres into the T3 IML. Colchicine was given ICV 4d later, and 24 h later animals were perfusion fixed. Tissue was sectioned at 4um. Using dual color immunofluorescence, adjacent sections were immunostained with a combination of a monoclonal rat SP antibody and a rabbit antiserum to either 5HT, sequence 160-169 of pre-pro-TRH or ENK.

ENK, 5HT, SP and TRH were each localized to IML projecting cells in ventral medullary nuclei, including N. reticularis magnocellularis pars alpha, N. reticularis paragigantocellularis lateralis and paraolivary N. Some cells in these nuclei that colocalized SP & 5HT; SP & TRH; or SP, TRH & 5HT also projected to the IML. Coexistence of ENK with the other neurochemicals in IML projecting cells occurred only rarely. These results indicate that ventral medullary sites important in cardiovascular regulation contain neurons that project to the IML and that can be subdivided based on their neurochemical content.

82.11

LOCALIZATION OF TYROSINE HYDROXYLASE (TH) AND PHENYLETHANOLAMINE N-METHYLTRANSFERASE (PNMT) CONTAINING CELLS IN THE MEDULLA OF THE DOG. G.A. Iwamoto, J.H. Mitchell*, M. Sadeq* and G.P. Kozlowski, Dept. of Veterinary Biosciences, Univ. Illinois, Urbana, IL. 61801 and Dept. of Physiology, U.T. Southwestern, Dallas, TX. 75235.

TH and PNMT containing cells of ventrolateral medulla are associated with cardiovascular control in many species. The TH cells are identified with the A1 area and associated with depressor responses while the PNMT cells (C1 area) are associated with pressor responses. Although it is often the species of choice for cardiovascular studies, no one had attempted to localize these areas in the dog. Using immunocytochemical methods, we found that the TH containing cells of the dog were localized much as they are in both cat and rat with two major concentrations of cells, one located in the ventrolateral and the other in the dorsomedial medulla. The ventrolateral group (A1) were located within 1.5-2mm of the ventrolateral surface and extended from the pontomedullary border to a few mm caudal to the obex. The dorsomedial group (A2) was concentrated in the region just medial to the solitary tract at levels ranging from the obex to the rostral pole of the inferior olive. The PNMT containing cells groups were different in the dog compared with the cat and rat. The ventrolateral cells (C1), as in the rat, but not the cat,were found in a column-like group which ranged from the level of the obex to the pontomedullary border. The dorsomedial group (C2) was far less extensive than either cat or rat, consisting of only a few cells in the area occupied by the A2 group. (Supported by HL 06296 and HL 37400)

82.12

FUNCTIONAL MAPPING OF THE CANINE ROSTRAL VENTROLATERAL MEDULLA (RVL) WITH L-GLUTAMATE AND PHENYLETHANOLAMINE N-METHYLTRANSFERASE (PNMT) IMMUNOHISTOCHEMISTRY. **KJ Dormer, DA Ruggiero, M Anwar* and SR Ashlock*. Physiol. & Biophy., Univ. Okla. HSC, Okla. City 73190 and Lab. Neurobiol., Cornell Univ. N.Y., N.Y. 10021.**

Coincident with kainic acid RVL lesions and their performance sequelea in the conscious dog it was necessary to match function with the analagous C-1 cell group. To demonstrate cardiorespiratory function we injected 100 mM L-glutamate into areas that electrically increased arterial pressue (AP) and respiration. Mongrel dogs (13-18 kg) were anesthesized with alpha-chloralose and instrumented to record AP, heart rate (HR) and respiration. Inductive plethysmography (Respitrace) monitored spontaneous chest and abdomenal excursions. Stereotactic injections of L-glu increased AP 12-35 mmHg and decreased HR 15-25 bpm. Respiratory rate and depth were increased 10-20% above resting control. Responses were limited to a region extending 2 mm rostral-caudal just ventral to the n. retrofacialis. Immunohistochemical staining of RVL for PNMT in other dogs revealed a diffuse population of cells extending in a 4+ mm horizontal column, 4 mm lateral to obex, including the retrofacial zone that elicited excitatory cardiorespiratory responses. Summary: only a limited portion of the diffuse PNMT-containing cells in the dog RVL has vasomotor function and these cells are intermingled with cells exhibiting respiratory control.

82.13

A NEW SYMPATHOINHIBITORY AREA IN CAT MEDULLA: EFFECTS ON CARDIOVASCULAR TONE AND BAROREFLEX. C.W. Dempesy and D.E. Richardson. Lab. of Neurosurgery, Tulane Univ. Sch. of Med., New Orleans, LA 70112

We report a new vasodepressor area (VDA) located superior to the rostral ventrolateral medullary area (RVL) that maintains vasomotor tone. Specifically, the VDA occurs at the level of the caudal pole of the nucleus paragigantocellularis lateralis, dorsal to the retrofacial nucleus, and medial to the infratrigeminal nucleus. Microinjections of glutamic acid (Glu, 5 nmol) into the VDA induce striking hypotension and bradycardia, and indicate that the cell group involved is narrowly confined. The Glu responses are unaffected by either atropine (Atr, 1.2mg i.v.) or bilateral vagotomy, but are blocked (90%) by combined propranolol & regitine treatment (1mg & 3mg respectively/kg) or cervical cord transection. Bilateral microinjections of either muscimol (100 pmol) or kainic acid at toxic dose (KA, 200 pmol) into the VDA induce long-term elevation of blood pressure tone. Baroreflex (BR) chemically induced with phenylephrine (30 ug, i.v.) was shown with Atr (i.v.) to be 80% (± 10%) sympathetically mediated. This part of the BR could be eliminated by bilateral microinjections of KA (toxic dose) into the VDA. These data suggest that the VDA provides inhibitory regulation of the RVL and functions as part of the baroreceptor reflex arc. The relationship between the VDA and sites reported to possess similar properties in the caudal ventrolateral medulla has yet to be determined.

82.14

BLOOD PRESSURE MEDIATED CHANGES IN EPINEPHRINE, NOREPINEPHRINE, ASCORBATE AND URATE RELEASE FROM ROSTRAL VENTROLATERAL MEDULLA OF RAT MEASURED BY IN VIVO DIALYSIS. D. Bhaskaran, P.A. Mason and C.R. Freed. Depts. of Med. and Pharm., Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

We have previously used in vivo electrochemistry (EC) to study blood pressure induced changes in neurochemical release from rostral ventrolateral medulla (C1 area) (Neurosci abst. 227.12, 1987). Phenylephrine (PHEN)-induced hypertension led to a fall in the catechol peak and an increase in the hydroxyindole peak while nitroprusside (NITRO)-induced hypotension was associated with an increase in the catechol peak and a reduction in the indole. Because of ambiguity in the signal measured by EC, we have repeated these experiments using in vivo dialysis to recover neurochemicals for assay by HPLC. Male Sprague-Dawley rats, 300-350 g, anesthetized with urethane had femoral artery and vein catheterized. An in vivo dialysis probe 300 μ diameter with tip length 500 μ was implanted in the C1 area and perfused with Ringer's at a rate of 1.5 μl/min. Blood pressure was changed with infusion of PHEN or NITRO for one hour. The dialysate was analyzed for epinephrine, norepinephrine, ascorbate and urate. Preliminary results showed that PHEN reduced norepinephrine and epinephrine release while NITRO increased the concentration of both catechols. Uric acid and ascorbic acid concentrations were unchanged by either treatment.

82.15

Pressor Systems Involved in the Maintenance of Arterial Pressure After Lesion of the Rostral Ventrolateral Medulla (RVLM). K.L. Cochrane* and M.A. Nathan. University of Texas Health Science Center, San Antonio, TX 78284-7764.

In an earlier study (Cochrane et al., 1988), we found that hypotension after electrolytic lesion of the RVLM is anesthetic-dependent. The current study was designed to examine the pressor systems which may support mean arterial pressure (MAP) after RVLM lesion in pentobarbital (50 mg/kg iv) anesthetized rats. In protocol 1, sequential administration of captopril (5 mg/kg, iv) reduced MAP from 103±5 mmHg to 87±5 mmHg and a vasopressin antagonist (AVPX, 30 ug/kg, iv) reduced MAP to 62±5 mmHg. Spinal transection (ST) further decreased MAP (39±2 mmHg). In protocol 2, sequential administration of AVPX lowered MAP from 117±5 mmHg to 102±7 mmHg and captopril further reduced MAP to 55±5 mm Hg. The MAP was subsequently reduced after ST (33±1 mmHg). In protocol 3, the kidneys were bilaterally denervated prior to RVLM lesion. Renal denervation decreased MAP from 112±5 mmHg to 89±3 mmHg. RVLM lesion subsequently decreased MAP to 64±5 mmHg. AVPX (51± mmHg), captopril (35±4 mmHg) and ST (26±3 mmHg) did not significantly decrease MAP. The results show that after RVLM lesion, normotension is maintained by the neural release of renin and the subsequent synthesis of angiotensin II. Supraspinal sympathetic activity is abolished after RVLM lesion and renal denervation. Therefore, an area lying outside of the RVLM maintains MAP via the neuroendocrine vasoconstrictor effects of the renin-angiotensin system (Supported by HL33635).

82.16

CAROTID ARTERIAL RESISTANCE REDUCED BY STIMULATION OF AN AREA DORSAL TO THE FACIAL NUCLEUS OF CATS. J. S. Kuo*, M. R. Wang* and C. Y. Chai* (SPON: M. J. Wayner). Dept. of Med. Res., Veterans Gen. Hosp., Dept. of Physiol. and Biophysics, Natl. Def. Med. Ctr. and Inst. of Biomed. Sci., Academia Sinica, Taiwan, Republic of China.

Electrical stimulation of or glutamate injection into a small area just dorsal to the facial nucleus (DFA) elicited an ipsilateral reduction in common carotid resistance (CCR-reduction) with no or minimal change in other cardiovascular parameters in chloralose-urethane-anesthetized and pancuronium-paralyzed cats. The maximal reduction was elicited by electrical stimulation at 20-80 Hz and 0.2-0.8 ms or by glutamate injection at dose of 50-10 nmol. The CCR-reduction was completely abolished by hexamethonium (5 mg/kg, i.v.). It was potentiated dose-relatedly by physostigmine (0.03-0.06 mg/kg, i.v.), but partially attenuated by atropine at a dose as high as 2 mg/kg, i.v.. It was largely reduced by ipsilateral section of the facial nerve root and completely abolished by additional interruption of the glossopharyngeal nerve root. However, CCR-reduction was not abolished by ipsilateral cervical sympathectomy. These findings suggest that DFA-induced CCR-reduction is mediated via facial and glossopharyngeal nerves, involving partially muscarinic and partially non-muscarinic mechanisms. CCR-reduction may not result from inhibition of sympathetic system.

82.17

THE EFFECT OF STIMULATION OF THE PERIAQUEDUCTAL GRAY ON CARDIOVASCULAR RESPONSES IN THE RABBIT. A. Wilson* and B.S. Kapp, Dept. of Psychology, University of Vermont, Burlington, VT 05405.

The midbrain periaqueductal gray (PAG) has been implicated in cardioregulation in the rabbit (Tan and Dampney, 1983), a finding consistent with the demonstration of a projection originating primarily from the posterior ventral PAG to the solitary complex in this species (Wilson and Kapp, 1987). This experiment was designed to determine the effects of electrical and chemical stimulation of the ventral posterior PAG on heart rate and blood pressure in the anesthetized rabbit.

Chloralose-anesthetized, paralyzed New Zealand rabbits were prepared with triple-barrel micropipettes through which electrical stimulation (75-150uA, 100 Hz, 1.0 sec), glutamate (1.0M; 100-200nl) or saline could be administered. Electrical stimulation of the posterior, ventral PAG invariably elicited bradycardia (40-60BPM, latency ≅ 1.0 sec) immediately preceding and accompanied by pressor responses (10mm Hg). Glutamate injected at the same sites elicited bradycardia (25-60 BPM, latency 7-12 sec) and typically depressor responses (15-30mm Hg). These results indicate that activation of ventral PAG cell bodies elicits bradycardia and that the differing blood pressure responses may reflect, in part, fiber activation by electrical stimulation. Supported by AHA grant 861277.

82.18

LESIONS OF THE PARABRACHIAL NUCLEI DEPRESS RECOVERY OF ARTERIAL PRESSURE FOLLOWING HEMORRHAGE. D.G. Ward and J.H. Ward*. H. M. Ward Memorial Lab., Valley Home, CA 95384.

The role of the parabrachial complex (PBN) in control of arterial pressure was examined in 53 conscious freely-behaving male rats. On Day-0, under ketamine anesthesia (150 mg/kg), a femoral arterial catheter was exteriorized to a spring tether attached between the scapulae. Bilateral lesions were directed toward either the lateral PBN or the medial PBN. One group of animals served as a control. Mean arterial presure (MAP), basal water intake and urine volume were measured daily for five days. On Day-2 mean arterial pressure (MAP) was measured for 20 min following a 15 ml/kg·3 min hemorrhage (HEM). The shed blood was reinfused and HEM was repeated on Day-5. MAP was significantly higher in lateral PBN animals than in medial PBN animals on Day-5 and Day-2. However, there were no significant differences within groups between Day-5 and Day-2 in recovery of MAP following HEM. Lateral PBN animals were associated with greater decreases in MAP following HEM than either medial PBN animals or controls.

	Day-5 PreHEM	Day-2 PreHEM	0 m post	20 m post
Control	110 ±9	115 ± 6	-49 ±15	-11 ± 8
Medial	102 ±9	107 ±11	-47 ±21	-14 ±10
Lateral	130 ±9	118 ±11	-62 ±15	-28 ±11

Water intake and urine volume were not significantly different among the three groups throughout the five days of measurement. Thus, in the freely-behaving rat neural elements in the lateral PBN play a demonstrable role in control of arterial pressure. (supported by USPHS HL36034)

82.19

Pressor Responses from the Parabrachial Complex: Lack of Correspondence Between Electrical and Glutamate Stimulation. F.J. Gordon, Dept.of Pharmacology, Emory Univ. School of Medicine, Atlanta, GA 30322.

The purpose of these studies was to determine if activation of neurons in the parabrachial complex (PBC) with L-glutamate would elicit cardiovascular responses similar to those obtained using electrical stimulation. Rats were anesthetized with urethane, paralyzed and respirated. In 11 rats, 30 sites in the PBC were tested using multibarrel glass pipettes containing a carbon fiber for electrical stimulation (20 sec trains, 100 uA, 0.5 msec). Microinjections (50 nl) of L-glutamate (20 and/or 200 mM) or artificial cerebrospinal fluid (ACSF) were then delivered from adjacent pipette barrels. Electrical stimulation (10-80 Hz) yielded frequency-dependent increases in mean arterial pressure (MAP) ranging from 7 ±2 to 54 ±3 mmHg. Microinjections of 20 and 200 mM L-glutamate increased MAP by only 4 ±1 mmHg (n = 25) and 5 ±1 mmHg (n = 14) respectively. These changes in MAP, although small, were significantly different ($p < .01$) from those produced by ACSF (1 ±1 mmHg; n = 10). Virtually identical results were obtained from a subset of sites in the Kolliker-Fuse nucleus (n=7). These results suggest that pressor responses evoked by electrical stimulation of the PBC are mediated predominantly by activation of fibers of passage because presumed activation of cell soma by L-glutamate produced only minimal changes in arterial pressure.

82.20

LOCUS COERULEUS NEURONS ARE ACTIVATED BY RIGHT ATRIAL STRETCH RECEPTORS. J.H. Jhamandas, S. Kaufman* and R.J. Reiffenstein. Depts. of Medicine and Pharmacology, University of Alberta, Edmonton, AB, Canada, T6G 2B7.

Previous studies have indicated that rat locus coeruleus (LC) neurons respond by reciprocal alterations in firing rate to changes in blood pressure. We have assessed the influence of activating cardiac atrial receptors on the excitability of LC neurons.

Extracellular recordings were obtained from identified LC neurons in urethane anaesthetized Long-Evans rats. These animals had been previously implanted with inflatable, indwelling balloon catheters with the tip located at the superior vena caval (SVC)/right atrial (RA) junction. Activation of right atrial receptors, consequent to inflation of the balloons with 0.05 ml of physiological saline, evoked a prompt but unsustained increase in the firing rate of 13 out of 17 LC cells without a significant change in mean arterial blood pressure. Histological verification of LC recording sites and the position of balloon catheters within the SVC/RA junction was performed in each animal.

Central projections of cardiac receptors beyond the level of the medulla need further study; the present results indicate that the LC may be an important site for transfer of cardiovascular-related information from right atrial baroreceptors throughout the CNS.

Supported by the MRC of Canada and Alta. Heart Fdn.

ALCOHOL II

83.1

EFFECTS OF ACUTE AND CHRONIC ETHANOL EXPOSURE ON CRAYFISH BEHAVIOR AND SYNAPTIC TRANSMISSION. J. A. Blundon and G. D. Bittner. Dept. Zoology and Inst. for Neurol. Sci., Univ. TX, Austin, TX 78712.

The effects of ethanol (EtOH) exposure on behavior and synaptic transmission were examined in crayfish (*Procambarus clarkii* and *P. similans*). Hemolymph (blood) concentrations of EtOH of 50 - 100 mM produced behavioral signs of intoxication in crayfish (i.e. significant increases in righting reflex times and significant decreases in tail-flip escape responses) within 6 hours. Crayfish showed signs of tolerance to 50 mM EtOH after 2 weeks as determined by a return to control behaviors. Crayfish exhibited withdrawal symptoms of hyperactivity following removal of EtOH from aquarium water after 4 weeks of exposure. Data previously collected in our laboratory (Friedman *et al.*, 1988) suggests that EtOH has a concentration dependent biphasic effect on synaptic transmission in the opener excitor neuromuscular junction of the crayfish walking leg. Acute applications of 10 - 100 mM to the opener neuromuscular junction produced an increase in the probability of neurotransmitter release as measured by an increased frequency of spontaneously released neurotransmitter quanta. EtOH concentrations of 300 - 600 mM resulted in a decrease in excitatory junction potentials due to decreased postsynaptic membrane resistance. We are currently comparing effects of acute EtOH application on synaptic transmission in crayfish showing signs of intoxication, tolerance and withdrawal. Supported by NIAAA grant #AA07746.

83.2

INCREMENT OF CORTICAL BRAIN MICROVASCULARISATION FOLLOWING AGING, CORTICAL INSULT AND CHRONIC ALCOHOLISATION. M. Gewiss*, Ch. Heidbreder* and Ph. De Witte (SPON :European Brain and Behavior Society). Lab. Psychobiology,Univ. Louvain, B-1348 Louvain-la-Neuve.

Among the experimental models used to maintain high blood ethanol levels in animals chronically, the inhalation procedure seems to be one of the most appropriate to obtain constant high blood ethanol level. After sejourning 2 to 4 weeks into the alcoholised chamber rats were then perfused with a nuclear emulsion allowing to reveal the brain vascularisation. All the vessels, including terminal and lateral branches were measured and the lengths were summed up (in squares of .000625 mm² of the cerebral cortex). These measurements were also performed on brain adult animals after lesioned by aspiration and on rat brain aged of 2 years old. Our results show that cortical microvascularisation network increases after 2 weeks staying into the alcoholized chamber to remain at the same hypervascularization level after 4 weeks alcoholization. It has to be noticed that animals present this cortical hypervascularization before preferring the alcoholized bottle in a free test (water vs. alcohol 10% v/v). A similar enhancement of the microvascularization network was observed on aged brain animals and at day 6 after a cortical insult. These results thus tend to show that alcoholised adult animals present the same vascular evolution when compared to chronological aged animals and to animals which were brain insulted.

83.3

REGIONAL BRAIN METABOLISM DURING ALCOHOL INTOXICATION IN HUMAN SUBJECTS MEASURED WITH PET. N.D. Volkow, A.P. Wolf*, R.H. Hitzemann, S. Dewey, J. Logan, B. Bendriem*, and S. Vitkin*. Brookhaven National Laboratory, Upton, NY 11973.

We investigated the effects of acute alcohol administration on regional brain glucose metabolism in healthy volunteers (n = 6). Measurements of regional brain metabolism were done using 5-6 mCi of ^{18}F-deoxyglucose (FDG) on the PET VI. Subjects were scanned twice on consecutive days. The first scan was done under baseline conditions (eyes open, ears unplugged). For the second scan, subjects were given 1 g/kg 98% ethanol p.o. FDG was administered 30 min later. Global glucose metabolism in the brain decreased an average of 10% following alcohol ingestion. Regional changes in brain metabolism showed that the decrease occurred in cortical areas and in the cerebellum. Relative changes in glucose metabolism were quantified by dividing the metabolic activity in the region by the metabolic activity in the central slices. The basal ganglia showed a relative increase in metabolism. The average relative values for left and right basal ganglia during baseline were 1.28 and 1.23 and, during intoxication, 1.45 and 1.40, respectively. The changes in metabolism after alcohol intoxication were different from the changes reported in cerebral blood flow (CBF), suggesting regional uncoupling of CBF and glucose metabolism by alcohol. Research supported by U.S. DOE-OHER NIH NS-15638 and Contract No. DE-AC02-76CH00016.

83.4

ETHANOL SUPPRESSES ACTIVITY OF RHYTHMICALLY BURSTING NEURONS OF THE MEDIAL SEPTAL AREA IN FREELY MOVING RATS. B.S. Givens and G.R. Breese. Univ. North Carolina Chapel Hill, NC 27514.

The medial septal area (MSA) is thought to play a role in the sedative effects of ethanol. In urethanized rats, ethanol suppresses firing in MSA cells and disrupts the rhythmically bursting pattern of activity. We report here the effects of ethanol on MSA neurons in freely moving rats.

Rats are fitted with head-mounted microdrives then placed on a rotorod apparatus and attached to standard recording equipment. After isolation of a single MSA cell, rats are injected with ethanol (0.75, 1.5, or 3.0 g/kg i.p.). There is a dose-dependent decrease in firing to ethanol in most neurons. The decrease is dose-related and lasts 30-120 minutes. The bursting pattern of activity is maintained in most cells at the 0.75 and 1.5 g/kg dose, but the burst frequency decreases from 6.2 to 5.0-5.7 bursts/second. At 3.0 g/kg, ethanol severely disrupts neural activity which then returns simultaneously with behavioral recovery.

Acute administraion of ethanol in freely moving rats results in similar changes in MSA neuronal activity as observed in the urethanized rat. Supported by NCARA fellowship #8611.

83.5

PROTEIN KINASE C AND ETHANOL-INDUCED UP-REGULATION OF CALCIUM CHANNELS IN PC12 CELLS.
R.O.Messing, A.B. Sneade*. Department of Neurology and Gallo Research Center, Univ. of California, San Francisco, CA 94110.

Long-term exposure to ethanol increases voltage-dependent Ca uptake and the number of binding sites for Ca channel antagonists in PC12 cells. Since activation of protein kinase C (PKC) modulates Ca currents in several tissues, we investigated whether PKC is involved in ethanol-induced Ca channel up-regulation in PC12. PC12 cells grown in 200 mM ethanol for six days showed an increase in ^{45}Ca uptake of 82 ± 5% over untreated control cells, whereas cells grown in ethanol plus the PKC inhibitor sphingosine (1 μM) showed an increase of only 40 ± 2% ($p < 0.002$ compared to ethanol-treated cells). N-Acetylsphingosine (1 μM), a sphingosine derivative that does not inhibit PKC did not significantly reduce the response to ethanol. Sphingosine alone did not alter ^{45}Ca uptake in cells cultured without ethanol. Exposure to 200 mM ethanol for 6 days increased the V_{max} for PKC in detergent-solubilized cell extracts by 66 % (148 ± 6 in control vs. 245 ± 9 pmol/min/mg of homogenate in ethanol-treated cells; $p < 0.02$). These findings suggest that Ca channel up-regulation during chronic ethanol exposure is mediated by PKC. Increased PKC-dependent phosphorylation could possibly result from an increase in the amount of PKC present in ethanol-treated cells.

83.6

CHRONIC ETHANOL INTOXICATION EFFECTS ON LIMBIC ELECTROPHYSIOLOGIC ACTIVITY.
R.K.Harper*, R.M.Harper and H.L. Lesse (SPON: S. Eiduson). Depts of Psychiatry and Anatomy, UCLA, Los Angeles, CA 90024.

We assessed the effects of acute and chronic ethanol administration on excitability of limbic structures by measuring the threshold and duration of electrically induced afterdischarges (AD) in kindled cats. Electrodes were placed in amygdala and hippocampus; jugular vein was cannulated. AD thresholds and durations were determined following acute injections of 1.6 g/kg 15% ethanol and during 10 day chronic ethanol periods (determinations at 1 and 24 h). Marked elevations in AD threshold and reductions in both AD duration and propagation occurred at 1 h only. Control condition tonic-clonic convulsions were replaced by minimal mouth movements and transient arrest reactions, demonstrating protective action against limbic seizures. On 4th, 6th, 8th and 10th day after onset of chronic ethanol, 1 h thresholds decreased and durations increased relative to saline and ethanol baseline tests; behavioral response was limited to brief automatisms. No 24 h effect. Following alcohol abstinence, anti-convulsant effects of ethanol recovered to 1 h baseline; 24 h tests showed no change. Behavioral signs of intoxication diminished in degree over chronic ethanol days and remained reduced throughout post-chronic challenges. Thus, ethanol exerts a short-term effect on limbic excitability; tolerance and sensitization attenuate ethanol's initial depressant actions.

83.7

EFFECT OF ALCOHOL AND NICOTINE ON CYTOTOXIC RESPONSE OF HUMAN LYMPHOCYTES.
M.P.N. Nair*, Z.A. Kronfol and S.A. Schwartz*. The University of Michigan, Ann Arbor, MI 48109.

The in vitro effect of ethanol (EtOH) and nicotine on natural killer (NK), antibody dependent cellular cytotoxic (ADCC), and lympokine activated killer (LAK) cell activities and immunoglobulin synthesis by normal lymphocytes was investigated. Lymphocytes precultured with EtOH at concentrations of 0.4 and 0.6% (V/V) produced significant suppression of NK (K562, U937 and HSB targets) and ADCC (SB target) activities. In target binding assays, EtOH decreased the target binding capacity of effector cells. The generation and lytic capacity of LAK cells were also significantly affected by EtOH when added at the initiation of culture. Nicotine at concentrations of 5 and 10 ug/ml, produced significant inhibition of NK activity. Nicotine (2 ug/ml) and EtOH (0.01, 0.1 and 0.2%) at noninhibitory concentrations when added separately, showed significant suppression of NK activity when used in combination. Addition of nicotine to a pokeweed mitogen induced B cell activation system resulted in suppression of Ig synthesis by normal lymphocytes. These studies suggest that EtOH and nicotine may have significant immunomodulatory effect on human lymphocytes that may be of clinical relevance.

83.8

GABA-MEDIATED INHIBITION OF CA1 POPULATION SPIKES IN HIPPOCAMPAL SLICES OF CHRONIC ETHANOL-TREATED RATS.
R. A. Palovcik, B. E. Hunter and D. W. Walker, Department of Neuroscience, University of Florida and V. A. Medical Center, Gainesville, FL 32610.

Hippocampal slices from chronic ethanol-treated (CET) and sucrose-fed control (SUC) rats, cut 400 microns thick from septal and temporal hippocampal poles, were maintained in a Haer slice chamber. CET rats were fed a nutritionally complete diet containing ethanol for 20 weeks followed by an eight week withdrawal period. SUC control rats were pair fed the same diet but with sucrose isocalorically substituted for ethanol. Stimulation of stratum radiatum (SR) with a bipolar electrode produced an evoked population spike, recorded extracellularly in stratum pyramidale (SP). The same recording, iontophoretic, and stimulating electrodes were used on slices from CET-SUC pairs to directly compare measured electrophysiological parameters.

Population spike responses in temporal pole slices of SUC rats were of lower amplitude than those in septal pole slices of SUC rats whereas CET rats had comparable amplitudes of evoked action potentials in both septal and temporal poles. Iontophoretic ejection of GABA into SP was more effective at blocking SR-evoked action potentials than ejection into SR or stratum oriens (SO) in both CET and SUC rats. There were no significant differences in the effect of GABA iontophoresis between CET and SUC rats in septal vs temporal hippocampal poles. Ejection of bicuculline enhanced total population spike amplitude more when applied to SP than SO or SR. Duration of bicuculline enhancement of population spikes was longest for temporal pole slices from SUC rats, whereas CET rats showed equivalent effects of bicuculline on septal and temporal poles. The overall effects of bicuculline on amplitude of population spikes were greater for CET than for SUC rats in both septal and temporal poles.

Alterations in responsiveness of ventral hippocampal slices between CET and SUC rats revealed by bicuculline reflect changes in GABA circuitry of the hippocampus that do not alter postsynaptic responsiveness of CA1 pyramidal neurons to GABA.

Supported by the Veterans Administration, Grants NIAAA AA00200 and RCDA AA00065 (BEH).

83.9

EFFECTS OF ETHANOL ON PURKINJE NEURONS IN RAT CEREBELLAR BRAIN SLICES.
William R. Proctor and Thomas V. Dunwiddie. Dept. of Pharmacology, Univ. of Colo. Hlth. Sci. Ctr. and Veterans Administration Med. Ctr., Denver, CO.

The acute effects of superfusion with ethanol were characterized on cerebellar Purkinje neurons recorded intracellularly in rat cerebellar slices (350 μm, sagittal sections from the vermis). Perfusion with 100 mM ethanol had minimal effects on the resting membrane potential (0.4 ± 0.2 mV hyperpolarization), and on the membrane input impedance (2.0 ± 2.7% reduction). Complete current/voltage curves for 2 cells examined before, during and after washout of ethanol were essentially identical. In addition, synaptic responses elicited by stimulation of the climbing fibers, showed only a small reduction in the epsp amplitude (10 ± 8.1%). A number of the neurons showed a characteristic pattern of firing consisting of periods of highly regular 30-60 Hz firing, which gradually became more irregular, and was then followed by a rapid hyperpolarization (6-10 mV). This was followed by a relatively long (5-10 sec) period of electrical silence during which the membrane gradually depolarized back to baseline, which was followed by a return to repetitive firing (see Llinas and Sugimori, J. Physiol. 305:171, 1980 fig. 11). Ethanol consistently modified this pattern by shortening the period of repetitive spiking. Passing a constant current through the intracellular recording electrode under control conditions to depolarize or hyperpolarize the cell did not reproduce the firing pattern produced by ethanol. The pattern of firing during the active periods, and the shape of the action potential showed no significant effect of ethanol treatment. These results suggest that ethanol treatment (100 mM) has little direct effect on the membrane potential or input impedance of Purkinje neurons, but might modify either the calcium or potassium conductances that underlie the periods of repetitive firing and the subsequent inhibition respectively.

Supported by Veterans Administration Medical Research Service.

83.10

CONTENTS OF SEROTONIN, DOPAMINE AND THEIR METABOLITES IN THE CNS OF HIGH- AND LOW-ALCOHOL-DRINKING LINES OF RATS.
J.M. Murphy, M.A. Gongwer*, W.J. McBride, L. Lumeng* and T.-K. Li* Inst. Psych. Res. & Regenstrief Inst., Indiana Univ. Sch. Med. & VA Med. Ctr., Indianapolis, IN, 46223.

This study was designed to test the hypothesis that voluntary, high alcohol intake is associated with an imbalance of serotonin (5HT) and dopamine (DA) systems in certain CNS regions. Levels of 5HT, DA and their metabolites (5HIAA, DOPAC and HVA) were assayed in CNS regions of two rat lines selectively bred from the N/Nih heterogeneous stock for high- or low-alcohol-drinking (HAD or LAD) behavior. Contents of 5HT and/or 5HIAA were lower ($p<0.05$) for the HAD compared with the LAD line (N=11-12/line) in 3 regions studied thus far: nucleus accumbens (ACC; 3.1±0.1 vs 3.6±0.1 nmol/g for 5HIAA); frontal cortex (7.3±0.1 vs 8.0±0.2 nmol/g for 5HT) and posterior striatum (4.6±0.2 vs 5.6±0.3 nmol/g for 5HT and 3.3±0.1 vs 4.1±0.2 nmol/g for 5HIAA). Only the ACC yielded a difference ($p<0.05$) between the two lines in the contents of DA (46.4±0.2 for HAD vs 51.1±0.8 nmol/g for LAD), DOPAC (5.1±0.2 for HAD vs 6.0±0.1 nmol/g for LAD), and HVA (2.4±0.1 for HAD vs 3.0±0.1 nmol/g for LAD). The data are consistent with the hypothesis that abnormal functioning of certain 5HT and DA systems are associated with high alcohol drinking behavior. (Supported by AA-03243 and AA-07462.)

83.11

ETHANOL, BRAIN DOPAMINE CONDENSATION PRODUCTS, AND DOPA IN THE DIET. M.A. Collins, N. S. Ung-Chhun*, D. Pronger* and B. Cheng*. Biochem., Loyola Med. Sch., Maywood IL 60153.

We suggest that diet may be responsible in part for reported increases in rat brain levels of salsolinol (SAL), a condensation product of dopamine (DA) and acetaldehyde (AcH), following chronic ethanol (EtOH). With capillary GC/MS and deuterated standards, we assayed SAL and related condensation products, as well as DA and DOPAC, in brain areas of rats ingesting EtOH (6 mo; 10-14 g/kg/d)--but in a nutritionally complete liquid diet rather than in water with rat chow, as per previous researchers. In contrast, EtOH resulted in either no changes or moderate decreases in regional levels of SAL and other condensation products, while striatal DA and DOPAC were reduced 1/3. Concerned that the diets with EtOH may be a reason for the discrepancy over SAL, we analyzed chow and liquid diet for DOPA, SAL and related amines. Quantities of DOPA and SAL were found in chow, but not in liquid diet. In the EtOH/chow studies, we suspect that sustained intake of rat chow DOPA provided, upon decarboxylation, a unique pool of DA which reacted in the EtOH-ingesting rat with circulating AcH. Of significance is that this may have occurred at the brain capillary endothelial barrier rather than within neurons, and EtOH might even form AcH at this site also. Rat chow SAL could further contribute, but dietary DOPA was probably the key factor. Supported by NIAAA.

83.12

THE SELECTIVITY AND SENSITIVITY OF DIMYRISTOYL-PHOSPHATIDYLCHOLINE LIPOSOMES TO PERTURBATION BY N-ALCOHOLS. R. Hitzemann and K. Dains*. Psychiatry Service-VAMC, Northport, NY 11768.

The partition coefficients "K" of a non-electrolyte "i" into two membranes "x" and "y" are related by, $\log K_{i,y} = \log K_{i,x} * S_{x,y} + R_{x,y}$ where S is the selectivity constant(see Diamond and Katz, J.Mol. Biol.17:121, 1974). When $S_{x,y}$ is < 1, membrane "x" is more hydrophobic(from a partitioning perspective) than membrane "y". We propose that since the perturbation of membrane structure induced by non-electrolytes is highly correlated to K, the above equation could be re-written, substituting P for K, where P is a measure of membrane perturbation. From this perspective, $R_{x,y}$ is a measure of the difference in membrane sensitivity, independent of the partitioning electrolyte. We have utilized this selectivity/sensitivity concept to analyze the perturbation of DMPC membrane order induced by C1 to C8 n-alcohols; order was monitored by fluorescence polarization with either DPH or TMA-DPH as the membrane probes. Comparing the domains monitored by DPH and TMA-DPH at 30 C, the DPH domain is nearly 10 times more sensitive to perturbation by the n-alcohols than the TMA-DPH domain; as expected the DPH domain is significantly more hydrophobic($S_{x,y}$=0.65+0.08).

83.13

EFFECT OF ETHANOL ON THE NEUROSTEROIDS CONCENTRATIONS IN THE RAT BRAIN. O.C.Vatier*, F.E.Bloom, Preclin.Neurosci. & Endocrin., Scripps Clinic & Research Foundation, La Jolla, CA 92037.

3ß-hydroxy-Δ5 steroids such as pregnenolone (P), dehydroepiandrosterone (D), and their sulfate esters (PS and DS) exist in rat brain at higher concentration than in blood, and persist after removal of gonads and adrenal glands. Although the role of these steroids in brain function is still unknown, previous studies have showed that the levels may be functionally regulated. This study sought to establish the relationship between blood alcohol levels (BAL) and the concentration of P,PS,D and DS in different structures of the rat brain. Sprague Dawley male rats (175g) were given ethanol (16%in saline,IP) to obtain BALs of 150mg%. Control were given a equal volume of saline. Animals were sacrified two hours later and the brain quickly dissected for steroids assays. The results (N=24-36) shows a averaged 75 % decrease of D+DS in the olfactory bulbs (OT), olfactory tubercules (OT), amygdalas (A), hypothalamus (H). The mean concentration of D+DS in these combined regions is: 0.78+0.24ng per wet weight in ethanol-injected rats and 3.24+0.44ng per wet weight in controls. There is approximately the same depletion in each regions studied (OB=average loss=75%; OT=80%; A=75%; H=60%). There were no significant changes observed in the concentrations of P and PS. In conclusion, the dehydroepiandrosterone (D) seems to be a reliable marker of the alcohol intoxication in the rat brain the possible differential effects of ethanol exposure on D versus DS are still under investigation as is the time courses of the recovery from intoxication. Additionals datas will be required to determine the metabolic implications of this decrease, and its possible physiological significance. (NIAAA Grant #06420).

83.14

ANESTHESIA-DEPENDENT EFFECTS OF ACUTE ETHANOL IN HIPPOCAMPAL CA3 FIELD ELECTRICAL ACTIVITY. J.R. Criado and L.P. Gonzalez. Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.

Studies have shown that ethanol and general anesthetics can alter membrane ionic conductance and therefore the electrical properties of neurons. To study the acute effects of ethanol and its possible interactions with general anesthetics, a field potential laminar analysis of the hippocampal CA3 field was performed under two different anesthetics: halothane (1%) and urethane (1.2 g/kg). Male Sprague-Dawley rats were surgically prepared and bipolar stimulating electrodes were positioned in the ventral hippocampal commissure contralateral to the hippocampal CA3 recording site. Glass micropipettes filled with 2% Pontamine Sky Blue in 0.5M sodium acetate were used for extracellular field potential recordings. Evoked field potentials were recorded at 100u intervals throughout the CA3 field. After baseline data were collected, a moderate dose of ethanol (1.5 g/kg) was injected. In general, ethanol produced a decrease in the amplitude of positive field potentials and an increase in negative field potentials in rats under halothane anesthesia. However, acute ethanol in the urethane preparation resulted in an increase in both the negative and positive field potentials.

Supported in part by NIAAA grant AA07254 and a Presbyterian Health Foundation Grant.

83.15

ARYLSULFATASE A ELECTROPHORETIC VARIANTS IN ALCOHOLIC PATIENTS. P. Manowitz*, R. Bradley*, R. Nora*, and S. Chokroverty* (SPON: J. Ravens). UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, and Veterans Administration Medical Center, Lyons, NJ.

The purpose of this study was to determine if electrophoretic variants of arylsulfatase A (ASA) occurred more frequently in alcoholic patients than controls. ASA catalyzes the degradation of sulfatides, which are part of the opiate receptor, the GABA receptor, and the Na^+-K^+ ATPase.

Twelve percent (17/143) of alcoholic patients have variant ASA's of the III_A, III_B, and IV_B pattern as determined by electrophoretic analysis of blood samples. While none (0/208) of the psychiatric patients without alcoholism have the III_A or III_B variants, two percent (5/208) of these patients have the IV_B variant. Only 0.6 percent (1/159) of the healthy control subjects have a variant of ASA, in this case a III_A variant.

These data support the hypothesis that the III_A, III_B, and IV_B variants of ASA are associated with alcoholism and may be predisposing factors for this disease.

As part of this study of ASA, a more sensitive enzyme assay using high performance liquid chromatography with an electrochemical detector has been developed with detection of p-nitrocatechol, the artificial enzymatic products, at a level of 0.3 picomole. (Supported by a grant from the UNICO Foundation.)

83.16

EFFECTS OF ETHANOL ON THE INHIBITORY GTP-BINDING PROTEIN-MEDIATED ADENYLATE CYCLASE INHIBITION IN RAT CEREBRAL CORTEX. S. Hatta*, T. Saito, F. Tsuchiya*, H. Ohshika* and N. Takahata*. Departments of Pharmacology and Neuropsychiatry, Sapporo Medical College, Sapporo 060, Japan.

Ethanol (EtOH) has been suggested to stimulate adenylate cyclase (AC) in vitro through an increased function of the stimulatory GTP-binding protein (Gs). However, effects of EtOH on the inhibitory GTP-binding protein (Gi)-mediated inhibitory pathway of AC still remain unclear. The present study was conducted to examine EtOH effects, in vitro, on the Gi-mediated AC inhibition caused by GppNHp in the presence of forskolin (100 μM) in rat brain cortical membranes (CM).

CM were prepared from male Wistar and Fischer 344 rats. The forskolin-activated AC was inhibited about 30% with GppNHp (0.01 - 0.3 μM) in CM from Wistar rats. The concentration of GppNHp required for half-maximal inhibition of AC ("$GppNHp_{50}$") was 84.8 nM and 34.9 nM in control and EtOH (500 mM)-treated CM, respectively. In CM from Fischer 344 rats, GppNHp-induced inhibition of the forskolin-activated AC was about 20%. The "$GppNHp_{50}$" value in control was 63.8 nM, and EtOH did not alter the "$GppNHp_{50}$" value.

These results suggest that EtOH in vitro may alter the Gi function in Wistar rat CM, besides its effects on the Gs function, and EtOH effects on the Gi function in CM are different between in Wistar and Fischer 344 rat CM.

83.17

EFFECT OF INTOXICATING DOSES OF ETHANOL ON HIPPOCAMPAL INHIBITORY PROCESSES ASSESSED IN THE RAT DENTATE GYRUS. Steven J. Henriksen, Yung Kim, and James Wiesner. Research Institute of Scripps Clinic, 10666 N. Torrey Pines Road, La Jolla, CA. 92037.

Low doses of ethanol affect cognitive processes thought to employ, in part, hippocampal circuitry. In an attempt to investigate the neurochemical substrates for the action of ethanol in this structure, we have studied the action of systemically, locally, and intraventricularly administered ethanol on a variety of electrophysiological measures of granule cell excitability in halothane anesthetized, and unanesthetized, rats. All routes of ethanol administration produce dose-dependent reversible decreases in the spontaneous discharge of randomly encountered single granule cells. Stimulation of the perforant path afferents to the dentate gyrus also elicits dose-dependent ethanol responses. Low doses of ethanol (0.5-1.5 gm/kg i.p.) produce no effect on "field-evoked" EPSPs recorded from the dendritic zone of termination. In addition, singly-evoked population spikes (PS) (quantified by input-output analysis), or conditioning PS (evoked in paired-pulse [PP] paradigms) were also unaffected by these ethanol doses. However, recurrent inhibition assessed by PP analysis demonstrated a significant increase following low doses of ethanol. This enhancement of PP inhibition by ethanol, but not after saline, was partially reversed by systemic administration of atropine. Therefore, cholinergic processes may play a role in the action of ethanol on recurrent excitation/inhibition observed in the dentate gyrus.

83.18

THE EFFECTS OF ETHANOL ON SYNAPTIC TRANSMISSION IN THE ABDOMINAL GANGLION OF APLYSIA CALIFORNICA. Alan J. Grant* and Steven N. Treistman. The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Earlier work from this laboratory has indicated that calcium currents within identified Aplysia neurons are reduced by clinically relevant concentrations of ethanol. Since calcium currents are known to play an important role in release at synaptic terminals, we might expect that low concentrations of ethanol will affect synaptic transmission. To test this, we examined the effects of ethanol on synaptic function in the Aplysia abdominal ganglion. Acetylcholine, serotonin, gamma-aminobutyric acid, and dopamine are known to be acting at the synapses which we studied, and some of the synaptic linkages examined were cyclic nucleotide-mediated. In most synapses, concentrations of ethanol lower than 100 mM had minimal effects on transmission. By simultaneously voltage-clamping cell L10 and recording postsynaptic potentials in follower cells during ethanol exposure, we were able to compare the changes in calcium current with changes occurring in cholinergic transmission, and these results will be discussed.

These studies were supported by a grant from the Public Health Service.

83.19

SPECIFIC BINDING OF [^{3}H]INOSITOL - 1, 4, 5 - TRISPHOSPHATE TO CEREBRAL CORTICAL AND CEREBELLAR MEMBRANE FRAGMENTS FROM ETHANOL SENSITIVITY (LS) AND RESISTANT (SS) MICE. T.L. Smith and E.C. Turbak. Veterans Administration Medical Center, Tucson, AZ 85723 and Department of Pharmacology, University of Arizona, Tucson, AZ 85724.

In a variety of tissues nanamolar concentrations of IP_3 stimulate the release of Ca^{+2} sequestered within the endoplasmic reticulum. Since ethanol affects CNS Ca^{+2} disposition, it was of interest to characterize [^{3}H]IP_3 specific binding in two different brain regions from mice specially bred for differential sensitivity to the hypnotic effects of ethanol. Membrane fragments from cerebral cortex or cerebellum of LS and SS mice were suspended in 50 mM Tris buffer (pH 8.3) containing NaCl, 20 mM KCl, 100 mM; EDTA, 1 mM. Tissue samples (350 - 500 µg protein) were incubated for 10 mins at 4° in the presence of 10 to 160 nM [^{3}H]IP_3 (20 Ci/mmol) in a final volume of 0.5 ml and the reactions stopped by rapid vacuum filtration. Scatchard analysis of the binding data from cerebral cortex of LS and SS mice yielded: (LS) Bmax, 256 +13 fmoles/mg protein; K_D, 18 ± 2 nM. (SS) Bmax, 229 + 30 fmoles/mg protein; K_D, 26 + 7 nM. Similar analyses of cerebellar membrane fragments from these mouse lines yielded: (LS) Bmax, 1142 + 250 fmoles/mg protein; K_D, 33 + 7 nM. (SS) Bmax, 1243 ± 260 fmoles/mg protein; K_D, 8 + 1 nM. Ethanol added in vitro up to 500 mM had no significant effect on [^{3}H]IP_3 specific binding to cerebellar tissues from either LS or SS mice. It is concluded that only differences in [^{3}H]IP_3 dissociation constants are observed in cerebellar tissues from LS and SS mice which may not generalize to other brain areas. (Supported by a Vet. Admin. Medical Research Grant).

83.20

ALCOHOL ADMINISTRATION REDUCES THE NUMBER OF IMMUNOREACTIVE VASOPRESSIN PERIKARYA. G.P. Kozlowski, J.H. de Schweinitz* and M. Sadeq*. Dept. of Physiology, Univ. of Texas Southwestern Med. Ctr., Dallas, TX 75235.

Vasopressin (VP) has been shown to enhance learning and memory which is negatively affected in alcoholics. We sought to determine the effects of 5% ethanol (ETOH) administration on VP-containing neurons using a simultaneous pair-feeding system of Israel et al. Twenty-five male Long-Evans rats were used. Two experimental groups (n=5/gp) received a Bio-Serv liquid diet containing ETOH for 5 or 15 days. Three groups of control animals were administered: (1) solid diet (Lab Chow) pellets and water ad libitum, (2) control liquid diet (isocaloric to the ETOH group) for either 5 or, (3) 15 days. The animals were perfuse-fixed and their brains processed for immunocytochemistry using rabbit anti-VP 710 and the ABC kit from Vector Laboratories. All of the VP-immunopositive perikarya having a nucleus were classified into 6 nuclear areas and manually counted. All three groups of control animals had significantly more VP-containing perikarya than the 15 ($p<0.01$) day ETOH group. The 6 nuclear areas studied were: anterior commissural (ACN), supraoptic (SON), suprachiasmatic, paraventricular, retrochiasmatic of the SON and anterior hypothalamic. Significant ($p<0.05$) decreases in numbers of VP-containing neurons (e.g. mean of 2339 for 15 da ctrl vs. 1647 for 15 da ETOH) occurred in all areas except for the ACN in 15 days ETOH group. Supported by AA-06014.

FEEDING AND DRINKING I

84.1

ANGIOTENSIN II MEDIATES DRINKING ELICITED BY HISTAMINE IN RATS. **F. S. Kraly and R. Corneilson*. Dept. of Psychology, Colgate Univ., Hamilton, NY 13346.**

Drinking elicited by s.c. histamine (H) was preceded by 10 min by s.c. captopril (CA; 100 mg/kg), to prevent the synthesis of angiotensin II (AII) in circulation and in brain, or 0.9% NaCl in Sprague-Dawley male rats in 1-hr tests for drinking. CA attenuated (72% inhibition) but did not abolish drinking elicited by the ED_{100} dose (20 mg/kg) for H. This inhibition was reversed by s.c. AII (0.12 mg/kg). Selective gastric vagotomy attenuated drinking elicited by H or by 1 mg/kg AII, and when combined with CA, vagotomy abolished drinking elicited by the ED_{100} for H. The ED_{100} for H and 0.12 mg/kg AII were additive in their effects on water intake. CA abolished drinking elicited by the ED_{50} (2.5 mg/kg) for H. This blockade was surmounted by s.c. AII. The ED_{50} for H and 0.5 mg/kg AII were additive in their effects on water intake. Inhibition of drinking by CA was not nonspecific; e.g., CA failed to inhibit drinking elicited by 0.5 mg/kg AII. Antagonism of H_1 and H_2 receptors (1 mg/kg dexbrompheniramine and 16 mg/kg cimetidine i.p.) for H abolished drinking elicited by H without inhibiting drinking elicited by 0.12 mg/kg AII. These findings are consistent with the hypothesis that the renal renin-angiotensin system through AII mediates histamine's dipsogenic effect in rats.

84.2

GABA DISRUPTION OF INTRACEREBROVENTRICULAR INFUSED ANGIOTENSIN II IN RATS. K.A. Roberts, J.W. Harding and J.W. Wright. Dept. of Psychology and VCAPP, Washington State University, Pullman, WA 99164

An initial experiment established a dose-response relationship between the intracerebroventricular (icv) infusion of angiotensin II (AII: 0, 0.1, 1, 10 and 100 pmol/2 ul aCSF/min) for 5 min and resulting water consumption in Spontaneously Hypertensive Rats (SHR), Wistar-Kyoto (WKY) and Sprague-Dawley (SD) normotensive controls during the 30 min measurement period. The results indicated an exaggerated intake by the SHR at the two highest doses in agreement with a previous report that utilized icv bolus injections of AII (Wright et al., 1987). From these data the 100 pmol dose of AII was selected for further study because it most reliably displayed this strain difference in consumption. A second experiment utilized icv pretreatment infusion of GABA (0, 10, 100 and 1000 nmol/2 ul aCSF/ min) for 5 min followed by the icv injection of AII(100 pmol in 2 ul aCSF) in SD rats. The results indicated progressive reductions in AII-induced water consumption with increasing dose of GABA, and confirm earlier reports of an interaction between central gabanergic and angiotensinergic systems (Unger 1983).

	n	Mean Water Intake (ml/30 min)		n	Mean Water Intake (ml/30 min)
aCSF	5	10	100 nmol	13	6
10 nmol	8	8	1000 nmol	11	2.2

84.3

HYDROMINERAL HOMEOSTASIS IN FISCHER 344 RATS: ROLE OF HYPOTENSION IN ANGIOTENSIN-RELATED WATER AND NACL INTAKE. F.A. Caputo*, N.E. Rowland and M.J. Fregly* (SPON: V.C. Pellis). Depts Psychol & Physiol, Univ of Florida, Gainesville, FL 32611.

Fischer 344 (F344) rats differ from many other strains by their absence of spontaneous preference for NaCl solutions. We have also found that they drink less in response to acute treatment with either the B-adrenergic agonist, isoproterenol (ISOP) or extracellular dehydration with polyethylene glycol. We now report that ISOP produces comparable elevations in plasma renin activity and heart rate in F344 and Sprague-Dawley (SD) rats. In contrast, ISOP produces less hypotension in F344 than SD, and the higher water intake in SD may thus relate to their lower blood pressure for a given plasma concentration of angiotensin (ANG) II. We are presently examining whether the reduced NaCl preference and appetite in F344 may be related to these cardiovascular differences. Acute administration of ANG II (200 ug/kg SC) induced intake of both water and 0.15M NaCl in F344 and SD rats, with no apparent strain difference. In contrast, ANG I (133 ug/kg, SC) produced water, but no 0.15M NaCl intake in F344 rats; SD rats drank both water and NaCl after ANG I. The ANGI-induced intakes of water and NaCl were not significantly altered in either strain by pretreatment with the ANG converting enzyme inhibitor, captopril (3 mg/kg).

84.4

CENTRAL NOREPINEPHRINE INFUSIONS RESTORE DRINKING TO ANGIOTENSIN II IN RATS WITH CATECHOLAMINE DEPLETIONS OF THE MEDIAN PREOPTIC NUCLEUS (MNPO) AND THE ORGANUM VASCULOSUM OF THE LAMINA TERMINALIS (OVLT). J.T. Cunningham & A.K. Johnson. Depts. of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa.

Our laboratory previously has shown that 6-hydroxydopamine (6-OHDA) injections into the MnPO and OVLT produce specific drinking deficits to angiotensin (ANG). This deficit can be reversed by tranplanting norepinephrine containing tissue into the injection sites. In the present study, we attempted to reverse the 6-OHDA-induced defict to ANG with central infusions of norepinephrine (NE). Male rats were injected with 6-OHDA (N = 8) or vehicle (VEH, N = 7) into the MnPO and the OVLT and guide cannulae were placed into each lateral ventricle. Drinking tests were conducted with carbachol (50 ng/2 ul) to ensure the patency of each cannula. The 6-OHDA group drank significantly less to saline infusions and bolus injection of ANG (S + ANG 50ng/2ul) than did the VEH group. Central icv infusions of NE (100 nmole/15 ul 0.5 ul/min) with saline bolus injection (NE + S) did not induce drinking in either the 6-OHDA or VEH groups. When ANG was injected during NE infusion (NE + ANG), the 6-OHDA group drank significantly more than the previous S + ANG and the NE + S treatments. NE replacement restores drinking to ANG in rats with catecholamine depletion of the MnPO and the OVLT.

84.5

ATRIAL NATRIURETIC PEPTIDE INJECTIONS INTO THE SUBFORNICAL ORGAN REDUCE DRINKING TO ANGIOTENSIN II. K.J. Ehrlich*, J.B. Simpson and D.A. Fitts. Dept. of Psychology, Univ. of Washington, Seattle, WA 98195.

Intracerebroventricular application of atrial natriuretic peptide (ANP) attenuates the water intake generated by central angiotensin II (ANG). One possible site for this antagonism might be the subfornical organ (SFO), a circumventricular organ that has high concentrations of receptors for both ANG and ANP. The SFO is a primary site for the dipsogenic action of circulating ANG, and thus is a logical site for antagonism of ANG-induced drinking by ANP. This study examined the effect of a pretreatment of ANP on ANG-induced water drinking at the SFO. Long-Evans rats were fitted with cannulas in the SFO under equithesin anesthesia. On each of three test days, the rats received two 0.5-μl injections into the SFO including a pretreatment followed 5 min later by 10 pmol ANG. On days 1 and 3 the pretreatment was a saline vehicle; on day 2 the pretreatment was vehicle (n=7), 100 pmol (n=6), or 230 pmol α-human ANP (n=7). Water intakes (ml/30 min ± s.e.m.) are shown below. ANP significantly reduced water intake generated by ANG at the SFO at both doses ($p<.05$). Thus, the SFO may be a site for the antagonism of drinking to ANG by ANP.

	Days		
Pretreatment	1	2	3
Vehicle	3.24±1.01	4.26±0.64	4.79±0.90
100 ANP	5.31±1.24	2.87±0.91	2.88±1.45
230 ANP	3.50±0.71	1.59±0.51	2.09±1.17

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84.6

SUBFORNICAL ORGAN: SITE OF DIPSOGENIC ACTION OF ANGIOTENSIN II. S. Cohen, M. Kadekaro, M.L. Terrell, H. Lekan, H. Gary, Jr. and H.M. Eisenberg. Div. of Neurosurg., Univ. of Tex. Med. Branch, Galveston, TX 77550

The subfornical organ (SFO) and the anterior ventral portion of the third ventricle (AV3V area) contain angiotensin II (AII) receptors and are putative sites of dipsogenic action of peripheral AII. The objective of this study was to identify the primary site of action of AII, employing the deoxyglucose (DG) technique. Male adult Sprague-Dawley rats (n=41) were used. Under Equithesin anesthesia the ventral stalk of the SFO was stereotaxically disconnected from the AV3V area using a knife with a retractable blade. For the sham operation, the knife was lowered into the brain but the blade was not extruded. The DG experiments were performed three days after the surgery. Rats in each surgical group were infused with either saline or AII (2.5 μg/min) for 45 min starting 10 min before the injection of DG. Lesioned animals (n=7) drank less than sham-operated rats (n=14), in response to AII [0.9 ml (SD 0.9) vs. 2.9 ml (SD 1.8)]. Glucose utilization in the SFO, however, increased in lesioned rats to the same extent as in the sham-operated animals in response to AII. In the AV3V area and in the hypothalamo-neurohypophysial system the responses to AII were also similar in both groups of animals. From these results, we conclude that the SFO is the main site of dipsogenic action of AII but that the activation of the hypothalamo-neurohypophysial system by the AII does not depend on inputs from the SFO.

84.7

INTRACEREBROVENTRICULAR AMASTATIN POTENTIATES DRINKING TO ANGIOTENSIN AND HYPERTONIC SALINE. **M. J. Sullivan, T. G. Beltz and A. K. Johnson. Departments of Psychology, Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242.**

The aminopeptidase A and leucine aminopeptidase inhibitor, amastatin, has been demonstrated to increase the half-life of iodinated angiotensins (ANG) in the ventricles of the rat. In this study, the effects of amastatin delivered into the lateral ventricle on central ANG-induced or carbachol-induced drinking and peripheral ANG-induced and hypertonic saline-induced drinking were evaluated. Rats were prepared with a cannula into the lateral cerebral ventricle. Central effects were examined by administering 100 nmoles amastatin alone, 100 pmoles ANG alone or both 100 nmoles amastatin and 100 pmoles ANG. Central effects of amastatin on drinking to carbachol (CARB) were testeded by administering 100 pmol amastatin alone, 50 ng CARB alone or both 100 nmoles amastatin and 50 ng CARB. Effects of central amastatin on drinking to peripheral ANG or hypertonic saline (HTS) challenges were assessed by delivering either artificial cerebrospinal fluid or amastatin (100 nmoles) into the ventricle and ANG (1.5 mg/kg) or hypertonic saline (1 ml 3% NaCl/100 gm) subcutaneously.

Amastatin potentiated the dipsogenic effects of centrally administered ANG, in all likelihood as a result of inhibition of degradation of the exogenously applied peptide. Amastatin had no effect on CARB-induced drinking. The dipsogenic actions of peripherally injected ANG and HTS were also significantly enhanced by the inhibitor. These data suggest the involvement of central peptidergic synapses in drinking to osmotic and hypovolemic stimuli.

84.8

EFFECTS OF IV AND/OR ICV CAPTOPRIL INFUSIONS ON THE DRINKING AND PRESSOR RESPONSES TO IV ANG I IN THE RAT. M. M. Robinson, G. P. McLennan* and A. K. Johnson. Dept. of Physiol., The Univ. of Western Ontario, London, Canada, N6A 5C1 and Dept. of Psychol. and Pharmacol. and The Cardiovascular Center, Univ. of Iowa, Iowa City, 52242.

We investigated the effects of IV and/or ICV infusions of Captopril (Capt.; 1 ug/min), on the drinking and pressor responses to IV Ang I (100 ng/min). IV Capt. (begun 30 min before IV Ang I) had no effect on the drinking response (6.5 ± 1.4 vs 5.3 ± 1.2 ml/90 min), whereas, ICV Capt. abolished drinking (1.0 ± 0.5 not significantly different from saline treated controls; 0.8 ± 0.3 ml/90 min). Both IV and ICV Capt., given separately, reduced the pressor response (Controls = 39.5 ± 2.1 mmHg vs ICV Capt. = 23.6 ± 2.7 mmHg or IV Capt. = 21.9 ± 7.3 mmHg; Mean change in MAP 90 min after start of IV Ang I). IV and ICV Capt., given together, further reduced the pressor response to 12 ± 1.1 mmHg. The effects of ICV Capt. were not due to leakage into the general circulation since a double dose of Capt. (2 ug/min), given IV, did not reduce the pressor response any more than a single dose (20.6 ± 3.1 mmHg). Neither IV nor ICV Capt. had any effect on the drinking or pressor responses to IV Ang II (100 ng/min). These results suggest that conversion of blood borne Ang I to Ang II, destined to act at brain sites to stimulate drinking or a neural pressor response, occurs in the brain rather than in the lung. (NIH Grant HL 14388)

84.9

SHAM DRINKING IN DEHYDRATED RATS: PHARMACOLOGICAL INHIBITION AND THE COMBINATION OF EXTRACELLULAR AND INTRACELLULAR STIMULI. N.E.Rowland, J.J.Salisbury* and M.J.Fregly*. Depts Psychol & Physiol, Univ of Florida, Gainesville, FL 32611.

Natural dehydration by fluid deprivation for 24hr is followed by a copious amount of drinking in rats with open gastric fistulas (sham drinking). In a 1hr test, about 80 ml is ingested, at an almost steady rate. In a first study we found that pretreatment with either the dopaminergic antagonist, spiroperidol (30 ug/kg), or the noradrenergic alpha 2 agonist, clonidine (12 ug/kg), reduced the sham drink by about 50%. The inhibition was comparable in each of the 15min segments of the 1hr test, and was at least as great as the inhibition of real drinking (closed fistula) at these doses. This suggests that the antidipsogenic action of these agents is not through modulation of post-ingestional signals, and also that a 'fatigue' or motor hypothesis is inadequate. Instead, it appears that these agents act directly on aspects of the neural circuitry involved in the quantitative expression of behavior.

In a second study, we examined whether the copious sham drinking after dehydration (a mixture of intracellular IC and extracellular EC depletions) could be mimicked by the combination of IC (NaCl) and EC (polyethylene glycol PEG) challenges. Surprisingly, neither stimulus alone nor the combination produced a robust sham drinking response: open and closed fistula rats drank comparable amounts.

84.10

SEXUAL DIMORPHISM OF NEED-FREE SALT INTAKE IN THE RAT R.R. Sakai*, J.A. Witcher*, N.T. Adler, and A.N. Epstein. (Spon: S. Frankman) Departments of Biology and Psychology, Univ. Pennsylvania, Philadelphia, PA 19147.

Need-free salt intake in rats is sexually dimorphic. Females, from a pre-pubertal age, ingest more salt than age-matched males. To determine whether salt intake is influenced by neonatal androgen, day-one male rats were orchidectomized and day-one females were androgenized (1 mg testosterone propionate-TP). Day-one orchidectomized males displayed exaggerated female-like salt intakes throughout life. Day one androgenized females displayed male-like salt intakes through 70 days, at which time their intakes rose to that of normal females.

To determine whether TP exposure during adulthood alters need-free salt intake gonadectomized (GNX) adult males and females were implanted with silastic tubing containing TP or cholesterol (CHOL). TP suppressed salt intake in both sexes. Repeated sodium depletions produced incremental increases in need-free salt intake for both CHOL males and females. TP suppressed these increases in both sexes. However, after removal of TP all animals displayed the elevated salt intakes that result from histories of sodium depletions.

A developmental role of androgen for salt intake is indicated since heterotypical salt intake is produced in genetic males throughout life by day one orchidectomy and in genetic females (through pubertal age) by day one androgenization. An activational role for androgen is indicated with the observed suppression in TP treated GNX adults. Androgen's suppressive effects on need-free salt intake following repeated sodium depletions are expressed only as long as androgen is present.(NSF NS03469 to ANE and NIH HD04522 to NTA)

84.11

THE EFFECTS OF VENTROMEDIAL HYPOTHALAMIC LESIONS ON DIURNAL DRINKING BEHAVIOR AND LIPID PEROXIDATION IN THE RAT. J. H. Peck and R. R. Jenkins. Depts. of Psychology and Biology, Ithaca College, Ithaca, NY 14850.

Ventromedial hypothalamic (VMH) lesions produce a variety of behavioral and physiological changes, the most notable of which is overeating which leads to obesity. It has been demonstrated that the obesity effects of the VMH lesion are a result of damage to the ventral noradrenergic bundle and not to the VMH itself.

Oxygen centered radicals are constantly formed during metabolism. Biological systems are normally protected against radical damage by a wide array of defenses. Under certain conditions the defense is overwhelmed and the resultant lipid peroxidation alters membrane functions and integrity.

Rats were either lesioned or sham operated and allowed to recover from surgery for 1 week. The rats were then placed in drinkometer cages where their daily drinking behavior could be monitored. After 8 weeks, the rats were sacrificed and heart, liver, kidney and skeletal muscle tissue was taken for the assay of catalase, superoxide dismutase, lipid peroxidation and percent fat. Results showed the expected weight gains and a disruption of the daily drinking rhythm. There was an apparent enzyme induction in lesioned animals. The catalase and superoxide dismutase enzymes were correlated with percent fat in the tissues studied. The mechanism is yet to be determined.

84.12

THE EFFECT OF DORSOMEDIAL HYPOTHALAMIC NUCLEI (DMN) KNIFE CUTS ON FOOD (FI) AND WATER (WI) INTAKE, BODY WEIGHT (BW) AND FEEDING RESPONSES TO 2DG, CCK AND NALOXONE. L.L. Bellinger. Dept Physiol, Baylor Coll Dent, Dallas TX 75246.

Rats with DMN lesions (Bellinger, Am J Physiol 252:R938, 1987), have reduced FI,WI,BW (but normal body composition) and altered ingestive responses to 2-deoxyglucose (2DG), cholecytokinin (CCK) or naloxone (NOX). In the present study, 150g male rats fed chow were given knife cuts either posterior (PC), lateral (LC), dorsal (DC), ventral (VC) or anterior (AC) to the DMN or sham (S) operations. Histology confirmed correct cut positioning in all groups (grps) (n=7-13) except AC. Rats with PC or VC were hypophagic and had reduced BW ($P<0.01$) compared to S. Plasma Na^+, K^+ and osmolality were similar in all grps. WI effects were variable with PC and LC being slightly attenuated. After receiving 2DG (300mg/kg, i.p.) all grps, except LC, increased ($P<0.05$) their FI at 1,2, and 3 h. Following i.p. CCK injections (3.0ug/kg) all grps, except PC, showed attenuation ($P<0.05$) of FI at 30 min, with the VC and LC grps having a greater ($P<0.05$) suppression than S. When tested with NOX (1ug/kg) the VC and S grps had reduced FI ($P<0.05$) at 1,2 and 3 h. Rats with PC showed no reduction of FI, while the DC and LC grps had attenuated FI ($P<0.05$) only at 2 h. The data show that loss of 2DG feeding and NOX suppression of FI can be disassociated from changes in daily FI or BW and that the reduced FI and BW of DMN lesioned rats involves tracts to/from the L and P DMN

Supported in part by BCD research funds.

84.13

CATCH-UP GROWTH IN RATS THAT WERE FOOD-RESTRICTED PRIOR TO LESIONS IN THE DORSOMEDIAL HYPOTHALAMIC NUCLEI (DMNL): THE FIRST 48 HOURS AFTER OPERATION AND REALIMENTATION. Lee Bernardis, Larry Bellinger, Marge Kodis* and Mary Jane Feldman*. VAMC Buffalo & SUNY/Buffalo, Buffalo, NY 14215

Forty-five day(d)-old male Sprague-Dawley rats were maintained ad libitum (AL) for 11 d. During the subsequent 21 d, some of the rats were continued AL whereas others were fed half of the amount eaten (RR) by the AL rats. At this point some of the RR and some AL rats received DMNL, whereas other RR and AL rats were sham-operated (CON). Following surgery, all rats were refed AL and killed 2 d later. Rats that were RR-DMNL had the same body weights as RR-CON. Whereas AL-DMNL rats showed the characteristic hypophagia, RR-DMNL rats ate as much as RR-CON, ate more than AL-DMNL and utilized food energy as competently as RR-CON. Testes growth/metabolic size (KU) was greater in RR than in AL rats, irrespective of brain manipulation. On the other hand, liver growth/KU was greater in RR rats only in the case of the DMNL rats. Plasma glucose was similar in all groups, whereas plasma insulin was higher in both RR groups vs. the two AL groups. Plasma growth hormone, glucose-U-C14 incorporation and body composition data will be presented at the meeting. The data are taken to mean that the processes underlying catch-up growth supervene in DMNL rats at the same time and to the same degree as in CON. With other data, this supports our contention of an "organismic" set point in DMNL rats. Supported by VA and Baylor funds.

84.14

NORMAL ULTRADIAN GROWTH HORMONE (GH) RHYTHM IN GROWTH RETARDED RATS WITH DORSOMEDIAL HYPOTHALAMIC LESIONS (DMNL). S.L.Byrd*and L.L.Bellinger (SPON: B. Hutchins). Dept. of Physiol., Baylor Coll. Dent., Dallas, TX 75246.

Rats with DMNL are hypophagic and have reduced linear growth and body weight (BW), but normal body composition (Bernardis and Bellinger, Brain Res Rev 12:321,1987). Growth related hormones are normal in DMNL rats when measured several wks after L. In the present study, male Sprague Dawley rats (180g BW) received jugular cannulas and on return to pre-cannulation BW 4.2±0.5 d they received bilateral electrolytic (1.0 ma, 5 s) DMNL or sham (S) operations. The rats were then bled ($\bar{x}$=6.9 d after L) every 15 min between 0600-1215 h; L:D 12:12, lights out 1430 h. Plasma was saved for assay, whereas the rat's RBC's were suspended in a 10% BSA solution and returned to the animal. GH was assayed using materials (rGH-I-5, S-5, RP-2) supplied by NIDDK and NHPP. DMNL (n=8) rats were hypophagic (6d $\bar{x}$ = 13.4±1.0 vs 16.8±0.9g, $P<0.05$) and weighed less (185.4±4.9 vs 201.4±4.8g, $P<0.05$) than S (n=9) at sampling. Total GH secretion as computed from the area under each rat's ultradian pattern was similar in both groups (DMNL vs S, 3986.4±481 vs 4363.2±556, ns) as was mean 6 h GH secretion (DMNL vs S, 22.0±2.6 vs 24.4±3.2 ng/ml, ns). Peak secretion intervals were similar in both groups (DMNL vs S, 3.7±0.2 vs 3.3±0.2 h) and DMNL rats had more peaks over 100ng/ml than S. The data suggest that the reduced growth of DMNL rats cannot be readily explained by a deficiency in GH secretion. Supported by BCD research funds.

84.15

CHOLECYSTOKININ-INDUCED CONDITIONED ODOR-PREFERENCE IS BLOCKED BY THE SELECTIVE ANTAGONIST L-364,718. A. Weller* and E.M. Blass. (SPON: E.C. Lotter). Dept. of Psychology, Johns Hopkins Univ., Baltimore, MD 21218

Cholecystokinin (CCK) octapeptide reliably produces single-trial conditioned odor-preference in neonatal rat pups. This was achieved by pairing exposure to a novel orange odor with saline (i.p.) or CCK (2 ug/kg in 5-day-old and 0.25 or 0.5 ug/kg in 11-day-old rats). After twenty-four hours, subjects that had previously received CCK remained significantly longer than controls on the side of a choice arena that contained an orange scent. This also holds for weaning age rats. In contrast, adult rats failed to show conditioned odor-preference.

This conditioned response to CCK can be blocked by a CCK antagonist. Pups received the selective antagonist of peripheral CCK receptors L-364,718 (1.4 or 14 ug/kg) or vehicle (DMSO), 45 minutes before they were exposed to orange odor paired with either CCK (0.06 ug/kg) or saline. Forty-eight hours later, the CCK group stayed significantly longer than saline controls over the orange side of the choice arena ($t(25)=2.229$; $p<0.035$). This was totally blocked by L-364,718 at both doses ($p>0.8$). On a molar basis, the lower dose's antagonism is at a 64:1 ratio (antagonist: ligand). This is within the range in which binding studies have shown blockade by L-364,718 of peripheral-type but not of CNS-type CCK receptors (Chang and Lotti, PNAS 83:4923, 1986). Therefore, our results suggest a peripheral site for CCK's initial action in causing conditioned odor preference in 11-day-old rats.

84.16

CHRONIC ALTERATIONS IN SEROTONIN METABOLISM DIFFERENTIALLY AFFECT FOOD INTAKE AND MEAL PATTERNS IN OBESE AND LEAN ZUCKER RATS. T.J. Kalogeris*, T.W. Castonguay, N.E. Rowland and J.S. Stern*. Food Intake Laboratory, Dept. of Nutrition, University of California, Davis, CA 95616.

In order to characterize the possible role of serotonin in the hyperphagia of the obese rat, the effects of chronic administration of d-fenfluramine, a 5-HT releaser, on meal patterns were compared in lean and obese Zucker rats. Six week old female Zucker rats (12 lean, 12 obese) were adapted for 3 wks to ad libitum access to semipurified diet (modified AIN 76) and water, and their meal patterns were monitored. They were then implanted subcutaneously with osmotic minipumps which delivered d-fenfluramine at 3 or 6 mg/kg/day. Food intake and meal patterns (meal size, MS; meal frequency, MF) were monitored for another 5 d. Rats were decapitated and their brains analyzed for 5-HT. The low dose of d-fenfluramine had no effect on meal patterns of either phenotype. At the high dose, lean rats depressed their intake by 38% the first 2 days after implantation. During this period, there was no clear change in MS, while MF was depressed. Average daily intake and MF returned to baseline by day 4. Obese rats showed a 32% depression in intake the first 2 days after implantation which returned toward, but never recovered to more than 85% of baseline intake. Obese meal patterns over the five day post-implantation period were characterized by a persistent depression in MS and no clear effect on MF.

84.17

CHANGES IN BRAINSTEM METABOLIC ACTIVITY ASSOCIATED WITH GASTRIC DISTENSION IN 6-DAY-OLD RAT PUPS: AN ANALYSIS OF AVERAGED 2-DEOXYGLUCOSE AUTORADIOGRAPHS. C.B. Phifer and W.G. Hall. Dept. of Psychol., Duke Univ., Durham, NC 27706.

In very young rat pups, gastric distension is the single gastrointestinal control mechanism for intake termination. Thus its neural substrate should be relatively easy to isolate and also of prime interest. Six-day-old rats with closed pyloric nooses and chronic gastric cannulas either rested without stimulation or received gastric infusions of isotonic saline during 1 hr of [14-C] 2-DG incorporation (30 uCi/100g,sc). Autoradiographic images corresponding to several levels in the brainstem were averaged for pups in each condition. Differences in activity between groups were revealed by subtracting one average image from another.

Difference images revealed that brains of pups with distended stomachs had increased metabolic activity in the nucleus tractus solitarius, particularly at the level of area postrema and in the medial portion of the nucleus. Increased activity was also apparent in the dorsal motor nucleus of the vagus. Relative to pups without gastric distension, pups with distension had less activity in several reticular areas, including medullary, caudal pontine and caudoventrolateral. These results reinforce earlier findings on gastric projections via the vagus and also suggest that gastric distension may cause depression in reticular areas with projections to ingestion-related motor nuclei. (Supported by NICHD Grant HD-17457).

84.18

SUCKLING-INDUCED CHANGES IN BRAINSTEM METABOLIC ACTIVITY: 2-DEOXYGLUCOSE AUTORADIOGRAPHIC DIFFERENCE MAPS. L.M. Terry and W.G. Hall. Department of Psychology, Duke University, Durham, NC 27706.

The topography of neural metabolic activity was compared in 6-day-old rat pups that were 1) resting quietly; 2) searching for a nipple on the ventrum of a dam; or 3) attached and suckling a nipple during a 1 hr [14-C] 2-deoxyglucose (2-DG) incorporation period (30μCi/100g BW, sc). Autoradiographic images corresponding to sections of the neural axis at several levels of the brainstem were selected for each experimental group and analyzed. Difference images were then generated by subtracting average images from one another.

Relative to pups at rest, pups searching for a nipple showed increased metabolic activity in spinal trigeminal nuclei (ventral portions of the interpolar and oral subnuclei) and in distinct reticular regions. Searching pups had decreased activity in the facial nucleus. In contrast, pups that had attached to a nipple and were suckling showed marked activity, relative to resting pups, in the spinal trigeminal nucleus (medial interpolar subnucleus), nucleus ambiguous and in distinct reticular nuclear regions associated with attachment. Suckling pups had decresed activity in the solitary tract nucleus. Together, these results reveal that two primary components of mammalian suckling behavior, searching and attachment, have different neural substrates.(NICHD Grant HD-17457).

84.19

ORAL INFUSION OF FAT OR SUCROSE PRODUCES OPIOID-MEDIATED SINGLE-TRIAL ODOR PREFERENCES IN NEONATAL RATS. D.J. SHIDE* & E.M.BLASS Dept of Psychology, Johns Hopkins University, Baltimore MD 21218.

Central and peripheral administration of morphine, paired with a novel odor in Day 5 rats, results in a preference for that odor when testing occurs on Day 10. Intraoral infusion of a sucrose, polycose, or corn oil solution in the Day 10 rats elevates pain threshold and reduces isolation-induced vocalizations; these effects are reversed through naltrexone pretreatment. The following experiments tested the hypothesis that oral infusions of substances implicated in opioid-mediated ingestive behavior could influence associations made with a novel odor in Day 6 rats. In Exp. 1, Day 6 pups were exposed to a novel orange odor for 30 min while either corn oil, 7.5% sucrose, 32% polycose, distilled water, 0.1% quinine, mineral oil, or nothing was infused through jaw cannulae into their mouths. When tested 2 days later, Day 8 pups that had received infusions of corn oil or 7.5% sucrose significantly increased time spent over orange-scented bedding versus plain bedding when compared to control pups. Exp. 2 investigated potential contributions of the endogenous opioid system to this conditioned preference. Prior to conditioning, Day 6 pups received either an IP injection of naltrexone (0.5 mg/kg) or isotonic saline. Pups were then exposed to orange-scented bedding while either corn oil, 7.5% sucrose, or nothing was intraorally infused. Day 8 pups that had received saline injections paired with infusions of corn oil or 7.5% sucrose showed a preference for orange bedding when compared to no infusion controls. Pre-treatment with naltrexone before conditioning abolished this effect. These findings suggest that intraoral infusions of corn oil or 7.5% sucrose are reinforcing when associated with a novel environmental stimulus, and this effect appears to be mediated through the endogenous opioid system.

85.1

DETERMINING THE BASIS OF ORIENTATION SELECTIVITY THROUGH COMPUTATIONAL MODELS OF CORTICAL CIRCUITRY.

M.A.V. Gremillion* and Bryan Travis* (SPON:D.Sinex). Center for Nonlinear Studies, Los Alamos National Lab, Los Alamos, NM 87545.

Orientation selectivity is a key feature of the physiological response patterns of visual cortex neurons. In order to understand the circuital computations that underlie functional behaviors in V1, we have simulated the circuitry of macaque primary visual cortex and its connections from the LGN. In this model a 3-D cortical module has layers 1-4B, 4C-alpha and 4C-beta, 5 and 6; cell types distributed among the layers include spiny stellates, pyramidal cells and five kinds of local inhibitory neurons. Ratios, distributions, connections and geometry of cell types were derived primarily from the work of Jennifer Lund and colleagues. Neuronal activity is determined through a modified membrane potential algorithm. The model allows us observe single neuron, planar, and columnar patterns of activity over time; theories of orientation selectivity can thus be tested.

Preliminary findings using this model suggest a new understanding of orientation selection. Our results indicate that *planes* of neurons may be the relevant computational unit for these properties. The density of cells per type as well as the ratio between types results in a functional geometry which contributes to the ability of cells to be orientation selective. In addition, while it is well-established that LGN parvocellular neurons project to layer 4C-beta, it is not known whether X-on and X-off cells project to the same or different populations of cells in that layer. We predict that X-on and -off cells project to the same inhibitory neurons but to two different sets of spiny stellate cells. Distributions of 'on' and 'off' spiny stellates would then form two interleaved mosaics that are out of phase with respect to each other. This difference in phase insures that on and off pathways remain separate but interact via common connections to inhibitory neurons, sharpening the edge between light and dark regions. Those patterns of activity are read by neurons in other layers, which then respond with "simple" or "complex" behavior depending on the degree of input from layer 4C.

85.2

SIMULATION OF EARLY PROCESSING OF VISUAL CONTOURS.

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The complex patterns of neural activity at primary cortical levels are studied via computer simulation based on a mathematical model of the first stages of visual information processing. Cortical cells were modelled as receiving information from a few X-LGN cell afferents whose receptive field sensitivity functions were characterized by Difference-of-Gaussians. We simulated four cortical cell types: Simple cells summed two inputs linearly and complex cells summed them non-linearly, while adding cells summed two ON-center X-cell inputs and differencing cells summed one ON-center and one OFF-center cell input. We found that different stimulus dimensions may be detected by different cortical cell types. The most efficient orientation detectors may be the adding cells, whereas the differencing cells may be the best edge and shape detectors.

85.3

TRANSMISSION EVENTS IN MAMMALIAN VISUAL CORTEX THAT INVOLVE NMDA AND NON-NMDA GLUTAMATE RECEPTORS.

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The participation of NMDA and of non-NMDA glutamate receptors in the ascending and tangential transmission of excitation in adult rat visual cortex was assessed by recording field potentials in slices taken from area 17. When stimuli were applied to infragranular layers or the white matter, pre- and postsynaptic (Ca++ dependent) population spikes and slower, radially oriented dipoles were observed both radial to the stimulus and tangentially displaced by up to 1.5 mm. The following pharmacological data were obtained while recording in the supragranular layers: All postsynaptic responses were profoundly antagonized by 0.5 or 1.0 mM kynurenic acid. In normal medium (1.2 mM Mg++), late potentials (later than 10 msec) were smaller and decayed faster when 2-amino-5-phosphonovaleric acid (APV, 20 to 50 uM) was applied, whereas closely paired stimuli and low Mg++ medium enhanced late responses. Low Mg++ also produced new response inflections suggestive of disinhibited postsynaptic activity. Both of these effects of low Mg++ were reversed by APV.

These data are indicative of distinct roles played by NMDA and non-NMDA glutamate receptors in visual cortex. They imply that NMDA receptor-mediated currents can substantially enhance the intracortical spread of excitation when the block present in physiological [Mg++] is lifted.

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85.4

DIRECTIONAL SELECTIVITY OF CELLS IN THE VISUAL CORTEX IS INDEPENDENT OF OTHER REPONSE PROPERTIES.

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For most neurons in the visual cortex, the strength of response varies according to the direction of stimulus motion. Cells in the cat's striate cortex are also characterized by other properties like orientation, spatial and temporal frequency selectivities. We have asked if direction selective cells in striate cortex constitute a homogeneous group with overall properties which can be clearly distinguished from those of cells that are not selective for direction.

Drifting sinusoidal gratings were used to characterize receptive field properties. A total of 102 simple cells and 90 complex cells were studied. A direction index (DI) defined as: DI= 1- response in the non-preferred direction/ response in the preferred direction was used. We find that 33% of simple cells and 37% of complex cells respond equally to both directions (DI<0.5). For all these cells, the two optimal directions were always 180 degrees apart, and the orientation bandwidth was identical in each direction (r=.99, p<0.001). Small proportions of simple (17%) and complex (12%) cells, having a DI between 0.5 and 0.7, were considered to be direction biased. We find similar proportions of simple and complex cells that are directionally selective (50% and 51%, respectively; DI>0.7). In addition, a substantial number of complex cells show a suppression of discharge below the spontaneous levels in the non-preferred direction (DI>1). Preliminary results indicate that this supression is maintained when the size of the grating is reduced to fill only the receptive field area.

We do not observe major differences between the properties of cells according to direction selectivity. In the group of simple cells, there is a small tendency for directionally selective cells to be more narrowly tuned for orientation (r=0.4, p<0.02). In the complex cell group, directional cells tend to exhibit lower degrees of modulation than non-directional neurons. These results indicate that direction selectivity is independent of other response characteristics of cortical neurons. (supported by EY01175 (RDF), Bourse Lavoisier de France (JPN) and MRC of Canada (CC))

85.5

EFFECT OF ELECTRICAL STIMULATION OF LOCUS COERULEUS ON THE ACTIVITY OF NEURONS IN THE CAT VISUAL CORTEX.

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To understand the functional role of the projection from locus coeruleus (LC) to visual cortex and the noradrenaline receptor types involved, we studied the effect of electrical stimulation of LC on the activity of area 17 neurons in conjunction with iontophoresis of α- and β-antagonists in the anesthetized and paralyzed cat. Bipolar stimulating electrodes were implanted in both LC stereotaxically.

Fiftynine cells were recorded in area 17. 66% (23/35) of the cells recorded in layers II,III and IV and 47% (7/15) of the cells in layer VI were suppressed. 67% (6/9) of the cells recorded in layer V and 53% (8/15) of the cells in layer VI were excited by stimulation of LC. The suppressive effect was antagonized by β-adrenergic antagonists and the facilitatory effect was antagonized by α-adrenergic receptor antagonists. α-antagonists suppressed the activity of cells. β-antagonists were predominantly facilitatory but in one third of cells they were suppressive. α- and β-receptor antagonists affected most neurons in all layers of area 17 which suggests that both α- and β-receptors affect the activity of most individual cells. Activation of LC did not improve the signal to noise ratio of the activity of neurons.

In summary, noradrenergic effects in area 17 are primarily facilitatory in layers that project to subcortical areas and primarily suppressive in layers that project to other cortical areas.

85.6

ORGANIZATION AND PROPERTIES OF RECEPTIVE FIELDS OF CORTICAL NEURONS (AREAS 17, 18, 19 AND PMLS) ARE TIME-VARIANT.

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From single cell responses to stationary small-field stimuli applied at different locations within a grid of subfields in random order we compiled temporal sequences of response planes (TSRP) and from the responses to stimulation of the rf-centre with bars of light of varying lenghts and orientations we compiled temporal seqences of tuning curves (TSTC) for bar lenght and bar orientation in steps of 20 msec.

The TSRP showed that each location within the rf exhibited its own temporal characteristic. This is a clear index that the spatial and temporal behaviour is not separable in cortical cells. Non-separability has some remarkable implications for rf properties. Time-variance has been observed for the rf dimensions and for orientation selectivity and hypercomplexity in the case of stationary stimulation. We found selectivity after an initial phase of non-selective excitation (24 %) and a change of selectivity over time (34 %). In the case of moving stimuli one has to expect strong influences of speed and direction of motion as well as of orientation and lenght of the stimuli (i.e. form) on the total response characteristics, which could be experimentally verified suggesting that rf properties are *time-* and *stimulus variant*.

The following aspects are closely connected to non-separabilty of space and time: 1. Non-separability can lead to the phenomenon of *nonlinear spatial summation* because of the complex temporal behaviour even if the cell is *linear* in the true sense of system theory. 2. Non-separable spatio-temporal filters might be optimal adapted for the analysis of moving stimuli. 3. In more than 50 % of the cells specificity is no longer correlated with spikerate. This is taken as strong evidence for the biological significance of a *time code*, in which a temporal modulation over a range of up to 500 msec carries stimulus information.

The experimental data and their interpretation are in close agreement with the outcome of a computer network model, based on a mean, lamina specific neuroanatomy (Krone et al 1986, *Proc Roy Soc* 226:421), which revealed high structured temporal response patterns due to intracortical positive feedback.

85.7

RETINOTOPIC REPRESENTATION OF MOVING STIMULI IN CAT VISUAL CORTEX. - SIMULTANEOUS RECORDINGS WITH AN ARRAY OF EIGHT MICROELECTRODES. H. Koch* & HRO. Dinse Dept. of Zoology III Biophys. Sect., University of Mainz, D-6500 Mainz, West Germany

We recorded single neurons with an array of 8 independent microelectrodes in the cat visual cortex simultaneously. The distance between each recording site varied between 1 and 2 mm. The recordings were performed in area 17/18 along the border at the vertical meridian. The overall visual field representations of the recording sites extended up to 30 degrees visual angle. After plotting the rf of each neuron, bars of light and background of visual noise were swept at various speeds along trajectories adjusted to stimulate each rf. On the basis of PSTHs, the resulting trajectories and the so-called *apparent velocity* for the spread of activity across the cortex were calculated off-line and compared with the actual trajectories of the stimulus.

When the *apparent velocity* was determined for pairs of neurons that were stimulated one after the other, there was no smooth motion of activity across the cortex, but motion typically either preceded or lagged behind the stimulus. In most cases, the observed *mean apparent velocity* was not identical with the speed of the stimulus. The absolute difference between *mean apparent velocity* and actual stimulus speed could amount up to 100 %. The responses to the forward and backward motion differed in that the trajectories of cortical activity were always shifted towards the direction of the stimulus, which resulted in an *offset* for the two directions in the range of 1 to 5 degrees visual angle.

While the retinotopic map was roughly preserved in a trajectory of activity across the cortex, there were a number of deviations on a finer scale (width of rf), that have to be discussed in relation to motion processing and the meaning of retinotopic and non-topographic mapping.

85.8

CLASSIFICATION OF NEURAL ACTIVITY IN V_1 OF THE AWAKE MACAQUE AS PRODUCED BY GANZFELD ILLUMINATION. T.M. Wengenack* C.M. Checkosky* and S.J. Bolanowski Jr. (SPON: S. Demeter). Dept. of Physiol., U.R. Med. Sch., Rochester, NY 14642.

Unlike the binocular case, continuously presented, monocular Ganzfelder fail to produce 2-C^{14}-deoxyglucose uptake in the cytochrome oxidase (CO) patches of layers II-III of macaque striate cortex (Bolanowski et al., SN Abstr. 10: 730, 1982). Thus, although neurons in these patches respond to diffuse illumination, they must also be involved in binocular vision. For the purposes of classification and to relate neural responses to the CO patches, we have measured single unit activity (n=163) in striate cortex of the awake macaque to achromatic monocular and binocular Ganzfelder. Several classes of responses have been identified: transient, 14%; slowly adapting, 10%; luxotonic, 36%; mixed, 10%; and not driven, 30%, with 47% being only monocularly activated. Of the units binocularly activated (23%, n=38), 29% show binocular summation of the monocular responses, 24% show averaging, 13% show inhibition, and 8% show suppression. The remaining 26% have mixed properties. Most (92%) of the units having binocular interactions were luxotonic. We note that some of these binocular response types correlate with psychophysical phenomena found under Ganzfeld conditions. For example, binocular brightness summation occurs in the Ganzfeld and the "blankout" effect produced with monocular but not binocular Ganzfelder may be controlled by the inhibitory and/or suppressive mechanisms.

85.9

EFFECTS OF A REMOTE STIMULUS ON CORTICAL CELL RESPONSES Molotchnikoff S. and C. Morin* and F. Leporé, département des Sciences biologiques, Université de Montréal. Montréal Québec, Canada. H3C 3J7.

It is well established that the introduction of a remote stimulus (RS) outside the boundaries of the classical receptive field (RF) influences the light responses of a unit which is stimulated by a circumscribed target positioned in its RF. This phenomenon has been demonstrated in cats and rabbits at the geniculate and collicular levels. Since both of these structures provide inputs (directly or indirectly) to the cortex, it is expected that responses of cortical cells will be influenced by the addition of a remote stimulus. The proposed experiments are designed to test this hypothesis, in anesthetized (Ketamine, N_2O/O_2 70/30%) and paralyzed rabbits. Animals are prepared for single cell recordings from area 17 (V1). The test stimulus is a slit sweeping across the RF, while the RS is a LED. Although the experiments are in an early stage it appears that 50% of cells (N = 18) had their responses modified by the RS if the receptive field is close to the optic axis, conversely only 30% (N = 10) of cells with an eccentric RF had their discharges altered by the RS. In addition, responses to horizontal movements are more affected than responses to vertical displacement. Thus, it appears that cortical cell responses differ if more than one stimulus is present in the visual field.

Supp. CRSNG, FCAR

85.10

INFLUENCE OF AFFERENTS ON SPATIAL- AND TEMPORAL-FREQUENCY PROCESSING BY NEURONS IN THE CAT'S POSTEROMEDIAL LATERAL SUPRASYLVIAN (PMLS) VISUAL CORTEX. L. Tong, W. Guido and P.D. Spear. Dept. of Psychology and Neurosciences Training Program, Univ. of Wisconsin, Madison, WI 53706.

The PMLS cortex receives converging visual afferents from thalamus and cortex. We investigated the extent to which spatial- and temporal-frequency processing by PMLS neurons depends on afferents from cortical areas 17 and 18.

Recordings were made from PMLS neurons in normal adult cats and in adult cats that had ipsilateral areas 17 and 18 removed 24 hr earlier. The cells were presented with drifting sine-wave gratings and responses were assessed with Fourier analysis. In normal cats, most cells had spatial resolutions from 0.1 to 1.6 c/deg and optimal spatial frequencies from 0.05 to 0.2 c/deg. Removing inputs from areas 17 and 18 had no significant effect on these properties or on contrast sensitivity. When tested at the optimal spatial frequency, most normal PMLS cells showed band-pass temporal-frequency tuning with 3-10 Hz optimal temporal frequency and 14-22 Hz temporal-frequency cutoff. Removing areas 17 and 18 had no significant effect on temporal-frequency cutoff but may have altered some of the tuning characteristics.

Previous studies have shown that PMLS neurons depend on inputs from ipsilateral areas 17 and 18 for elaboration of direction selectivity. The present results suggest that spatial- and temporal-frequency selectivity of PMLS neurons are generated largely on the basis of thalamic inputs.

85.11

ANATOMICAL AND ELECTROPHYSIOLOGICAL INVESTIGATION OF THE INSULAR VISUAL AREA IN THE CAT. M.Norita, T.P.Hicks, G. Benedek* and Y.Katoh*. Fujita-Gakuen Health Univ. School of Med., Toyoake 470-11, Japan.

Electrophysiological recordings of extracellular unit responses were performed using carbon fiber-containing glass micropipettes in cats anesthetized with barbiturate and immobilized with gallamine triethiodide. After identifying a point in the insular visual area physiologically, WGA-HRP was injected at that site. The anatomical results indicated that retrogradely labeled cells were found not only in a variety of cortical regions but also in some subcortical structures. Cortically, densest labeling was found in AEV and LS (mainly in ALLS, PLLS and DSL). Moderate labeling was observed in the cingulate and parasplenial gyri and in areas 8, 20, 35 and 36. Weaker labeling was seen in agranular insular cortex. Subcortically, a number of labeled cells were observed in the LM-Sg complex, some in the VM complex and the intralaminar nuclei of the thalamus and in the lateral amygdaloid nucleus, and a few in the claustrum and basal nucleus. The insular visual area has been shown to receive afferents from structures belonging to the extrageniculate visual system (e.g. AEV, LS, LM-Sg complex) as well as to those belonging to the limbic system (e.g. cingulate gyrus, amygdala), suggesting that this insular area may be involved in higher levels of cerebral functioning.

85.12

VISUAL, AUDITORY AND OCULOMOTOR PROPERTIES OF SINGLE NEURONS IN THE POSTERIOR ECTOSYLVIAN GYRUS OF THE ALERT CAT. E.M. Bowman, W.A. Thomas* and C.R. Olson. Psych. Dept., Princeton Univ., NJ, 08544.

The cortex of the cat's posterior ectosylvian gyrus (p.e.g.) contains two districts dominated respectively by auditory and visual afferents (Bowman & Olson, J. Comp. Neurol., in press). These districts are probable homologues of areas occupying the superior temporal gyrus and sulcus of primates. In order to examine sensory and oculomotor activity in the p.e.g., we have recorded from single neurons in cats trained to make eye movements to visual and auditory targets.

Sixty-four neurons were studied during performance of this task. Of these, 19 responded only to visual stimuli, 19 responded only to auditory stimuli and 4 were bimodal. Nine of the visual neurons exhibited enhanced responses to a saccade target when it was accompanied by an isoazimuthal tone burst. In 6 of 10 auditory neurons, the response to a noise burst was attenuated when it was presented during fixation of a visual target.

The angle of the eye in the orbit influenced the activity of 21 of the neurons examined in the saccade task. Dependence of this effect on orbital position was confirmed in 4 neurons by repeating the task under reduced ambient illumination or with the head rotated relative to the stimulus. The activity of 17 neurons was altered during saccades, but, in all cases, this activity could be explained by visual reafferent stimulation.

We conclude that neurons of the posterior ectosylvian gyrus: (1) exhibit bimodal responses; (2) modulate their activity according to gaze angle; and (3) do not participate phasically in the generation of saccades.

85.13

SENSORY AND OCULOMOTOR PROPERTIES OF SINGLE NEURONS IN THE POSTERIOR CINGULATE CORTEX OF THE ALERT CAT. S.Y. Musil, M.J. Consuelos* and C.R. Olson. Psychology Department, Princeton University, NJ 08544.

Recent studies from our laboratory have demonstrated that the posterior cingulate cortex (CGp) of the cat is linked by strong, reciprocal and topographic pathways to cortical areas with sensory and oculomotor functions. These include area 7p (Olson and Lawler, J. Comp. Neurol. 259:13-30, 1987) and the medial frontal eye field (Olson and Jeffers, J. Comp. Neurol. 266:73-94, 1987). The visual and oculomotor connections of CGp far outweigh the limbic connections stressed in traditional accounts.

To test the hypothesis that CGp is directly involved in sensory and oculomotor processes, we have recorded from single neurons in cats implanted with scleral search coils and trained to make saccadic eye movements to visual-auditory targets. All phases of the experiment were under computer control.

Out of 98 neurons studied to date, 68 underwent a consistent change of activity in connection with the task. Firing was modulated according to the position of the eye in the orbit (43 neurons), during the perisaccadic period (44 neurons) or throughout the task (17 neurons). Only 6 of 98 neurons were activated by onset of the fixation or saccade target. With stimuli other than those employed in the task, however, 26 of 67 neurons tested exhibited somatosensory responses and 11 of 52 neurons tested exhibited auditory responses.

These results support the view that CGp is involved in sensory and oculomotor processes.

85.14

SENSORY AND OCULOMOTOR PROPERTIES OF SINGLE NEURONS IN AREA 7 OF THE ALERT CAT. C.R. Olson. Psychology Department, Princeton University, Princeton, NJ 08544.

In cat, as in monkey, visual information is relayed over transcortical pathways to a large set of interconnected oculomotor and association areas including area 7 (Olson and Lawler, J. Comp. Neurol. 259:13-30, 1987). To clarify the functions of this widespread cortical network, we have begun to carry out single-neuron recording experiments in alert cats trained to make saccadic eye movements to visual and auditory targets. We describe here results obtained from area 7 and compare them to results obtained in parallel studies of posterior cingulate cortex and the posterior ectosylvian visual belt (see accompanying abstracts).

Out of 40 area 7 neurons studied to date, all were activated during some phase of the saccade task. Ninety percent fired bursts in conjunction with saccades; firing commonly began before the saccade and persisted in the dark. Fifty percent underwent prolonged increases or decreases of activation during the entire task or certain phases of it; the determinants of the tonic firing were complex and never reduced simply to the angle of gaze. Thirty percent fired phasically in response to onset of a foveal or peripheral target. When tested outside task context, more than half of the neurons exhibited visual, somesthetic or auditory responsiveness, often with convergence. Suppression of auditory responses during fixation of a visual target was observed in 2 of 3 cells tested.

Area 7 differs markedly from posterior cingulate cortex in that many more neurons fire phasically to visual target onset; moreover, firing before and during saccades is more robust and more sharply time-locked to eye movements. Area 7 differs from the visual belt in containing neurons that carry saccade-related motor signals.

85.15

VENTRAL V3 IN THE CEBUS MONKEY: VISUAL TOPOGRAPHY AND PROJECTIONS TO V1 **R.Gattass, A.P.B.Sousa*, M.G.P.Rosa* and M.C.Piñon*. Instituto de Biofísica Carlos Chagas Filho, Rio de Janeiro 21941 Brazil.**

The visual topography of ventral extrastriate cortex was studied in four anesthetized and paralized Cebus apella, by means of multi-unit recordings. Ventral V3 (V3v) is a strip of cortex, anterior to V2, which contains a topographically organized representation of the upper visual quadrant up to 60 degrees eccentricity. In V3v, the representation of the horizontal meridian is located posteriorly and is congruent with that of V2, while that of the upper vertical meridian is located anteriorly. The foveal representation is located laterally, in the inferior occipital sulcus, while the periphery is represented medially and anteriorly, in the tentorial surface. The cortical magnification factor (CMF) decreases with increasing eccentricity and is highly anisotropic. For a given eccentricity, CMF along isopolar dimension is about 3 times greater than that along the isoeccentric one. After injections of fluorescent tracers in V1, labelled cells are found, mostly in the infragranular layers, within visuotopically corresponding portions of V3v. Projections to V1, similar to those from V3v, are also observed from a dorsal area, anterior to V2 in the lunate sulcus. This area contains, at least, a partial representation of the lower visual quadrant, and may correspond to the dorsal extension of V3v.

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85.16

VISUAL TOPOGRAPHY OF AREA TEO IN MACAQUES. D. Boussaoud*, L.G. Ungerleider, and R. Desimone. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Previous studies have shown that the inferior and ventral occipitotemporal cortex contains the upper visual field representations of areas V2, V3, and V4. To delineate any additional visual areas in this region, we mapped it with multiunit recordings in three anesthetized, paralyzed rhesus monkeys over repeated recording sessions. Anatomical tracers were injected at the end of the recordings. Anterior to the V4 border, which corresponds roughly to the representation of the horizontal meridian, we found a crude representation of the central 40-45° of the upper quadrant of the contralateral visual field. We tentatively term this area TEO, following Bonin and Bailey. Like V4, TEO appears to form an elongated dorsal-to-ventral band, and the representation of visual eccentricities seems to parallel that of V4. The representations of the foveal and parafoveal visual fields are located on the inferior convexity of the hemisphere, adjacent to area TE, and the representation of the upper periphery lies on the ventral surface of the hemisphere, adjacent to visually unresponsive cortex. The location of label following injections in TEO suggests that it receives inputs from area V4 and projects in turn to area TE.

85.17

Analysis of object motion in the ventral part of the macaque MST area. K. Tanaka, M. Fukumoto*# and H. Saito. NHK Sci. & Tech. Res. Labs. and #Univ. of Tokyo, Tokyo, Japan.

To infer the functional subdivision within MST, we studied response properties of cells in the ventral part of MST in paralyzed monkeys (M. fuscata) anesthetized with N_2O. Only directional cells which responded to a straight equidistant movement clustered there. The size of the excitatory receptive field ranged wide, whereas a movement within a small field (5-20 degs in diameter) generally evoked larger responses than that over a wide field. Such preference to a small-field movement is also shown by a half of cells in MT, the area which projects to MST. What is new in the ventral MST is that some cells behaved as if they responded to a relative movement of an object and its background. They did not respond to a movement of a wide textured field, but they started to respond to it if a small stationary mask was placed anywhere within the receptive field. The direction in which they responded was opposite to the preferred direction for a small-field movement. This reversal of effective direction can not be explained by a directionally antagonistic center-surround organization, since the response to the annular stimulus disappeared when the fringe of the mask was made blurred by remoting the mask from the screen. The vanishment and emergence of texture components at the fringe may be crucial for the response. With our previous results for the dorsal MST, we propose that information of object motion and that of field motion are processed separately in the ventral and dorsal MST, respectively.

85.18

BINOCULAR DISPARITY SENSITIVITY OF CELLS IN AREA MST OF THE MONKEY. H. Komatsu, J. P. Roy, and R. H. Wurtz Laboratory of Sensorimotor Research, National Eye Institute, N.I.H., Bethesda, MD 20892.

Neurons in the medial superior temporal area (MST) of extrastriate visual cortex frequently show a directionally selective response to moving stimuli, and many cells in a subregion of this area respond preferentially to motion of a large field patterned stimulus rather than to motion of a single spot. In the present experiments we determined whether these cells also prefer motion at selected depths within the visual field. Awake monkeys were rewarded for fixating on a tangent screen 86 cm in front of them; the positions of both eyes were monitored using the magnetic search coil technique. Disparity within a moving random dot pattern was produced by using a red-green color anaglyph projected onto the screen and viewed by the monkey through a red or green filter over each eye. With stimulation of the central region of the receptive field, many cells had broadly tuned disparity sensitivity. Most cells preferred near stimuli (crossed disparity). These cells also were generally sensitive to stimulus motion viewed monocularly, and the effect of disparity was to reduce the response to motion at certain disparities (mainly for far stimuli - uncrossed disparities). The cells also responded to changing disparity (motion in depth), but for most of the cells studied to date the response has been the same for motion toward and away from the monkey, suggesting that the response is due to the changing disparity stimulus passing through a range of fixed disparities for which the cells are selective.

85.19

ANATOMICAL SEGREGATION OF NEURONS SENSITIVE TO FACE EXPRESSION AND IDENTITY IN MACAQUE TEMPORAL CORTEX.
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Neurons which respond to faces have been described in both the superior temporal sulcus and the inferior temporal gyrus of the macaque monkey. These responses may convey information about face identity (Baylis et al., 1985, Brain Res. 342: 91.) or expression (Hasselmo et al., 1986, Neurosci. Lett. 26:S571). Models of human face recognition based on neuropsychological and psychophysical evidence postulate separate processes for the encoding of expression and identity (Bruce and Young, Brit. J. Psych. 77: 305).

To determine if expression and identity are encoded independently by face response neurons, 45 neurons in two macaques were tested on a stimulus set depicting three monkeys with three expressions each. The responses were analyzed with a two-way ANOVA. Of the 45 neurons tested, nine (20%) showed significant differences of response to expression independently of identity, and fifteen (33.3%) showed significant differences of response to identity independently of expression. Only three neurons showed a significant effect of both expression and identity.

Neurons sensitive to expression were found primarily in the cortex in the superior temporal sulcus, while neurons responsive to identity were found primarily in the inferior temporal gyrus. The difference in distribution was shown to be highly significant. Multidimensional scaling is used to describe differences in the representation of expression and identity in the two regions.

85.20

SEPARATE NEURONAL CODING OF STRUCTURE FROM BIOLOGICAL MOTION AND TRANSLATION IN THE MACAQUE TEMPORAL CORTEX.
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Neurones within the temporal cortex (area TPO) of the macaque monkey respond selectively to the sight of body movements of other animals (Bruce, C.J., et al., J. Neurophysiol., 46:369, 1981). The sensitivity of such cells was investigated to the form of the body defined through different types of motion. 31% of cells (19/61) selective for body movements under normal lighting continued to discriminate body form under "biological motion" conditions where the only visual information available is the pattern of movements of a small number of lights attached to points of limb articulation. These cells were selective for body view and were less responsive to random moving lights. For the remaining cells insensitive to biological motion stimuli, 73% (14/19) were selective for body form of 3D or 2D stimuli translating without articulation. The study indicates the existence of separate motion pathways for defining structure from translation (common fate) and non-rigid articulation (biological motion). Further evidence was found that descriptions of form, based on static and dynamic visual information, converge on cells within the temporal cortex.

85.21

FACE NEURONS AND NEURONS RESPONSIVE TO HUMAN FACES IN THE INFEROTEMPORAL CORTEX OF THE MONKEY.
S. Yamane*, K. Kawano, S. Kaji*, and H. Komatsu. Electrotechnical Lab., Umezono, Tsukubashi 305, Japan

Single neurons were recorded in the inferotemporal cortex (IT) of a monkey trained to discriminate three human faces from other stimulus. We used 30 faces, a square and an ellipse as the discrimination stimuli, and a small green spot, appearing after one of these stimuli, as a response cue for the monkey. We examined 446 single neurons in the anterior and posterior banks of the superior temporal sulcus, and the gyrus of the IT. There were 86 neurons responsive to faces. Most of them also responded to the green spot (41/86), and the square and/or the ellipse (24/86). 21 neurons responded only to the faces, and all of them showed selective responses to the 30 faces. Most of them (18/21) were located in the gyrus of the IT. To know the orign of the selectivity, we analysed the correlation between neuronal responses and facial features. 5 of these neurons showed highly correlated responses to the combinations of distances between facial parts. For example, one neuron showed responses correlated to both the amount of the hair above the left eye and the distance between the eyes and the mouth. Its responses to montaged faces, constructed with two of the 30 faces, confirmed the neuron's preferrence to the combination of facial features. The fact that the neurons detected essential features in faces leads us to call each a face neuron.

85.22

SACCADE-RELATED ACTIVITY IN AREA LIP.
S. Barash, R. Andersen, M. Bracewell, J. Gnadt, L. Fogassi. Dept. Brain & Cog. Sci., M.I.T., Cambridge, MA 02139.

Many cells in area LIP, a subdivision of the macaque posterior parietal cortex, are excited by visual stimuli but are also active in relation to saccades. The properties of the saccade-related activity were studied in two tasks in which the stimulus and motor responses were temporally isolated.

In the first task the animal was required to memorize the location of a briefly presented target and make a saccade to that remembered location after a variable time period. Generally saccade activity was found to begin 50 to 150 msec before the initiation of the saccade, to peak within 0 to 50 msec after the peak velocity of the saccade, and to decay exponentially back to baseline 100 to 200 msec after the eye movement. Especially interesting are a subclass of cells that remain active in the dark in the interval between the offset of the visual target and the eye movement, which, in the memory task, can be as long as 2.7 seconds. In previous experiments we have shown that this memory linked activity is coding the direction and amplitude of the impending saccade in motor coordinates and thus represents the intended movement of the animal.

The nature of the intended movement activity was further analysed using a double saccade task. In this paradigm the second saccade was made to a location within the motor field of the neuron without the target light ever falling within the receptive field of the neuron under study. The onset of the intended movement activity was very tightly linked to the first eye movement, generally beginning during the movement. On the other hand, the offset of the intended movement activity decayed relatively slowly, requiring on average 200 msec to return to baseline after the completion of the second eye movement. Interestingly, if a light was turned on at the target location of the second saccade during the interval between the two saccades, the activity of the cells was considerably boosted, and the saccades themselves had higher peak velocities. These results suggest that area LIP is involved in high level visual-motor integration including aspects of motor planning.

NEUROETHOLOGY II

86.1

TEMPORAL ANALYSIS OF HORMONE INDUCED CHANGES IN ELECTRIC ORGANS AND ELECTRIC ORGAN DISCHARGES.
E.G. Freedman, J. Olyarchuk*, M.Marchaterre, and A.H.Bass. Section of Neurobiol. and Behavior, Cornell Univ. Ithaca, N.Y. 14853.

Gonadal steroids have been shown to increase the duration of the electric organ discharge (EOD) and to increase the surface area of the anterior face of the cells responsible for producing the dishcarge (electrocytes). This study followed the time course of the changes in amplitude and duration after testosterone treatment in order to understand the structural basis for the observed changes. Fish (*Brienomyrus sp.*) were implanted intraperitoneally with gonadal steroid hormones (11Keto-testosterone or 17-a-methyl-testosterone), and EODs were monitored for 27 days after treatment. The duration of all three phases of the triphasic waveform began to increase in duration three days after implantation. In treated animals the duration of the first phase increased linearly for about six days after which the duration remained constant. The durations of the second and third phases increased linearly for the duration of the experiment. Changes in surface area of the anterior and posterior faces (the membranes responsible for the second and third phases of the EOD respectively) of the electrocytes were measured. The surface area of both the anterior and posterior faces was found to increase after treatment. Increased surface area suggests an increase in the total capacitance of the anterior and posterior faces of the electrocytes, and such capacitative changes can account for the observed changes in duration of the second and third phases of the EOD. The amplitude of the second phase of the EOD increased as the total duration of the EOD increases; conversely, the amplitude of the third phase of the EOD decreased with increasing total EOD duration. These changes in amplitude may be due to the interaction of currents flowing across the two excitable faces in opposite directions. No change in first phase amplitude was observed. Electrocytes were measured from the outer edge of the anterior face to the outer edge of the posterior face, and their width was found to increase. The increase in electrocyte width and the increase in duration of the first phase of the EOD followed the same time course.

In summary, the way in which the EOD changes after exposure to gonadal steroid hormones is temporally linked with changes in the morphology of the electrocytes. We suggest that these morphological changes are indeed the structural correlates for changes in the EOD.

Supported by NIH Grants GM07469 and NS19942.

86.2

PATTERNS OF THE ELECTRIC ORGAN DISCHARGE DURING COURTSHIP AND SPAWNING IN THE MORMYRID, *POLLIMYRUS ISIDORI*.
B.O. Bratton and B. Kramer. Zoologisches Institut, Universitat Regensburg, W. Germany.

The weakly electric, African fish, *Pollimyrus isidori* proved an excellent subject to study 1) the intraspecific, individual variability of electric organ discharge (EOD) pulse characteristics as related to questions of mate recognition; and 2) reproductive behavior and electric signalling by EOD interval patterns. Pulse rhythms were examined in socially interacting fish, including the behaviors of courtship and spawning. Characteristic patterns are also described for resting, hiding, hovering, swimming and aggressive behaviors.

Male and female EOD interval patterns were not found to be sex specific before the time of courtship and spawning. Just prior to the onset of courtship, however, females displayed a "spawning readiness pattern" of a medium uniform rate consisting of fairly regular pulse intervals (8-12 Hz), contrasting with the much more irregular proceeding EOD activities. During the male-female engagement of courtship/spawning the male displayed a similar pattern of regularized intervals (about 10 Hz) for each 15-25 s bout. Between bouts, males displayed a higher rate, variable EOD pattern. The relative timing of EOD interval patterns associated with reproductive behaviors suggest an instrumental function of the EOD with the occurrence of courtship and spawning.

86.3

MEASUREMENT OF THE ELECTRIC FIELD OF *GNATHONEMUS PETERSII* AND RECONSTRUCTION OF FIELDS GENERATED DURING EXPLORATORY ACTIVITY. B. Rasnow[1], M.E. Nelson[2], and J.M. Bower[2]. Depts. of Physics[1] and Biology[2], Caltech, Pasadena, CA 91125.

We are investigating the role of body position and the timing of the electric organ discharge (EOD) in exploratory behavior of the weakly electric teleost, *Gnathonemus petersii*. We believe the electric fish is an excellent model system for studying the process of active repositioning of sensory structures during exploration and the possible role of the cerebellum in the control of this process. In order to evaluate the electrosensory consequences of body position and EOD timing in the fish, we have developed a computer-based finite-element simulation which allows us to calculate the fish's self-generated electric fields and the resulting transdermal potentials for arbitrary body positions in complicated environments. The accuracy of the simulation is dependent on the electrical properties of the fish's skin and body and we have calibrated the simulation by measuring the unperturbed fields generated by immobilized fish. To reconstruct the transdermal potential profiles generated during exploratory activity, we provide the simulator with accurate body position and EOD discharge data from real fish. This is obtained from videotape records of the trajectory and spatial orientation of freely moving fish and synchronized analog recording of the firing times of the electric organ in response to novel objects placed in the environment. In this way, we can simulate the sequence of peripheral electrosensory images established by the fish during its active exploration of the environment. (This work supported by the Joseph Drown Foundation and NIH Grant NS22205.)

86.4

SENSORY GUIDANCE OF PASSIVE ELECTROLOCATION IN ELECTRIC FISH. C.D. Hopkins, E. A. Davis* and E. Mutisya*. Neurobiology & Behavior, CORNELL UNIVERSITY. Ithaca, New York 14853.

•*Passive electrolocation* occurs when an electroreceptive organism uses exafferent information to locate the source of electric signals from animate or inanimate sources in the environment. It serves an important function in electric communication by orienting and guiding a receiver's responses to electric organ discharges (EODs) from other fish. • We observed territorial *Gymnotus carapo* approaching and attacking artificial EODs played through dipole or bipole electrodes in their home tanks. Six electrode configurations were used to generate different stimulus geometries; the observations were made in the dark with infrared video. Although superimposed paths were different for each electrode geometry, in each case the fish adopted a simple strategy for finding the active electrodes: they aligned their body axis parallel to the axis of the local electric field (a vector), and while swiming forward, maintained a constant alignment until they reached the electrode(s). "Hunting" motions of approximately 30° to the left and right of the electric field axis were common. The fish showed a slight preference for approaching the "head" end of the electric model. When the stimulator is a slowly-rotating horizontal dipole, the fish makes loops to follow the rotating vector. For rapid rotations, the fish follows the mean vector direction. The fish are *not* disoriented by homogeneous electric fields. • We modeled the passive electrical properties of the fish's body interior, its skin, the tank, and the electrodes using a two dimensional resistive mesh and iteratively solved for the DC voltage at each point in the mesh on the Cornell Super Computer. Both elliptical and disk-shaped fish models could use the position of the maximum transepidermal voltage as a cue to the local electric field vector direction. By left to right hunting, elliptical fish models could use the absolute magnitude of the maximum transepidermal voltage. Left side/right side comparison of transepidermal voltage seem a particularly potent cue for alignment of the body axis to the field vector. • Partial support by NIMH grant MH37972to CDH.

86.5

NEURAL ACTIVITY IN THE NUCLEUS EXTEROLATERALIS ANTERIOR (ELa) OF MORMYRID ELECTRIC FISH. S. Amagai*, C.D. Hopkins and G.D. Harned* (SPON: B.R. Johnson). Neurobiology & Behavior, Cornell University, Ithaca, NY 14853

The tuberous Knollenorgan electroreceptors of the Mormyrid electric fish are implicated in detecting the electric organ discharges (EOD) of other fish. The primary afferent terminates ipsilaterally in the nucleus of the electrosensory lateral line lobe (nELLL), and receives a strong inhibition by the electric organ corollary discharge, preventing throughput of reafference from the fish's own EOD. The sole output of the nELLL projects bilaterally to the mesencephalic nucleus exterolateralis anterior (ELa), which is thought to be a processing area for timing information, and terminates on large intrinsic neurons as well as small output neurons which are the only types of cells known in this nucleus (Mugnaini, E. and Maler, L., Anat. and Embryo., 176:313, 1987). Glass microelectrodes were used to make single unit intra- and extracellular recordings from ELa in a pulse Mormyrid, Brienomyrus brachyistius.

Most of the units encountered were spontaneously active and responded to a brief electrical pulse applied transversly across the fish with a single spike at a short latency ranging from 2.5-3.5ms for different units. Variations in each unit's response latencies were typically less than 100μs. Less common were units which fired double or triple spikes, both spontaneously and in response to electrical stimuli. The number of spikes occuring close together usually remained constant for a given unit and was independent of stimulus intensity. Other units had yet more complex characteristics, including bursting, stimulus intensity dependence and loose time-locking.

Post stimulus time histograms for these units were generated from the time of the spinal cord electric organ command discharge. This allowed the presumed inhibition at nELLL to be visualized as silent time periods and was used to identify Knollenorgan inputs. Considerable variation was observed in both the onset and the duration of inhibition, not only for different types of units but also among units which appeared to be similar in their behavior. Supported by NIMH grant MH37972(CDH).

86.6

SUPPRESSION OF THE ELECTRIC ORGAN DISCHARGE BY DESYNCHRONIZATION OF PACEMAKER NEURONS IN THE ELECTRIC FISH HYPOPOMUS. W. Heiligenberg, M. Kawasaki and J. Nissanov. UCSD, La Jolla CA 92093.

The gymnotiform electric fish, Hypopomus, produces pulse-like electric organ discharges (EODs) of up to several tens of Hz. EODs are triggered individually by single command pulses of the medullary pacemaker nucleus. This nucleus contains electrotonically coupled 'pacemaker cells' which generate the rhythm, as well as 'relay cells' which transmit the command signals to the motor neurons of the electric organ. Normally, these two populations of cells fire in synchrony with a phase difference of approximately 1 msec. Iontophoretic stimulation of a brain site close to the prepacemaker nucleus, which projects to the pacemaker nucleus, caused an interruption of the EOD. Simultaneous intracellular recordings in the pacemaker nucleus revealed that while relay cells show high-frequency firing with reduced amplitude, pacemaker cells continue to fire with normal amplitude and a slight reduction in frequency regularity. In contrast to Eigenmannia, a gymnotiform electric fish of higher EOD frequency in which both pacemaker and relay cells cease firing during interruption of the EOD, Hypopomus apparently is able to break the coupling between pacemaker cells and relay cells.

86.7

PACEMAKER MODULATIONS IN THE ELECTRIC FISH STERNOPYGUS. C.H. Keller, M. Kawasaki and W.Heiligenberg, Scripps Institution of Oceanography, UCSD, La Jolla CA 92093

The weakly electric fish, Sternopygus, produces quasi-sinusoidal electric organ discharges (EODs) at a regular rate in the range of 50 to 200 Hz. EODs are commanded by a medullary pacemaker nucleus. This nucleus contains electrotonically coupled 'pacemaker cells' which generate the rhythm, as well as 'relay cells' which transmit the command pulse to the motor neurons of the electric organ. Rapid and transient frequency increases as well as brief interruptions are produced during courtship and aggression. These two distinct forms of discharge modulations can be elicited by iontophoretic stimulation with L-glutamate at separate midbrain sites. Investigations underway demonstrate different changes in firing patterns within pacemaker and relay cells during each form of EOD modulation. Pacemaker cells recorded intracellularly during interruptions in the EODs continued firing at their regular frequency although with increased jitter. Field potentials recorded in the vicinity of the relay cells, however, were greatly reduced in amplitude during EOD interruptions, suggesting that interruptions are generated by either silencing or desynchronization at the level of the relay cell.

86.8

DIFFERENT GLUTAMATE RECEPTORS MEDIATE DISTINCT BEHAVIORS IN A SINGLE BRAINSTEM NUCLEUS IN WEAKLY ELECTRIC FISH. J. Dye, W. Heiligenberg, K. Keller and M. Kawasaki. Scripps Institution of Oceanography, Neurobiology Unit, UCSD A-002, La Jolla, CA 92093.

The weakly electric 'wave-type' fish possess an electric organ which emits a regular, quasi-sinusoidal discharge. They are capable of behavioral modulations for purposes of communication (e.g. brief, transient accelerations in frequency, called 'chirps') and preservation of electrolocating abilities (the jamming avoidance response or JAR: a much slower frequency change). The electric organ is controlled by the medullary pacemaker nucleus.

The pacemaker nucleus of one wave species, Apteronotus, continues to fire rythmically in vitro. It can also be induced to chirp in vitro by stimulation of afferents from the prepacemaker nucleus. Glutamate, aspartate and various agonists of glutamate receptors were found to cause transient accelerations when applied to the in vitro nucleus; among the agonists, kainate and NMDA appeared to be the most potent. Glutamate antagonist, PDA, blocked the chirps in vitro, whereas NMDA antagonist, APV, did not, but in some trials blocked a slow relaxation to baseline seen after trains of stimuli. Examination of the effects of these agents in vivo confirmed that PDA reversibly blocks all frequency modulations in the pacemaker nucleus while APV selectively and reversibly eliminates the JAR.

86.9

ANATOMICAL AND PHYSIOLOGICAL PROPERTIES OF THE PREPACEMAKER NUCLEUS OF EIGENMANNIA. G. Rose, M. Kawasaki, W. Heiligenberg, and L. Maler. Scripps Inst. Ocean., UCSD, La Jolla, CA 92093 and Univ. of Ottawa, Ottawa, Ontario K1H8M5.

Eigenmannia, a weakly electric fish from South America, produces rhythmic electric organ discharges (EODs) for the purpose of spatial orientation and social communication. The EOD rhythm follows that of an electrically coupled group of cells in the medullary pacemaker. This nucleus is innervated exclusively by diencephalic prepacemaker neurons. Abrupt and slow modulations in the EOD frequency occur in the context of social behaviors and can be induced by L-glutamate stimulation of ventrolateral prepacemaker locations, occupied by large cells, and dorsomedial locations, containing small cells, respectively. Accordingly, intracellular recordings show that the large neurons generate abrupt modulations. Extracellular recordings presumed to be from the small cells suggest that they are decision-making units situated at the top of a neuronal hierarchy for the control of the Jamming Avoidance Response. These neurons receive the convergence of phase information required to achieve temporal hyperacuity.
Grants: NS22740, NS22244-02, BNS84161115

86.10

SINGLE ELECTROSENSORY NEURONS RESOLVE TEMPORAL DISPARITIES OF ONE MICROSECOND. Masashi Kawasaki, Gary Rose & Walter Heiligenberg. Neurobiology Unit, Scripps Institution of Oceanography, University of California at San Diego, A-002, La Jolla, California 92093.

Behavioral studies have revealed that animals are capable of resolving temporal disparities in the microsecond range. Since this resolution is far superior to that of individual receptors, it must be achieved through central neuronal mechanisms. It is unclear, however, whether such sensitivity exists at the level of single neurons, or only at the behavioral level through the collective action of many less sensitive neurons. We have found that single neurons in the prepacemaker nucleus of a weakly electric fish are sensitive to temporal disparities as small as 1 microsecond, the highest temporal sensitivity ever observed at the single-neuron level. The remarkable temporal resolution of these prepacemaker neurons results from a high degree of spatial convergence of afferent inputs. These neurons represent the final elements of a sensory hierarchy and directly control a behavior.

86.11

PHYSIOLOGICAL AND ANATOMICAL CORRELATES OF THE STEROID-SENSITIVE EOD OF *STERNOPYGUS*. A. Mills, H. H. Zakon and A.H. Bass^. Dept. of Zoology, Univ. Texas, Austin, TX 78712; ^Section of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

The electric organ discharge (EOD) of the wave gymnotid *Sternopygus macrurus* is continually produced as a regular series of pulses, resulting in a quasi-sinusoidal waveform. The duration (dur.) of each pulse is longer in fish of lower EOD frequencies (freq.) and shorter in fish of higher freq. The pulse broadens after treatment with dihydrotestosterone (DHT), as freq. lowers. Since the basis of each EOD pulse is the simultaneous action potentials (a.p.s) in the electrocytes of the electric organ, it is likely that a.p. durs. recorded from fish with long pulse durs. are long, and that a.p. dur. increases following DHT treatment. To test the first assumption, intracellular recordings in electrocytes were compared to the externally-recorded EOD. The resting potential ranged from -40 to -104 mV (mean=-71±17mV), and threshold from -10 to -60 mV (mean=-31±13mV). The shape of each intracellularly-stimulated a.p. was very similar to the EOD pulse, with a faster rise to peak; this may be due to the absence of current shunting through acetylcholine channels. A.P. dur. ranged from 4.0 to 11.3 msec in fish with pulse durs. from 4.8 to 9.0 msec, respectively. The dur. of the a.p. was not identical to the pulse dur., but was highly correlated (r=0.88, N=7 fish), with most a.p.s slightly longer (mean a.p. dur. 12% greater than mean pulse dur.). Thus the basis of the DHT-induced increase in pulse dur. is probably an increase in electrocyte a.p. dur. To investigate the anatomical basis for the differences in a.p. dur., we studied the morphology of the tubular-shaped electrocytes using electron microscopy. Transverse sections 0.5-0.65µm thick were studied to determine overall electrocyte size, while longitudinal semi-thin sections were used to examine the surface invaginations characterizing the anterior and posterior faces. Although examination of field-preserved fish indicated a sex difference in electrocyte size (larger in males), DHT-treatment of lab-maintained fish did not induce a significant increase in size in comparison to controls. There also appear to be no significant changes in the extent of invaginations along either face. Supported by NIH grant to AHB and NSF grant to HHZ.

86.12

ULTRASTRUCTURAL ANALYSIS OF SYNAPTIC CONTACTS ON HRP LABELED SPHERICAL-LIKE NEURONS IN STERNOPYGUS. B. Losier* and J.A. Matsubara (SPON: M. Yoon), Dept. Anatomy, Dalhousie University, Halifax, Nova Scotia, Canada.

The weakly electric fish *Sternopygus*, a low frequency wave species, does not possess a JAR and previous physiological recordings in the electrosensory lateral line lobe (ELLL) revealed that certain cells are able to encode moving objects even in the presence of jamming signals. The aim of this study was to characterise the morphology and distribution of synapses on a morphologically unique cell type in the ELLL of *Sternopygus*. This cell is like the spherical cell found in other species in its location in the deep neuropil layer (DNL) and projection to the torus semicircularis (TS). However, it is different in that it possesses an extensive dendritic bush which arborizes in the primary afferent zone of the ELLL. We studied retrogradely labeled, spherical-like neurons at the ultrastructural level. The perikarya consisted of an electron lucent cytoplasm richly endowed by cytoplasmic organelles. Asymmetrical chemical synapses with round vesicles (30-40 nm) were seen on the cell soma and dendrites. Thus, the synaptology of DNL neurons differs remarkably between species, with electrotonic gap junctions observed on spherical cells of *Eigenmannia* and asymmetrical chemical synapses on spherical-like cells of *Sternopygus*. The relationship between the intrinsic organization of the ELLL and the non-jammable electrolocating abilities in *Sternopygus* will be discussed. (This work was funded by NSERC).

86.13

A TECHNIQUE FOR ANALYZING NEWLY-SYNTHESIZED PROTEINS WITHIN THE ELECTRIC ORGAN OF STERNOPYGUS. J. M. Patterson, and H. H. Zakon Dept. of Zoology, University of Texas, Austin, Texas. 78712

Weakly electric fish of South America, the Gymnotiforms, produce a peroidic, quasi-sinusoidal electric field which they use for electrolocation and social behavior. This electric field is generated by synchronous activity of electrocyte cells located in the tail. Previous studies have shown that administration of dihydrotestosterone (DHT) lowers the electric organ discharge (EOD) frequency and increases the pulse duration of the EOD. Since steroids often exert their effect via protein synthesis we have attempted a biochemical analysis of this tissue. We report here a technique for detecting newly-translated proteins within the electric organ, and our preliminary findings using one-dimensional gel electrophoresis.

Electric organ from Sternopygus was obtained by removing a segment of tail posterior to the anal fin, and dissecting away skin and spinal cord. The tissue was transferred to a tube containing 60 uCi ^{35}S-methionine (NEN) in 1 ml. of Hickman's saline and incubated at 25 °C for 1 hr. The organ was then frozen on dry ice, homogenized in an SDS/BME sample buffer, placed in a 100 °C water bath for 3 min., centrifuged at 10,000xG for 5 min., and frozen at -20 °C. Twenty ul./lane of sample was analyzed using 1-D SDS/PAGE. Gels were run at constant voltage (125v. for 4-5 hr.), stained with Coomassie Blue, destained, and treated with PPO phosphor for fluorography. Fluorograms were exposed 10-20 days, and consistently showed 20-30 labelled bands.

We have made several observations in our early work with this technique. 1. Electric organ translates proteins in vitro at a rate comparable to liver tissue measured under the same conditions, and at a much higher rate than skeletal muscle, from which it is derived embyrologically. 2. In vivo injections of labelled methionine confirm that no artifacts are generated by the in vitro procedures. 3. No significant differences between DHT-treated fish and control fish can be detected with the resolution offered by this technique, indicating that DHT induces small changes in protein synthesis, rather than major translational changes. Two-dimensional gel electrophoresis will be required to resolve such differences.

86.14

NEURAL CORRELATES OF MAGNETIC FIELD DETECTION IN THE MARINE MOLLUSC TRITONIA DIOMEDEA. K. J. Lohmann and A. O. D. Willows. Dept. of Zoology, University of Washington, Seattle, WA 98195.

Behavioral experiments have indicated that the nudibranch mollusc Tritonia diomedea can detect the magnetic field of the earth (K. J. Lohmann and A. O. D. Willows, Science, 235: 331-334, 1987). Intracellular recordings from a semi-intact whole animal preparation demonstrated that the large, visually identifiable neuron Left Pedal 5 (L Pe 5) responds with enhanced electrical activity to changes in earth-strength magnetic fields. Responses of L Pe 5 to a periodically imposed magnetic stimulus were characterized by an increase in spiking frequency occurring 6-12 minutes after the first field rotation. The same stimulus fails to elicit a response from L Pe 5 in isolated brain preparations. Cobalt fills and electrophysiological data indicate that neurites project from L Pe 5 through left pedal nerves 2 and 3. The function of L Pe 5 is not currently known. The neuron might be a primary sensory receptor, an interneuron receiving synaptic input from one or more primary receptors, or a motoneuron exerting a subtle, visually undetectable influence on a behavioral (e.g., turning) response to the stimulus.

87.1

COMPARATIVE NEUROPHARMACOLOGY OF IPSAPIRONE, FLUPRAZINE AND (+)-8-HYDROXY-DIPROPYLAMINOTETRALIN. B. A. McMillen, S. M. Scott* and H. L. Williams*, Dept. Pharmacol., Sch. Medicine, East Carolina Univ., Greenville, NC 27858.

The potential anxiolytic aryl-piperazine drugs, ipsapirone and fluprazine, were compared to DPAT for acute effects on monoaminergic neurotransmission. Ipsapirone, 10 mg/kg i.p., caused a small increase in mouse striatal dopamine (DA) metabolism without alterations in frontal cortex. Serotonin (5HT) metabolism decreased slightly (not significant) in both areas. In contrast, anti-aggressive doses of fluprazine or DPAT reduced 5HT metabolism. Both ipsapirone and fluprazine could reduce stimulation of striatal DA autoreceptors by apomorphine in vivo. Ipsapirone or trifluoperazine (TPZ) was added to the rats' drinking water for 6 weeks. Ipsapirone did not alter D2, 5HT2 or β receptor binding. TPZ increased D2 receptor Bmax: an effect that could be reversed by simultaneous infusion of ipsapirone, 1.0 mg/kg/day s.c., for 2 weeks prior to removal of all drugs. These data demonstrate that fluprazine and ipsairone have less pronounced dopaminergic effects than buspirone and more closely resemble gepirone in their pharmacology. Decreased serotonergic activity is the most likely explanation for the anti-aggressive effects of these drugs and possibly for their anxiolytic effects. Whether ipsapirone reversal of antipsychotic-induced increased D2 receptor binding has a therapeutic correlate is unclear.

87.2

FOOTSHOCK-INDUCED FREEZING IN RATS AS A MODEL FOR ASSESSING ANXIOLYTICS. L.H. Conti*, C.R. Shaw*, J.W. Rzeszotarski*, M.E. Guzewska*, S. Ellenberger*, J.W. Ferkany and M.E. Abreu* (SPON: L. Steranka). NOVA Pharmaceutical Corp., Baltimore, MD 21224

Soon after receiving electric footshock, rats assume a defensive posture referred to as freezing. We examined whether drugs with known anxiolytic action reduced the amount of time which rats spend in the freeze posture following mild footshock. Rats (male Sprague Dawley, 250g) were given an injection (ip) of a drug or vehicle 30 min prior to testing. Testing was conducted during a 6.5 min observation session during which rats received 2 mild footshocks (.5mA, .5sec). The amount of time which animals spent freezing following the second footshock served as the primary index of anxiety.

Diazepam, CPP (an excitatory amino acid antagonist with an anxiolytic profile), buspirone, and NPC 12626 (a novel excitatory amino acid antagonist) reduced time spent freezing in comparison to controls. Neither CPP, buspirone, nor NPC 12626 stimulated locomotor activity in experiments in which no shock was delivered. In a further control experiment, it was found that amphetamine (0.5mg/kg) did not reduce freezing. These data indicate that the footshock-induced freezing paradigm may be a sensitive method with which to assess anxiolytic potential of drugs with various mechanisms of action.

87.3

THE EFFECTS OF BUSPIRONE ON COMPARABLE RATES OF PUNISHED AND NONPUNISHED RESPONDING. C.V. Boley*, S. Izenwasser, S.I. Dworkin (SPON: G.F. Guerin). Dept. of Psychiatry, LSU School of Med., Shreveport, LA 71130.

The effects of buspirone were determine using a procedure that can evaluate the punishment-specific effects of drugs. Seven littermate pairs of male F-344 rats responded under this procedure which generated comparable rates of punished and nonpunished responding. One subject of each pair was maintained on a random-ratio schedule of food presentation. The interreinforcement intervals from this subject were used to generate a yoked variable-interval schedule of reinforcement for a littermate. A random-ratio schedule of electric footshock was then added to the random-ratio schedule of food presentation. The addition of the punishment contingency resulted in similar rates and patterns of responding by both the punished and yoked unpunished rats. The effects of buspirone (0.56-10.0 mg/kg, i.p.) were determined after stable baselines of responding had been obtained.

Chlordiazepoxide increased punished responding in 4 of the 7 rats studied. Maximal effects occurring at the two lowest doses evaluated. There was, however, little effect of the drug on nonpunished responding. These findings suggest the rate increasing effects of buspirone can be punishment-specific.

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87.4

CALCIUM CHANNEL ANTAGONISTS AND PSYCHOLOGICAL DISORDERS Y.Watanabe*, J.W.Spain*, H.Honda*, S.Emanuel*and T.Shibuya Univ. Ill. College of Medicine, Rockford, IL 61107 and Tokyo Medical College, Tokyo 160 JAPAN

The potential of calcium channel antagonists as a novel treatment of psychological disorders was investigated using the spontaneously hypertensive rat (SHR) exposed to psychological stress. The physiological characteristics, with respect to blood pressure, of the SHR and its normotensive control strain, Wistar Kyoto rat (WKY), have been well documented. Furthermore, there exists several differences in behavioral response to stimuli. We observed significantly higher spontaneous locomotor activity in SHR, when compared to age-matched WKY, during the first 30 min of exposure to a novel environment. Activity counts for SHR were roughly 60% higher than WKY. Both 10 day chronic and acute treatment with nitrendipine (10 mg/kg, s.c.) resulted in a 25% reduction in activity. Chronic treatment ended 24 hr prior to testing, ruling out the possibility of a direct sedative action of nitrendipine causing reduced activity. We suggest that hyperactivity during acclimation to a novel environment may, at least partially, be caused by anxiety resulting from unfamiliarity. In support of this hypothesis, we observe that chronic or acute treatment with a novel anxiolytic derivative of buspirone (SM-3997) produced similar decreases in activity. The results suggest that calcium channel antagonists may be clinically useful in the management of stress-induced anxiety.

87.5

EXPERIMENTAL ANXIETY MODEL EMPLOYING THE INTRASPECIES EMOTIONAL COMMUNICATION: CORTICOSTREONE RESPONSE OF RATS IN THE COMMUNICATION BOX METHOD. N. Ogawa, M. Ishikawa* and C. Hara. Dept. Pharmacol., Ehime Univ. Sch. of Med., Ehime 791-02, Japan.

The communication box (CB) consists of both electric shock (ES) and non-ES compartments. In the CB, the animals placed individually into non-ES compartments (responder; R) could receive emotional cues (EC) from the animals subjected to ES in the ES compartments (sender; S). Therefore, "R" were considered to be in psychosocial anxiety. In order to establish the method as a tool for inducing psychosocial anxiety, the present study examined corticosterone response of "R". Male Wistar strain rats (9 weeks old) were used. They were housed in an air-conditioned room with 12:12 LD cycle (lights on 0700) under free access to food and water. Blood was collected from tail vein before and after the exposure of EC. Corticosterone was assessed by the modified protein-binding radioassay of Murphy. In the daytime and nighttime exposures to EC for 6 hr in the CB, the corticosterone level of "R" increased to have a peak time at 1 hr after the exposure. In the daily exposure to EC for 1hr in the daytime under the condition renewing "S" to intact animals daily, the pre-level of corticosterone of "R" just before the EC exposure progressively increased, and was significantly higher than the initial level of Day-1 on Day-5. These results suggest that the increased pre-level is applicable for a marker of anxiety.

87.6

PRECLINICAL PHARMACOLOGICAL STUDIES OF WY-48,624 (ENCIPRAZINE), A NOVEL ANXIOLYTIC AGENT. J.T. Haskins, D.E. Jones*, R. Scerni*, E.A. Muth and T.H. Andree. Wyeth-Ayerst Research, Princeton, NJ 08540.

WY-48,624 (4-(2-methoxypheny1)-α-[(3,4,5-trimethoxyphenoxy)methyl]-1-piperazine ethanol, dihydrochloride was synthesized and tested as an anxiolytic agent and is now in phase II clinical trials. In in vivo behavioral studies the anxiolytic potential of WY-48,624 (ENCIPRAZINE) is predicted in antiagression testing procedures. In these tests Enciprazine is similar in potency to diazepam. In vitro neurochemical studies indicate that Enciprazine has the highest affinity (although weak) for alpha-1 adrenergic receptors (Ki=163 nM), and has little or no affinity for opiate, benzodiazepine, D-2, muscarinic cholinergic, alpha-2 adrenergic, or beta adrenergic receptors. Enciprazine is also weak at inhibiting the uptake of NE, DA, and 5-HT and does not inhibit monoamine oxidase activity. In neurophysiological studies, intravenous injections of Enciprazine, like buspirone, inhibited serotonergic neuronal activity although its effects were weaker than those of buspirone. Enciprazine and buspirone activated noradrenergic neurons although Enciprazine was again weaker than buspirone. Enciprazine, although having an unremarkable neuroreceptor profile, has behavioral effects predictive of anxiolytic activity and also has neurophysiological effects similar to the nonbenzodiazepine anxiolytic buspirone.

87.7

PSYCHOPHARMACOLOGICAL PROFILE OF WY-47,846 - A PUTATIVE NONBENZODIAZEPINE ANXIOLYTIC AGENT. **J. A. Moyer, T. H. Andree, J. T. Haskins, M. Abou-Gharbia and E. A. Muth. Dept. of Experimental Therapeutics, Wyeth-Ayerst Research, Princeton, NJ 08543**

Wy-47,846 (3a,4,4a,6a,7,7a-hexahydro-2-[4-[4-(2-pyrimidinyl)-1-piperazinyl]-butyl]-4,7-etheno-1H-cyclobut [f] isoindole-1,3(2H)-dione), a compound with structural and biochemical similarities to buspirone, was examined in a series of preclinical tests to determine efficacy and side effect potential as a nonbenzodiazepine anxiolytic agent. Wy-47,846 suppressed avoidance responding and increased escape performance in conditioned avoidance procedures at doses which did not cause animal debilitation. As several nonbenzodiazepine agents have similar profiles in this test, these results indicate Wy-47,846 may have preclinical anxiolytic activity. Like most nonbenzodiazepines, Wy-47,846 lacked anticonvulsant activity and anxiolytic activity in anticonflict models. With respect to side effect liability, Wy-47,846 was more potent in antagonizing apomorphine-induced climbing behavior than stereotyped behavior. Wy-47,846 was considerably weaker in producing benzodiazepine-associated side effects (sedation, ataxia, drug interaction effects) than diazepam. These studies indicate Wy-47,846 may have preclinical anxiolytic activity with a favorable side effect profile. Behavioral results will be discussed in terms of neurochemical and neurophysiological effects of Wy-47,846.

87.8

DISCRIMINATIVE STIMULUS PROPERTIES OF GEPIRONE. V. Guanowsky and P.A. Seymour, Central Research Division, Pfizer, Inc., Groton, CT 06355

The anxiolytic buspirone has been demonstrated to possess discriminative stimulus properties in pigeons (Mansbach and Barrett, JPET, 240: 364, 1987), but has been found to produce only a poor discriminative stimulus in rats (Hendry et al., Pharmacol. Biochem. Beh. 19: 97, 1983). This may be due to the fact that doses required to produce effective stimulus control are behaviorally disruptive. Since gepirone appears to be less disruptive, a group of rats was trained to discriminate gepirone (3.2 mg/kg, i.p.) from saline. Following acquisition, administration of gepirone resulted in drug-appropriate responding (ED50 = 1.1 mg/kg). Additional compounds with high affinity for the 5-HT1A receptor were then tested in generalization experiments. The anxiolytic buspirone and the putative anxiolytic, SM-3997, both were found to completely substitute for gepirone (ED50 = 1.32 and .69 mg/kg, respectively). The 5-HT1A agonist, 8-OH-DPAT also completely substituted for gepirone, with an ED50 of .05 mg/kg. These data support recent behavioral and biochemical studies suggesting that 5-HT1A receptors may be involved in mediating the effects of this novel class of anxiolytic agents.

87.9

COMPARATIVE EFFECTS OF ZACOPRIDE, GR38032F (ODANSERIN), BUSPIRONE, AND DIAZEPAM IN THE MOUSE LIGHT/DARK EXPLORATORY MODEL. R. Young and D. N Johnson. Dept. of Pharmacology, A. H. Robins Co., Richmond, VA 23261.

The effects of various anxiolytic agents and putative anxiolytic agents were assessed in mice in the two compartment light-dark chamber (Costall et al., Br. J. Pharmac. 90:257D, 1987). Significant increases in lit area activities (e.g., time spent in the lit area, locomotor activity, rearing behavior) are presumed to be indicative of anxiolytic action. The benzodiazepine, diazepam, the 5-HT1A agonist, buspirone, and the 5-HT3 antagonists, GR38032F and zacopride, produced significant increases in time spent in the lit area, rearing activity, and/or locomotor activity over a tenfold (1-10 mg/kg, IP), threefold (3.16-10 mg/kg, IP), ten thousandfold (.0001-1 mg/kg, IP), and over a one hundred fifty thousandfold (.0001-17.8 mg/kg, IP) range of doses respectively. In addition, buspirone and zacopride were compared for oral potency and for duration of action after IP and PO administration. Buspirone and zacopride produced significant increases in lit area activities over a fivefold (10-56.2 mg/kg, PO) and a one hundred thousandfold (.001-100 mg/kg, PO) range of doses respectively. The time course effect of buspirone was 2-4 hours after IP or PO administration, while zacopride was active for more than 8 hours by either route of administration. The positive results obtained for the three types of compounds suggest that the light-dark test is a useful procedure for identifying known and putative anxiolytic agents.

87.10

THE EFFECTS OF A CANDIDATE COGNITION ENHANCING AGENT, BMY 21502, ON CELL MEMBRANE PROPERTIES AND SYNAPTIC PLASTICITY IN CA_1 NEURONS OF RAT HIPPOCAMPAL SLICES. V.K. Gribkoff, L.A. Bauman*, and C.P. VanderMaelen. CNS Biology, Bristol-Myers Co., Wallingford, CT 06492.

The neuronal actions of a putative cognition enhancing compound, BMY 21502, were assessed with intracellular and extracellular recording techniques in CA_1 neurons of normal, young-adult male rats.

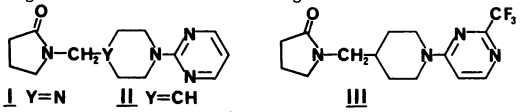
Bath application of BMY 21502 (500 nM-100 μM) did not produce consistent effects on cell membrane properties in intracellularly recorded neurons. Cell membrane potential, membrane resistance, spike frequency adaptation, and burst after-hyperpolarizations appeared to be unaffected by this agent. Synaptic potentials, evoked by Schaffer-collateral stimulation, were reduced in BMY 21502 at concentrations above 30 μM.

Using the population synaptic potential recorded in the apical dendrites as a measure of synaptic efficacy, the effect of BMY 21502 on short-term (1-10 min.) and long-term (15-30 min.) potentiation of these responses was determined following single 100/sec, 1 sec stimulus trains. The identity of the solutions were unknown to the experimenter, and data analysis was performed blind. While BMY 21502 did not have a noticeable effect on short-term potentiation, a moderate enhancement of long-term potentiation was noted in 1.0 and 10 μM, but not in 25 μM BMY 21502.

87.11

SYNTHESIS AND AMNESIA-REVERSAL ACTIVITY OF A SERIES OF NOVEL PYRROLIDINONE DERIVATIVES: IDENTIFICATION OF BMY 21502 AS A POTENTIAL TREATMENT FOR COGNITIVE DISORDERS. Ronald J. Mattson*, Eric P. Loukas*, Joseph P. Yevich, and Michael S. Eison. CNS Research, Bristol-Myers Company, 5 Research Parkway, Wallingford, CT 06492.

Several substituted pyrrolidinones, such as piracetam, aniracetam, and pramiracetam, have been found to reverse electroconvulsive shock-induced amnesia in animals. The initial screening of a series of novel pyrrolidinone derivatives identified an aminal, BMY 20046 (I), which also reverses ECS-induced amnesia in a step down, passive avoidance task. As an aminal, I possesses limited acid stability. The amnesia reversing activity of I could not, however, be explained by the hydrolysis products, since these were inactive in this paradigm. An acid stable analog of I, BMY 20764 (II), was subsequently found to be even more potent in reversing ECS-induced amnesia. A series of heteroaryl analogs of II have been screened for amnesia reversing activity. From the results in these and other tests, BMY 21502 (III) was chosen as a clinical candidate and is currently in Phase I clinical trials as an agent for the treatment of cognitive disorders.



I Y=N II Y=CH

III

87.12

BMY 21502: A POTENTIAL COGNITION ENHANCER THAT IMPROVES MEMORY AND ENHANCES GLUCOSE METABOLISM IN THE AGED HIPPOCAMPUS. S.L. Moon, R.J. Mattson*, F.D. Yocca, D.P. Taylor, J.P. Yevich, M.S. Eison, L.A. Riblet, D.L. Temple, Jr. Bristol-Myers Co., CNS Research, Wallingford, CT 06492.

BMY 21502 is chemically designated 1-(1-(2-(trifluoromethyl)-4-pyrimidinyl)-4-piperidinyl)methyl)-2-pyrrolidinone. It is active in models of learning and memory.

Sites of metabolic alteration were determined through the use of ^{3}H-2-deoxyglucose (2DG) autoradiography. The hippocampal formation of the young untreated rat was characterized by bands of high metabolic activity found in the pyramidal layer and in the dentate gyrus. In the hippocampus of some of the vehicle-dosed aged animals, the activity in CA1 and dentate gyrus was diminished. In the BMY 21502-dosed aged rats, there were bands of high activity in the hippocampal formation, so that the resultant pattern of 2DG in the BMY 21502-treated hippocampus of aged rats resembled the pattern seen in the young adult.

BMY 21502 did not inhibit in vitro binding to any neurotransmitter receptor tested, (cholinergic, aminergic, amino acidic, CCK, μ opiate, sigma). Additionally, BMY 21502 showed no activity at serotonergic and adrenergic reuptake sites, high affinity choline uptake sites, nor calcium channels.

BMY 21502 is a potential cognition enhancer for the therapeutic treatment of disorders that are characterized by learning and memory impairment.

87.13

[3H]TETRAHYDROAMINOACRIDINE BINDING SITES IN RAT CNS MEMBRANES. E. E. Mena, Department of Neuroscience, Central Research Division, Pfizer, Inc., Groton, CT 06340.

Oral administration of 9-amino-1,2,3,4-tetrahydroaminoacridine (THA) is reported to result in significant improvement in cognitive function in patients with Alzheimer's Disease (AD). THA is a potent acetylcholinesterase (AChE) inhibitor. However, since little is known about the complete pharmacological profile of THA, it is possible that effects on other neuronal systems may contribute to these clinical results.

In order to identify other potential actions of THA in the CNS, rat brain membranes were examined for [^{3}H]THA binding sites. [^{3}H]THA binding (5 nM) reached equlibrium after 2 min at 0°C and was rapidly reversed upon the addition of 50µM nonradioactive THA. Analysis of kinetic data (LUNDON software) showed that [^{3}H]THA bound with Kd and Bmax values of 420 ± 56 nM and 42.7 ± 12 fmoles/ mg protein, respectively. The THA binding site was distinct from the AChE enzyme since the relative levels of THA binding and AChE activity in membranes prepared from the striatum, hippocampus, cortex and cerebellum differed greatly. Pharmacological analysis of the binding site revealed that compounds which interact with receptors for monoamines, ACh, glutamate, GABA, diazepam and sigma opiates or bind to Ca^{2+} and K^+ channels were inactive at displacing [^{3}H]THA.

The [^{3}H]THA binding site appears to be a novel CNS binding site distinct from the AChE enzyme and the binding sites of several neurotransmitters/neuromodulators. It is possbile that the function(s) mediated by this site may contribute to the clinical effects of THA.

Acknowledgments: I would like to thank Dr. M. Desai, Department of Medicinal Chemistry, Pfizer, Inc., for generously supplying [^{3}H]THA and Ms. M. F. Gullak for technical assistance.

87.14

EVALUATION OF BENZOYL ESTERS OF DOPAMINE AS POTENTIAL PRO-DRUGS FOR DOPAMINE IN THE CNS. **M. Hauptmann*, S.M. Tejani-Butt, A. D'Mello*, A. Frazer, & D.J. Brunswick. Dept. of Psychiatry, Univ. of Pennsylvania & VA Med. Ctr. Phila, PA 19104.**

For a compound to be a useful pro-drug of dopamine (DA) in the CNS, it is necessary that it both penetrate the brain as well as break down to the parent compound once in the brain. In this study the monobenzoyl (MBDA) and dibenzoyl (DBDA) esters of DA were evaluated. These compounds were radiolabelled and their uptake into brain measured. ^{14}C-DBDA penetrated the brain rapidly; 0.28% of the injected dose per gram tissue was taken up into the brain at 5 minutes. However DBDA did not produce measurable increases in DA levels in the brain. ^{14}C-MBDA on the other hand was found not to penetrate the brain, but upon its i.c.v. administration to rats caused DOPAC levels to increase significantly both in the striatum and in the rest of the brain; the increase in the striatum was 3 to 10 fold greater than that seen in the rest of the brain. In rats pretreated with the MAO inhibitor, pargyline, MBDA given i.c.v. caused increases in DA levels in both the striatum and in the rest of the brain with the increase in the striatum about 25 fold greater than that in the rest of the brain. From these results, it is inferred that MBDA is being hydrolyzed in vivo in the brain to form DA which is then taken up into dopaminergic neurons. Given this, it seems likely that an ester pro-drug of DA can be obtained that will have sufficient lipophilicity to penetrate the brain as well as a rate of hydrolysis that will produce increases in DA in the brain. **(Supported by Research Funds from the Vet. Admin. and USPHS grant GM34781).**

87.15

EFFECTS OF NICOTINE AND PBA-415 ON CALORIC INTAKE. R.M. Keenan*, M.E. Carroll* and S.T. Lac* (SPON: D. Burkhardt). Dept. of Psychiatry, U. of Minnesota Med. Sch., Minneapolis, MN 55455.

Past research has shown nicotine to be effective as a suppressant of caloric intake. Also, nicotine withdrawal leads to increased caloric consumption. Accordingly, cigarette smokers cite their weight gain after cessation of use as a major factor for relapse. PBA-415 is a nicotine analogue without the undesirable cardiovascular side effects which may help smokers combat nicotine withdrawal during cessation.

Six rats were given i.v. nicotine (12 mg/kg/day), PBA-415 (24 mg/kg/day) and saline for alternating five day periods. Their daily intake of food, glucose plus saccharin solution, total fluids and total calories were monitored. The results show that PBA-415 (in comparison to nicotine) is equally effective in suppressing daily food intake and total daily caloric intake when compared to saline levels ($p<.01$). Water intake was also increased on the PBA-415 days when compared to nicotine and saline days ($p<.01$). There was a significant suppression of glucose plus saccharin intake as a result of removing nicotine ($p<.01$).

The present results showed that i.v. nicotine and PBA-415 are able to suppress caloric intake when compared to saline. From this, it appears that PBA-415 has nicotine-like qualities with respect to caloric intake and may serve as an effective therapeutic agent in the treatment of cigarette smokers.

This research was supported by Pharmaco Behavioral Associates, Inc. 7120 Valley View Road, Edina, Mn 55435.

87.16

ELTOPRAZINE, A DRUG THAT REDUCES AGGRESSIVE BEHAVIOR, BINDS SELECTIVELY TO 5-HT$_1$ RECEPTOR SITES IN RAT BRAIN. H.Sijbesma*[1], J.Schipper[1] and E.R.de Kloet[2]. Depts Pharmacology, Duphar B.V., P.O.Box 2, 1380 AA Weesp[1], and Rudolph Magnus Institute, Utrecht[2], Holland

Eltoprazine, a new serenic, specifically reduces aggressive behavior in animal aggression models. The distribution and characterization of [^{3}H]eltoprazine binding sites were studied with in vitro autoradiographic techniques. Specific binding of [^{3}H]eltoprazine was found in many brain areas. The distribution was very similar to that of [^{3}H]5-HT binding sites. However, brain areas enriched in 5-HT$_{1a}$ sites showed relatively more [^{3}H]5-HT binding, whereas in 5-HT$_{1b}$ receptor dense areas [^{3}H]eltoprazine binding was more pronounced. Competition for [^{3}H]eltoprazine by several "selective" serotonergic compounds supported the finding that [^{3}H]eltoprazine selectivity binds to the 5-HT$_1$-receptor type. In addition, the affinity of eltoprazine for the different 5-HT$_1$ receptor subtypes was investigated. Eltoprazine showed relatively high affinity for 5-HT$_{1b}$ > 5-HT$_{1a}$ > 5-HT$_{1c}$ sites. (IC_{50}: 28nM, 37nM and 240nM, respectively). These results indicate that eltoprazine selectively binds to the 5-HT$_1$-receptor subtypes. The binding profile of eltoprazine together with its behavioral properties, strengthen the importance of serotonin in the modulation of aggressive behavior.

87.17

CHANGES IN SOCIAL INTERACTION PRODUCED BY COMPOUNDS WITH SEROTONERGIC SITES OF ACTION. A.L. Johnston* and S.E. File. The School of Pharmacy, University of London, London WC1N 1AX.

The following compounds, which have been proposed as putative anxiolytics, were investigated in the social interaction test of anxiety in the rat: ipsapirone (0.5 - 2 mg/kg, i.p.), a 5-HT$_{1A}$ agonist; ritanserin (0.05 - 5 mg/kg, i.p.), a 5-HT$_2$ antagonist; GR 38032F, BRL 43694 and ICS 205-930 (0.01 - 1 mg/kg, p.o.), 5-HT$_3$ antagonists. Chlordiazepoxide (CDP) (7.5 mg/kg, p.o.) was included as a positive control.

Increases in the time spent in social interaction were displayed by: ipsapirone (1 mg/kg) in a low light unfamiliar and a low light familiar condition; ritanserin (5 mg/kg) in a low light familiar condition; BRL 43694 (1 mg/kg) and ICS 205-930 (0.1 mg/kg) in a high light unfamiliar condition; CDP in a low light unfamiliar and a high light unfamiliar condition. No other treatments significantly affected social interaction.

The increases displayed may indicate anxiolytic activity, however, these were confined to single doses and were often weaker, inconsistent or different in profile from those typically displayed by benzodiazepines.

87.18

THE EFFECTS OF GEPIRONE AND 1-(2-PYRIMIDINYL)-PIPERAZINE ON RAT CORTICOSTERONE LEVELS. G.K. Matheson, D. Guthrie, G. White, J. Rhoades & D. White. Neurobiology Laboratory. Indiana University School of Medicine.Evansville,IN 47714

The major metabolite of gepirone is 1-(2-pyrimidinyl)-piperazine (1-PP). The effects on the hypothalamic-pituitary-adrenal axis (HPAA) produced by gepirone and 1-PP were indicated by changes in plasma corticosterone (CS) concentration. The rats were maintained in a light (on 0600-1800 hrs), temperature and humidity controlled environment. Control levels of CS were 14.1 ± 1.2 S.E. µg/dl (n=43). A greater maximum facilitating effect on CS levels was seen 45 minutes after gepirone administration (+283%; 50 mg/kg, i.p.) than after 1-PP (+211%). Gepirone was also more potent than 1-PP since the ED_{50}'s were 2.6 mg/kg (6.4 µmol/kg) for gepirone and 13 mg/kg (65.4 µmol/kg) for 1-PP. The duration of the effect produced by these agents on the HPAA was determined by measuring the plasma CS levels 1/2, 1, 2, and 4 hours after their administration. At a dose of 10 mg/kg gepirone increased CS levels 244% for a period of one hour, but 1-PP did not produce any measurable change in CS levels over the four hour test period. Animals subjected to a one minute spinning stress (30-60 rpm) 15 minutes before decapitation had stress control levels of 35.6 ± 1.6 µg/dl CS (39). This stress and gepirone's effects were additive increaseing CS levels to 58.3 ± 1.5 µg/dl (14) after one hour of drug treatment.

88.1

DEVELOPMENT OF THE SEROTONERGIC SYSTEM IN THE CENTRAL NERVOUS SYSTEM OF THE TOBACCO HORNWORM MANDUCA SEXTA. N.A. Granger*, W.A. Radwan* and J.M. Lauder (SPON: R. Weinberg). Dept. Cell Biol. and Anat. Univ. of N.C., Sch. Med., Chapel Hill, NC 27599

Development of the serotonergic (5-HT) system in the brain and ventral nerve cord of Manduca sexta embryos, larvae and pupae was studied in whole mounts with an antiserum to 5-HT. Immunoreactivity (IR) was first detected in fibers in the head region at 40-50% of embryonic development prior to differentiation of the brain. 5-HT IR cell bodies were seen at 60% development in both the brain and ventral ganglia. By 75% development, eight IR cell groups (38-40 cells), four commissures, and five regions of arborization were visible in the brain. The same pattern was observed in fifth instar larvae and early pupae. These results indicate that embryonic 5-HT neurons are conserved during development. By 75% development, there were 94 IR neurons in the ventral ganglia, including segmentally homologous pairs of interneurons in each of the thoracic and abdominal ganglia, and efferent neurons in thoracic and posterior abdominal ganglia. Previously intense ganglionic arborizations of IR fibers had regressed by the end of embryogenesis, as did arborizations in the neuropil of metamorphosing larval brain, suggesting that a reorganization of 5-HT innervation had occurred. The early appearance of 5-HT IR in the central nervous system of Manduca suggests that 5-HT may play a role in neurogenesis in this species.

88.2

A SEROTONERGIC SUB/SUPRAEPENDYMAL AXONAL NET IN CONJUNCTION WITH A SUB/SUPRAEPENDYMAL MASS OF MESENCEPHALIC TRIGEMINAL NEURONS OF SHARKS. M.F. MacDonnell. Dept. Biol. Sciences, Rutgers Univ., Box 1059, Piscataway, N.J. 08855.

Light microscopy with silver stains and electron microscopy reveal a sub/supraependymal system of varicose axons in the tectal midline ridge formation of sharks, a region shared by a sub/supraependymal mass of mesencephalic trigeminal (Mes V) neurons in these animals. The relationship of axonal varicosities and terminals to both CSF and to sub-and supraependymal Mes V somas is such as to suggest a largely non-synaptic association. Immunocytochemical experiments show that the sub/supraependymal axonal network seen in the tectum is part of a large serotonergic sub/supraependymal axonal network involving the entire rim of the tecto-tegmental ventricle and arising in the midline tegmentum. The shared topography of the sub/supraependymal serotonergic and the sub/supraependymal mass of mesencephalic trigeminal neurons and the details of their association suggest a nonsynaptic relationship which may serve to modulate the powerful jaw reflexes of the shark.

88.3

SEROTONIN IMMUNOREACTIVE PERIKARYA AND PROCESSES IN THE MEDULLA OF THE NEW WORLD PRIMATE, CEBUS CAPUCINUS: CONTRASTS WITH RODENT, *V.A. Pieribone, E.J. Van Bockstaele, G. Go*, M. Springston* and G. Aston-Jones*, Dept. Biol. & Cntr. for Neural Sci., New York Univ., NY 10003.

Several studies have examined serotonergic perikarya in the medulla of primates using histofluorescence, but few have used the more sensitive and stable immunocytochemical methods. Additionally, there has been only limited study of serotonin immunoreactive (5-HT-IR) fibers and terminals in the primate medulla.

We have examined 5-HT-IR profiles in the medulla of Cebus capucinus using PAP immunocytochemistry followed by a gold-substituted silver procedure that substantially increases staining sensitivity. Sections containing 5-HT-IR structures were compared to adjacent sections stained for Nissl and with complementary sections of the rat medulla similarly processed for 5-HT immunocytochemistry.

Serotonin-positive somata were distributed throughout the midline raphe system, but additional prominent groups were identified in the ventrolateral medulla and within the nucleus of the solitary tract.

Patterns of 5-HT-IR fibers in primate medulla differed in many respects from those in rodent. For example, while the Xth and XIIth nuclei are only moderately innervated in rat, these structures receive heavy, basket-like innervation in primate. Nucleus commissuralis of the solitary tract is densely innervated in rat, but only lightly so in monkey. The inferior olive in rat contains homogeneously light 5-HT innervation, while in monkey this nucleus exhibits marked regional differences in density of innervation.

Distinct classes of 5-HT-IR fibers and termination patterns were identified in the primate medulla. Thick, beaded fibers that provide basket-like innervation of individual neurons were present in some of the cranial nerve nuclei, while thin axons containing numerous punctate varicosities homogeneously innervate subaspects of the inferior olivary complex. Overall, there was greater regional variation in fiber types and innervation patterns in primate compared to rodent. Supported by NINCDS grant NS24698, ONR contract N00014-86-K-0493, and the Air Force Office of Scientific Research.

88.4

IDENTIFICATION OF SEROTONIN NEURONS PROJECTING TO THE SUBSTANTIA NIGRA IN THE RAT USING A COMBINED TECHNIQUE OF IMMUNOHISTOCHEMISTRY AND FLUORESCENT RETROGRADE TRACING METHOD. S. Mori* and Y. Sano*(SPON: K. Toyama). Dept. of Anat., Kyoto Pref. Univ. of Med., Kyoto 602, Japan.

The origin of serotonin nerve fibers distributed in the rat substantia nigra was studied by a combined technique of immunohistochemistry using the serotonin antibody and a retrograde fluorescence Fluoro-Gold labeling method on the same tissue sections. After the unilateral injection (0.15 µL) of the Fluoro-Gold (2% solution) into the substantia nigra, the confined appearance of Fluoro-Gold-labeled neurons in the raphe nuclei was demonstrated in the dorsal raphe nucleus. A majority of these labeled neurons (56%) were serotonin-immunoreactive, but the rest (44%) were nonimmunoreactive. Fluoro-Gold-labeled as well as serotonin-positive neurons were localized mainly in the ipsilateral lateral and dorsomedial aspects. Additionally, after chemical axotomy of serotonergic fibers by an intraventricular injection of 5,7-dihydroxytryptamine, the Fluolo-Gold was similarly injected into the substantia nigra. Retrogradely labeled neurons were still observed in the dorsal raphe nucleus, however, a majority of these neurons (94%) were serotonin-negative. These findings indicate that a majority of serotongic fibers projecting to the substantia nigra originate mainly in the ipsilateral lateral and dorsomedial aspects of the dorsal raphe nucleus, and these raphe nigral pathways contain both serotonergic and non-serotonergic fibers.

88.5

LOW SYNAPTIC INCIDENCE OF SEROTONIN AXON TERMINALS IN THE FRONTAL, PARIETAL AND OCCIPITAL NEOCORTEX OF ADULT RAT. P. Séguéla, K. C. Watkins* and L. Descarries. Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Québec, Canada H3C 3J7.

PAP-immunocytochemistry with an antiserum against serotonin (5-HT-glutaraldehyde-protein conjugate, kindly donated by M. Geffard) was used to analyze the ultrastructural relationships of 5-HT axon terminals (varicosities) in the frontal (Fr1), parietal (Par1) and occipital cortex (Oc1M-Oc2) of adult rat. One hundred and fourty five immunostained varicosities from Fr1 (54 in layers I-II, 91 in layers V-VI), and 97 each from the upper layers of Par1 and Oc1M-Oc2, were examined in groups of serial thin sections (mean number of sections in series: 3.4 to 7.2). The terminals were of comparable size in all 3 regions, averaging 0.66 ± 0.16 µm (mean ± s.d.) in diameter. Their junctional complexes measured 0.24 ± 0.09 µm. The proportion of varicosities endowed with synaptic junctions was evaluated by linearization of the relationship between the observed junctional frequency and the number of thin sections per varicosity available for examination. Reliability of the sampling was evidenced by high correlation coefficients ($r > 0.9$) in each cortical region. Extrapolated synaptic incidence amounted to 30% in both the superficial and deep frontal cortex, and to 45% and 35% in the superficial parietal and occipital cortex, respectively. The differences between the 3 regions were not statistically significant. These 5-HT synapses were exclusively made with dendrites. They were equally distributed between dendritic spines and shafts in the frontal and the parietal cortex, but appeared more frequent on dendritic spines (71%) than shafts (29%) in the occipital cortex. It may be concluded that the 5-HT input is only partly synaptic throughout the neocortex of adult rat, which reinforces earlier views of a divergent afferent system capable of widespread and perhaps long-lasting influences in this part of the brain. (Supported by MRC grant MT-3544).

88.6

REGIONAL DIFFERENCES IN THE SEROTONIN INNERVATION OF RODENT CEREBRAL CORTEX: DIFFERENTIAL DISTRIBUTION OF TWO MORPHOLOGICALLY DISTINCT AXON TYPES. M.E. Blue, B.E. Kosofsky and M.E. Molliver. Dept. of Neurosci. Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205 and Dept. of Neurology, MGH, Boston, MA 02114

Morphologically dissimilar serotonergic (5-HT) axon types in rat cerebral cortex have separate origins in the midbrain: axons from the dorsal raphe (DR) are fine with small, pleomorphic varicosities; median raphe (MR) axons are coarse with large, spherical (beaded) varicosities. In the present study, we show, using 5-HT immunocytochemistry, that fine and beaded 5-HT axons have different regional patterns of distribution. Numerous fine axons are distributed throughout cerebral cortex with regional and laminar differences in density. Beaded axons are much less abundant than fine fibers and are highly concentrated in restricted regions and layers of cortex. In neocortex, higher densities of fine 5-HT axons are found in frontal areas of cortex than in parietal or occipital areas. Somatosensory cortex (SI) exhibits the most highly differentiated laminar distribution of 5-HT axons in neocortex. Although fine 5-HT axons are found in all layers of SI, they are particularly abundant in layer V_a where they form a dense band. Beaded axons appear to be distributed in clusters which are found predominantly in layers I-III and VI of SI. Similar clusters of beaded axons are found in occipital and auditory cortex. Beaded axons form distinctive patches or bands in other cortical areas including posterior cingulate cortex (layer II), hippocampus (the molecular layer of CA1, along the dentate granular layer and in CA3) and in the perirhinal and entorhinal cortex. In contrast, fine fibers are distributed relatively uniformly throughout these areas of cortex. The differential distribution of fine vs beaded axons in each area of cortex demonstrates that there is regional specialization of the 5-HT innervation to areas of cortex that have different cytoarchitecture and function. These results suggest that the 5-HT projection from the DR and MR may exert a different and possibly selective effect in each area of cortex. [Support: NIH NS15199 and HD19920]

88.7

THE ANATOMIC ORGANIZATION OF SEROTONERGIC PROJECTIONS TO NEOCORTEX IN THE PRIMATE. M.A. Wilson and M.E. Molliver. Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Serotonin (5HT) neurons of the midbrain raphe nuclei project widely upon the cerebral cortex in primates, however, there is evidence of specificity in the organization of this projection. In macaque monkeys, there are differences in the density and laminar distribution of 5HT axons in different areas of the cerebral cortex. In addition, the two classes of 5HT axon terminals which have been described in primates, fine and beaded, vary in density in different areas and laminae of cerebral cortex, and are differentially vulnerable to certain neurotoxic psychotropic drugs.

The present study examines the distribution of neurons in the raphe nuclei which project to selected areas in macaque cerebral cortex. Retrogradely transported fluorescent dyes (or WGA-HRP) were injected into primary motor, somatosensory, or visual cortex or into parietal or frontal association cortex in macaques, and labeled neurons were mapped in the midbrain raphe nuclei. 5HT immunocytochemistry was used to determine whether labeled neurons were serotonergic. After injections in different cortical areas, different, partially overlapping distributions of labeled neurons were observed in the raphe nuclei. For example, cells projecting to motor cortex are found in both rostral and caudal parts of the dorsal raphe nucleus (DR), with few in the median raphe nucleus (MR). Cells projecting to somatosensory cortex are found predominantly in the caudal part of the DR, with few in the rostral part of the nucleus and some in the MR. Neurons projecting to visual cortex are found in the MR and in the caudal part of the DR, with almost none in the rostral part of the DR. Raphe-cortical projections have an intricate organization, with different cortical areas innervated by different, but partially overlapping, sets of raphe neurons. These sets differ in their rostro-caudal distributions, and in their ratios of DR to MR neurons. (Support: NIH NS21011, T32NS07179, and the L.P. Markey Fund.)

88.8

SELECTIVE NEUROTOXIC EFFECTS OF (+)FENFLURAMINE UPON 5-HT AXONS IN RAT BRAIN: IMMUNOCYTOCHEMICAL EVIDENCE. D.C. Molliver* and M.E. Molliver. (Spon: E. O'Hearn). Depts. of Neuroscience and Neurology, The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Fenfluramine - a halogenated amphetamine derivative that is used as an anorectic agent - has been reported to cause lasting decreases in brain levels of 5-HT in the rat. The changes are similar to those of other substituted amphetamines that have neurotoxic effects upon 5-HT projections. In this study, we have used immunocytochemistry to determine whether 5-HT neurons are structurally damaged by fenfluramine and if there is a selective loss of axons similar to that caused by p-chloroamphetamine (PCA) and methylenedioxy-amphetamine (MDA). Rats were given (+)fenfluramine in single or repeated doses (10, 20 or 40 mg/kg s.c. q12h for 4 days) and studied at 5 hours, 2 weeks or 4 weeks after the first dose. At all time points there is a profound loss of 5-HT immunoreactive axon staining, with no change in catecholamine axons. There are regional differences in the effects, with neocortex and striatum most depleted and hippocampus partially spared. Sparing of 5-HT axons is seen in olfactory bulb and basal forebrain. There is little decrease in the brainstem while 5-HT staining is lost in the medial but not lateral hypothalamus with the ventromedial nucleus most affected. In all forebrain regions, fine 5-HT axons are selectively lost while beaded axons that are distinguished by large spherical varicosities are spared. The loss of 5-HT immunoreactive staining at 5 hours indicates massive depletion of 5-HT; the persistent loss at 2 and 4 weeks is indicative of degeneration of 5-HT axon terminals. The results show that in large repeated doses (+)fenfluramine is neurotoxic to one class of 5-HT axons and has the same pattern of selectivity as does PCA and MDA. The regional differences in effect may be due to a selective action of these drugs on fine axons (presumably of dorsal raphe origin). The anorectic action of fenfluramine may be related to release of 5-HT in the ventromedial hypothalamic nucleus. [Support NIDA #DA04431]

88.9

CONTRASTING *in vitro* and *in vivo* EFFECTS OF p-CHLOROAMPHETAMINE (PCA) ON 5-HT AXONS: IMMUNOCYTOCHEMICAL STUDIES IN HIPPOCAMPAL SLICES. M.E. Molliver, K. Stratton, P. Carr*, R. Grzanna and J. Baraban. Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Systemic administration of PCA elicits a profound depletion of brain serotonin associated with degeneration of 5-HT axons. To help define the neurotoxic role of PCA and its metabolites, we have examined the effects of PCA on 5-HT axons *in vitro* by applying 5-HT immunocytochemistry to the rat hippocampal slice preparation. Hippocampal slices, incubated in physiologic buffer, were immersion fixed in paraformaldehyde and frozen sections prepared for 5-HT immunocytochemistry. Slices from control rats, maintained for up to 6 hours *in vitro*, exhibit a dense plexus of 5-HT axon terminals identical to that seen with perfusion fixation. Incubation of slices for 1-2 hours in buffer containing PCA (50 μM) does not induce loss of axonal staining. In slices taken from rats treated *in vivo* with PCA (10 mg/kg i.p.) 2 hours prior to sacrifice and immediately fixed after slicing, profound depletion of 5-HT was observed; fine 5-HT axons were not detected while varicose (drug-resistant) fibers remain intensely stained. Incubation of these depleted slices in standard buffered saline for 1/2-2 hours led to progressive recovery of 5-HT immunoreactivity; nearly all 5-HT axons appear intact with normal morphology. In rats treated with PCA 16 hours prior to slice preparation, there is initial depletion of 5-HT with much less recovery of axon staining after 2 hours *in vitro*. These results indicate that in the early phase of 5-HT depletion following PCA administration, the axons have not degenerated and the terminals initially retain the ability to synthesize and store 5-HT. The additional finding that *in vitro* incubation of slices in PCA does not induce 5-HT depletion supports the hypothesis that PCA itself is not the active, neurotoxic agent, but that a peripheral metabolite of PCA is likely to produce 5-HT depletion and axon degeneration. [Support: NIDA DA-04431 and DA-00266]

88.10

MORPHOLOGICALLY DISSIMILAR SEROTONERGIC AXON TYPES IN RAT CEREBRAL CORTEX ARE DIFFERENTIALLY VULNERABLE TO THE NEUROTOXIN p-CHLOROAMPHETAMINE (PCA). L.A. Mamounas, C. Mullen* and M.E. Molliver. Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Morphologically dissimilar types of serotonergic (5-HT) axons have been described in forebrain: fine axons with small, pleomorphic varicosities and coarse axons with large, spherical (beaded) varicosities. Using immunocytochemical methods, we have previously shown that systemic administration of the neurotoxin PCA (10 mg/kg; 2-week survival) produces selective ablation of fine 5-HT axon terminals in forebrain, while sparing beaded 5-HT axons. In the present study, we demonstrate that this differential neurotoxicity is observed over a wide range of doses and survival times. The density of fine axons in cerebral cortex is progressively reduced at doses of PCA ranging from 2-10 mg/kg, with almost no fine axons remaining at 20 mg/kg; in contrast, beaded axons are consistently spared at doses up to 40 mg/kg. Moreover, the differential neurotoxicity of PCA on the two 5-HT axon types is not a transient event: up to five months after PCA administration (10-20 mg/kg), few fine axons are found in cortex, whereas beaded axons remain unaffected. Furthermore, at short survival times (4 hours), PCA causes profound depletion of 5-HT from fine axons but not from beaded axons, reflecting, most likely, differential release of 5-HT from the two axon types. We have recently shown that fine and beaded 5-HT axons arise from separate nuclei in the midbrain - the dorsal and median raphe nuclei, respectively (Kosofsky and Molliver, 1987) and that the dorsal raphe projection to neocortex is selectively eliminated by PCA (Mamounas and Molliver, 1988). Since other psychotropic amphetamines (MDA and MDMA) also affect the same type of 5-HT axons (O'Hearn et al., 1988; Mamounas et al., 1988), we propose that the dorsal raphe projections may function in the regulation of affective state. [Support: NIDA #DA-0443; NIMH # MH-09538]

88.11

ELECTRON MICROSCOPIC CYTOCHEMICAL AND IMMUNOCYTOCHEMICAL LOCALIZATION OF TYPE-SPECIFIC MONOAMINE OXIDASE (MAO) IN RELATION TO IDENTIFIED ENTERIC SEROTONERGIC NEURONS M.D. Gershon, J.E. Pintar and D.L. Sherman*. Dept. Anat. & Cell Biol., Columbia Univ., New York, NY 10032.

Intrinsic neurons of the guinea pig gut contain MAO-B, but little or no MAO-A, which is located in extrinsic sympathetic axons. MAO-B-rich neurons have been found to contain neuropeptide Y and calcitonin gene related peptide, but not 5-hydroxytryptamine (5-HT). We have now studied the distribution of MAO at the ultrastructural level in order to examine the possibilities that MAO-A or MAO-B may be present in the terminals of serotonergic neurons or in the post-synaptic elements of serotonergic synapses. MAO-B immunoreactivity was visualized by using a monospecific antiserum and an immunoperoxidase technique. Type-specific MAO activity was also localized by a coupled peroxidation method following preincubation with deprenyl (to inhibit MAO-B) or clorgyline (to inhibit MAO-A). Serotonergic elements were simultaneously identified by radioautography following incubation with tritiated 5-HT. Neither MAO-B immunoreactivity (I) nor activity were found in enteric glia. More neurons and processes were demonstrated immunocytochemically than cytochemically; therefore, either the immunocytochemical technique is more sensitive for the detection of MAO-B or not all of the immunoreactive molecules are enzymatically active. Individual cells varied greatly in the degree of immuno- or cytochemically demonstrable MAO-B, which was most concentrated on the outer membranes of mitochondria. MAO-B-I (but not cytochemical activity) was found on mitochondria in some serotonergic perikarya. In contrast, mitochondria in most serotonergic axon terminals displayed both MAO-B activity and immunoreactivity. Neurites receiving serotonergic synapses often, but not invariably, contained MAO-B-I and activity. It is concluded that serotonergic neurons contain relatively little MAO-B, that it is not present on all mitochondria, and that MAO-B-reactive mitochondria are preferentially located at terminals. Supported by NIH grants #NS 12969, NS 07062, and HD 18592.

88.12

DETERMINATION OF THE ACTIVITIES OF THE SUBTYPES OF MONOAMINE OXIDASE WITHIN SEROTONERGIC NERVE ENDINGS OF RAT BRAIN IN VITRO AND EX VIVO. S.S. Shim and R.E. Becker. Dept. of Pharmacology and Dept. of Psychiatry, Southern Illinois Univ., Springfield, IL 62794-9230.

Subtypes of monoamine oxidase (MAO) A and B in 5-hydroxy-tryptamine (5-HT) synaptosomes were determined using clorgyline and l-deprenyl at 100 nM of 14C 5-HT. 90% of the deamination of 14C 5-HT was inhibited by clorgyline at 0.1-30 nM, 10% inhibited by l-deprenyl at 1-30 nM, 90% inhibited by l-deprenyl at 100nM-100 uM, indicating that 90% of 14C 5-HT transported into 5-HT synaptosomes was deaminated by MAO A, 10% by MAO B. Results from ex vivo studies agreed with the in vitro. Deamination of 14C 5-HT was inhibited by increasing concentrations of clorgyline in presence of l-deprenyl at 30 nM. L-deprenyl increased the inhibition of deamination of 14C 5-HT only at concentrations of clorgyline at which 90% of the deamination was already inhibited. We suggest that deamination of 14C 5-HT by MAO B occurs after cytoplasmic concentrations of 14C 5-HT are increased by inhibition of MAO A. It appears that MAO A plays major role for inactivating 5-HT transported into 5-HT synaptosomes. MAO B is active only at high cytoplasmic concentrations of 5-HT due to its low affinity for 5-HT and may prevent excessive accumulation of 5-HT.

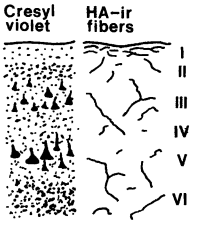
88.13

TRYPTAMINE IMMUNOREACTIVITY WITHIN THE RAT CENTRAL NERVOUS SYSTEM. F.TISON*, M.GEFFARD and H.DABADIE* (SPON : F. CLARAC). Lab. Neuroimmuno., CNRS-IBCN, 33077, Bordeaux, France. In order to define chemoanatomy of tryptamine (T) an immunocytochemical procedure was applied using a polyclonal antisera against T (TAS). TAS were raised in the rabbit with T-glutaraldehyde-BSA (T-G-BSA). TAS affinity and specificity tests carried out in competition experiments (ELISA method) showed apparent IC.50 of $2.5_{4}10^{-9}$ M and cross reactivity ratios of 1:50, 1:60 respectively for 5.hydroxy T (5.HT-G-BSA), 5.methoxy T (5.MT-G-BSA) conjugates. A G-mediated histological fixation procedure of Wistar adult rat brains and spinal cords was used. 50 µm thick vibratome transverse and sagittal sections were incubated with TAS (1:10, 000) followed by a peroxydase-anti peroxydase staining method. Sections were also incubated with 5.HT rabbit antisera (5.HT AS) for comparative neuroanatomy. Control sections were : rabbit non immune serum incubation, preadsorption of TAS with T-G-BSA, 5.HT-G-BSA, 5.MT-G-BSA from 10^{-8} to 10^{-9}M. Neurons were observed within the B1 to B9 raphe cell groups of Dahlström and Fuxe. Localisation and morphology of these neurons were comparable with those stained with 5.HT AS and distribution is consistent with L-aromatic-aminoacid decarboxylase localisation (Jaeger, C.B., *Neurosci.*, 11:691, 1984). Neuronal density was however about 30 % less than with 5.HT AS and intra-cellular immunoreactivity about 3 times less seeming to show a sub-cellular fractioning. 5.HT and T containing fibers also showed comparable distribution : strongest staining was seen within the spinal cord (anterior, intermediolateral and posterior horns), area postrema, locus coeruleus, interpedoncular nucleus, caudal substantia nigra and ventral tegmental area, arcuate nucleus, amygdalo-hippocampal structures, paraventricular thalamic nucleus, parts of caudato-putaminal complex and globus pallidus, accumbens, lateral septum, olfactory tuberculs and bulbs and in nearly all levels of median forebrain bundle and supra-ependymal plexus. Control sections showed a good specificity of TAS for T. Our results are comparable with those obtained by [^{3}H]-T binding studies (Mc.Cormack, J.K., *J.Neurosci.*, 6:94, 1986) and show a similar regional chemoanatomy of T and 5.HT.

88.14

HISTAMINE-IMMUNOREACTIVE NEURONS AND NERVE FIBERS IN HUMAN BRAIN. P. Panula, M.S. Airaksinen, U. Pirvola* and E. Kotilainen* Dept. of Anatomy, University of Helsinki, 00170 Helsinki, and Dept. of Neurosurgery, University of Turku, 20520 Turku, Finland.

Distribution of histamine-immunoreactive (HA-ir) nerve fibers was examined in samples of adult human frontal and parietal cortex removed in operations of astrocytoma in 3 patients. Hypothalamic areas were examined in postmortem brains after autopsy. All tissues were fixed with carbodiimide as described earlier (Panula et al., J. Histochem. Cytochem. 36:259-269, 1988). Cortical fibers were also examined after pre-embedding immunocytochemistry electron microscopically. A large number of neurons exhibited HA-ir in the tuberomammillary nucleus of basal hypothalamus. They sent long smooth and varicose fibers arranged as bundles. A widespread network of fibers was seen in the cortex (figure). HA-ir fibers were seen in all layers of the grey matter. They were densest in lamina I, where they ran mostly parallel to the overlying pia mater. The large number of HA-ir neurons in the basal hypothalamus and a well-organized network of fibers in the cerebral cortex suggest that histamine may be involved in the regulation of cortical functions in human brain.



88.15

ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL LOCALIZATION OF HISTAMINE IN CARBODIIMIDE-FIXED GUINEA PIG CNS NEURONS AND ADRENAL MEDULLARY MAST CELLS. M.S. Airaksinen and P. Panula. Department of Anatomy, University of Helsinki, Finland.

The aim of this work was to locate the compartments of histamine immunoreactivity (HA-ir) in the guinea pig hypothalamic neurons, their axon terminals and in the adrenal medullary mast cells by optimizing the effects of fixation and incubation parameters on preservation of antigenicity and ultrastructure. For preembedding electron microscopy (EM) perfusion with carbodiimide, followed by carbodiimide and aldehyde immersion was adequate for vibratome sectioning. PAP-immunocytochemistry was applied with specific HA antiserum on saponin-permeabilized sections, followed by postfixation with glutaraldehyde and osmium. The location of HAir in the cells was dependent on ultrastructural preservation. The reaction product was found in large cytoplasmic granules, but also diffused in the cytoplasm. Many axon terminals containing small HAir vesicles were found in CNS areas like nucleus accumbens, periventricular gray and neurohypophysis. In the adrenal medulla HA-ir was found in the cytoplasm of apparent mast cells around the vessels. HA in these cells may have a local role in liberating catecholamines.

88.16

CNS LEVELS OF HISTAMINE IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS. Edward L.Orr. Dept. Anatomy, Texas College of Osteopathic Medicine, Ft. Worth, TX 76107.

We have postulated that CNS and/or peripheral mast cells may be important in the development of experimental autoimmune encephalomyelitis (EAE). To begin testing this hypothesis, we have measured , using a radioenzymatic assay, the levels of histamine (Hm; a major mast cell product) in the brains and spinal cords of adult Lewis rats inoculated with adjuvant (Control) or adjuvant + guinea pig spinal cord homogenate (EAE). Rats were assessed daily for behavioral signs of EAE, and groups of 5 Control and 5 EAE rats were sacrificed on days 7, 9, 11, and 13 post-inoculation (pi). Compared to Controls, CNS levels of Hm were unchanged until days 11 (and 13) pi, when significantly increased levels of brain and spinal cord levels developed. Behavioral signs of EAE appeared on day 10 or 11 pi. Further, when EAE was attenuated by two/day injections of prazocin (i.p., 2 mg/injection) on days 9-11 pi, the behavioral signs of EAE and the increase in CNS levels of Hm (as seen in saline-treated EAE rats) did not occur in prazocin-treated EAE rats (all rats were sacrificed on day 12 pi). These results suggest that mast cells may be in the development of EAE, and support the report by Hinrichs, et al. (In: **Immune Regulation by Characterized Polypeptides**, 1987, pp.541) that agents which block or attenuate mast cell release, block or attenuate EAE. (Supported by NMSS grant #RG-1914-A-1).

88.17

RAPID TURNOVER OF HISTAMINE (Hm) IN RAT BRAIN DEMONSTRATED BY TISSUE FIXATION WITH IN SITU PERCHLORIC ACID (PCA) PERFUSION. W. L. Russell* and D. P. Henry* (SPON: L. Lemberger) Dept. of Pharmacology, Ind. Univ. Sch. of Medicine, Eli Lilly & Co. Indianapolis IN 46202.

A rapid turnover pool of Hm in rat brain has been difficult to examine since rapid tissue fixation by microwave irradiation or liquid nitrogen artifactually elevates brain Hm levels by the liberation of Hm from dural mast cells. Fixation of rat tissue with aldehydes would degradate Hm. We have developed a technique which simultaneously inactives brain enzymes and stabilizes brain Hm. Rats were anesthetized with ether. Next, 20 ml of chilled saline (0-4°C) was perfused to the brain from a cardiac puncture which was followed by the perfusion with 20 ml of PCA (0.1 N, 0-4°C). Rats were then decapitated. Whole brain Hm was unaffected by ether anesthesia or saline perfusion, but was higher after PCA perfusion.

HISTAMINE IN RAT BRAIN AREAS (ng/g tissue)

	Decapitation	PCA Perfusion (mean ± SEM)
Brain Stem	21.0 ± 0.6	24.2 ± 2.5
Hypothalamus	378.9 ± 17.8	484.0 ± 36.3 *p<0.05
Thalamus	106.5 ± 10.9	122.5 ± 12.5
Striatum	48.6 ± 2.7	58.3 ± 7.8

Histaminergic neurons are localized in the hypothalamus. Among all brain regions, PCA perfusion elevated hypothalamic Hm suggesting that the fast Hm turnover was terminated by acid fixation and the low temperature. PCA perfusion may be useful with neurotransmitters with a fast turnover in brain.

88.18

ELEVATED LEVELS OF HISTAMINE METABOLITES IN LUMBAR CSF OF AGING, HEALTHY HUMANS AND OF NEUROLOGICAL PATIENTS. G.D. Prell, J.K. Khandelwal*, R.S. Burns*, P.A. LeWitt* and J.P. Green. Dept. of Pharmacology, Mount Sinai School of Medicine, CUNY, New York, NY 10029 and Section on Experimental Therapeutics, NIMH, Bethesda, MD 20205.

The metabolites of histamine, tele-methylhistamine (t-MH) and tele-methylimidazoleacetic were measured in lumbar cerebrospinal fluid of healthy, normal subjects aged 20-31 (n=4) and 60-72 (n=8) by GC-MS. Mean levels (pmol/ml) of t-MH, t-MIAA and the sum of t-MH and t-MIAA (2.9, 6.4 and 9.4, respectively) were significantly higher in CSF from older subjects than from younger subjects (1.1, 4.5 and 5.5, respectively). Another older subject had yet higher levels of metabolites (6.7, 15.1 and 21.8, respectively). The sum of the levels of the known metabolites of histamine in brain, t-MH + t-MIAA, did not overlap between younger and older subjects. Levels of pros-methylimidazoleacetic acid (p-MIAA), an endogenous isomer of t-MIAA not derived from histamine, did not differ between groups. A larger sample (n=43) of patients and controls aged 20-74, showed significant ($p<0.05$) correlations (Pearson [α=1]) between age and lumbar CSF levels of t-MH, t-MIAA, and the sum of t-MH + t-MIAA, but not p-MIAA. These findings contrast with results of similar studies of metabolites of other transmitters which generally decline with increasing age. (Supported by NIMH grant 31805).

88.19

MULTIPLE [^{3}H]-IMIPRAMINE BINDING INHIBITORS IN CALF BRAIN EXTRACTS. A. Strijewski*, J. Chudzik*, D. Helmeste, S. Cheung* and S.W. Tang. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada M5T 1R8.

In order to isolate the endogenous ligand for imipramine (IMI) binding sites in the brain, combined calf caudate and frontal cortex were extracted with acetic acid and then with methanol. Methanol extract was delipidated with chloroform and chromatographed on Bio-Gel P-2 column. The elution profile as measured by inhibition of platelet ^{3}H-IMI binding shows four major distinctly separate peaks (A, B, C,D). None of these fractions contained salt or 5HT. All four fractions also inhibited platelet [^{3}H]-paroxetine binding and [^{3}H]-5HT uptake. Specificity of these fractions was further tested for their inhibition of β-adrenergic (^{3}H-dihydroalprenolol) and dopamine D_2 (^{3}H-spiperone) receptor binding. Fractions A and C had a substantial inhibitory effect on both [^{3}H]-DHA and [^{3}H]-spiperone binding while fraction B inhibited ^{3}H-DHA binding only. Fraction D had no significant inhibitory effect for either type of binding and therefore is the most specific ^{3}H-IMI inhibiting fraction of the four. Further TLC analysis of fractions A and B revealed a complex ninhydrin positive composition. Fraction C did not contain any detectable ninhydrin positive components. Antibodies raised against IMI revealed the possible presence of IMI-like compounds in fraction D while the HPLC results are so far non-conclusive. Further investigation should lead to the purification and identification of the [^{3}H]-IMI binding inhibitors.

88.20

TRYPTOPHAN HYDROXYLASE ACTIVITY IN HYPOTHALAMUS AND BRAINSTEM OF NEONATAL AND ADULT RATS TREATED WITH HYDROCORTISONE, FLUOXETINE OR PARACHLOROPHENYL-ALANINE. D.H. Park, H. Paivarinta, A.C. Towle and T.H. Joh. Lab. Mol. Neurobiology, Cornell Univ. Med. Coll., Burke Reh. Ctr., White Plains, NY 10605.

Glucocorticoids have been implicated in the regulation of the activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme in serotonin biosynthesis. In order to understand the mechanisms governing TPH activity in brain and superior cervical ganglion (SCG) of neonatal and adult rats, we determined whether chronic hydrocortisone treatment causes any change in TPH activity in hypothalamus, brainstem and SCG of 7-day-old or adult rats. In addition, the effect of fluoxetine, a serotonin uptake inhibitor or p-chlorophenylalanine (PCPA), a TPH inhibitor, on TPH activity was studied. Our results indicate that 1) hydrocortisone treatment increases only TPH activity in brainstem of 7-day-old rat and has no effect on TPH activity in other brain regions or SCG in neonatal and adult rats; 2) fluoxetine treatment also leads to a significant increase in TPH activity only in the brainstem of neonatal rats; 3) PCPA results in a 50% decrease in activity in SCG and 80-100% decrease, in the brain. Supported by NIH grants, MH 24285 and 1PO1MH 42626-01.

88.21

OPPOSITE EFFECTS OF SEROTONIN (5HT) AND CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE (cAMP) ON TRANSFERRIN SECRETION BY ENRICHED EPITHELIAL CELLS OF RAT CHOROID PLEXUS IN CULTURE. M. Tsutsumi*, M.K. Skinner*, and E. Sanders-Bush. (SPON: S.E. Mayer). Dept. of Pharmacology and Psychiatry, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

The choroid plexus secretes the cerebrospinal fluid into the ventricles of the brain. Messenger RNA for plasma proteins including transferrin, transthyretin, and ceruloplasmin have been localized in intact choroid plexus. We have previously shown that enriched epithelial cells of the rat choroid plexus in culture synthesize and secrete transferrin based on radioimmunoassay. Using this assay, we examined the effects of 5HT, dibutyrl cAMP and 8-bromo-cAMP on transferrin secretion by epithelial cells. Transferrin secretion increased with increasing concentrations of 5HT while cAMP analogs induced a concentration-dependent decrease. These data suggest that both 5HT and cAMP play a regulatory role in the choroid plexus. The level of transferrin in cells changes in concert with that in the medium, suggesting that the neurohumoral-induced changes in transferrin reflect alterations in the synthesis of the proteins rather than the secretory process. These findings show that choroid plexus synthesizes and secretes nutrients that are essential to the central nervous system, and that this function may be regulated by various neurotransmitters. (Supported by USPHS Grants MH-34007 and HD-20583.)

88.22

SENSITIVE HPLC METHOD FOR SEROTONIN IN CEREBROSPINAL FLUID. T.J. Tolliver,* S.J. Huang,* I.N. Mefford,* N.A. Garrick, and D.L. Murphy* (SPON: J.M. Tolliver). LCS, NIMH, Bethesda, MD 20892.

Serotonin (5HT) has been implicated in several psychiatric disorders. To evaluate serotonergic function in human disorders, we developed a method for quantitating 5HT in human lumbar and monkey cervical cerebrospinal fluid (CSF) using a sensitive (10 pg/ml lower detection limit) HPLC procedure with electrochemical detection. Use of a long chain ion pairing agent, sodium dodecyl sulfate (SDS), and high acetonitrile content coupled with microbore technology allowed for the "non-eluting matrix" approach for directly injecting large sample volumes onto the column. N-methyl 5HT was used as the internal standard. The mobile phase was 0.25 m NaH_2PO_4 containing 200 mg/l SDS, 100 mg/l Na_2 EDTA, and 20% acetonitrile. The system consisted of an Applied Chromatography System 400-02 pump, Rheodyne 7125 injector fitted with a 100-µl loop, and a Bioanalytical Systems electrochemical detector with glassy carbon working electrode. The working electrode potential was set at 0.55 V vs. Ag/AgCl. The column was 15 cm x 1.0 mm I.D. packed in house with 3 µm C_{18} ODS Shandon Hypersil. Levels of 5HT in human lumbar CSF ranged from < 10 to 793 pg/ml, with a median value of 16.2 (n = 108). Levels of 5HT in monkey cervical CSF ranged from < 10 to 882 pg/ml, with a median value of 35.9 (n = 223). In most cases, the highest 5HT values were found in CSF samples that appeared blood tinged.

88.23

REGULATION OF STRIATAL SEROTONIN AND HIPPOCAMPAL NORADRENALINE RELEASE BY THE LATERAL HABENULA AS STUDIED BY IN VIVO MICRODIALYSIS. P. Kalén*, R.E. Strecker, E. Rosengren* and A. Björklund. (SPON: B.D. Boss) Dept. Med. Cell Res., Biskopsg. 5, S-22362 Lund, Sweden.

Extracellular levels of serotonin (5-HT) and noradrenaline (NA) were monitored with the microdialysis technique in halothane anaesthetized rats. Striatal 5-HT was recovered through a new double-loop probe with a substantially improved recovery rate, with an uptake blocker added to the perfusion medium. NA was collected through a single loop probe implanted in the hippocampus. Samples were collected every 15 min. Electrical stimulation of the lateral habenula (LHb; 15 Hz, 0.5 mA, 0.5 ms) for 15 min increased the 5-HT levels by 70 %. It is suggested that this effect was mediated by a direct excitatory amino acid projection from the LHb to the dorsal raphe nucleus (NRD), and that this projection was modulated by GABA synapses at the raphe level, mainly since: (1) transection of the LHb efferents, but not lesion of the interpeduncular nucleus, abolished the effects of stimulation; (2) NRD injections of kynurenic acid blocked the effect of LHb stimulation, whereas bicuculline injections increased the 5-HT levels by 70 %, and potentiated the stimulation effect by 50 %. In the hippocampus LHb stimulation increased the NA release 3-fold. It is suggested that this effect was specifically mediated by the descending projections of the LHb, but that it was not relayed over the serotonergic component of the rostral raphe nuclei, mainly since: (1) transection of the dorsal bundle, or transection of the LHb efferents, abolished the stimulation effect; (2) no reduction in NA release was seen after neurotoxic lesion of the 5-HT system.

88.24

ENDOGENOUS RELEASE OF SEROTONIN AND NOR-ADRENALINE IN THE RAT AS STUDIED BY INTRA-CEREBRAL DIALYSIS. A. Björklund, P. Kalén*, R.E. Strecker, E. Rosengren*, M. Kokaia* and O. Lindvall*. Dept. Med. Cell Res., Biskopsgatan 5, S-22362 Lund, Sweden.

Extracellular levels of serotonin (5-HT), 5-HIAA and noradrenaline (NA) were monitored in the striatum and hippocampus of the rat, and analyzed by HPLC with fluorimetric detection (indols), or a radioenzymatic assay (NA). In awake freely moving animals the steady state output of 5-HT and NA in the hippocampus were 35 and 50 fmole/30 min, respectively, whereas during resting or sleep these levels were reduced by approx. 25 %. Halothane anaesthesia further reduced the output. In 5-HT and NA denervated animals the basal levels were below the limit of detection 2-3 weeks post lesion, but returned to the levels of intact animals after prolonged survival. KCl added to the perfusion fluid substantially increased the 5-HT and NA levels, whereas TTX reduced the output. P-chloroamphetamine and pargyline injected i.p. increased the 5-HT levels, whereas omission of Ca^{++} from the perfusion fluid, or i.p. injections of the 5-HT receptor agonist N,N-dimethyltryptamine, reduced the 5-HT release. Several of these manipulations were found to induce the same percentage changes also when an uptake blocker was added to the perfusion medium in order to increase the extracellular levels of 5-HT and NA, and thereby allow for shorter sampling times.

It is concluded that intracerebral microdialysis provides a useful method for the study of extracellular levels of 5-HT, 5-HIAA and NA, and that changes in extracellular level of 5-HT and NA are closely related to changes in synaptic activity, whereas 5-HIAA primarily reflect intraneuronal metabolism.

88.25

COMBINED *IN VIVO* ELECTROCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES OF MONOAMINE OVERFLOW FROM RAT HIPPOCAMPAL SLICES. Mei-Tsu Su*, Thomas V. Dunwiddie and Greg A. Gerhardt. (SPON: G. Kindt) Depts. of Pharmacology and Psychiatry, U.C.H.S.C., Denver, CO 80262, and VA Medical Center, Denver, CO 80220.

In vivo electrochemical measurements of chronoamperometric recordings from Nafion-coated electrodes were used to investigate monoamine overflow from selected regions of the rat hippocampal slice. Concurrent electrophysiological measurements of CA1 pyramidal cell population spike amplitude were used to characterize changes in the electrical activity of the slice during various treatments. Superfusion with elevated potassium (50 mM, 5 min) elicited consistent increases in the electrochemical response recorded from the dentate gyrus. At the onset of potassium perfusion, there was an initial increase in the population spike response, followed by electrical silence, which usually lasted 5-10 min and required 20-30 min for complete recovery of the response. The electrochemical signal always increased following the decline in the electrophysiological response, suggesting that release occurred only following a high degree of depolarization, and the electrochemical signal usually returned to baseline much before the electrophysiological response (usually within 5 min). Cocaine pretreatment (10-50 uM) caused a dose-dependent augmentation of the electrochemical responses. Local pressure ejection of potassium via a micropipette elicited dose-dependent increases in the electrochemical signals that were of relatively brief duration. The present experiments demonstrate the feasibility of conducting simultaneous electrophysiological and electrochemical experiments in an isolated preparation of brain such as the hippocampal slice.

(Supported by USPHS grants AG06434 and DA02702, and by the Veterans Administration Medical Research Service).

88.26

INHIBITION OF SEROTONIN METABOLISM BY OPENING SODIUM CHANNELS. L.S. Sorkin, M.G. Hughes*, W.D. Willis and D.J. McAdoo, Marine Biomed. Inst. and Dept. of Anat. & Neurosci., Univ. of Texas Med. Branch, Galveston, TX 77550.

We are using dialysis fibers to administer drugs and collect released neurotransmitters within the dorsal horn of the spinal cord of α-chloralose anesthetized cats. Released amines and metabolites are subsequently assayed by high pressure liquid chromatography-electrochemical detection. We found that depolarizing agents caused an increase in 5-hydroxytryptamine (5HT) levels in the extracellular fluid. The same treatments caused a several-fold decrease in the 5HT metabolite 5-hydroxyindole acetic acid (5HIAA). Sodium channel openers veratridine and aconitine both produced 5HIAA decreases that persisted for at least 2 hours. The sodium channel blocker lidocaine prevented the drug-induced decreases in 5HIAA levels. Neither the monoamine oxidase inhibitor pargyline nor the 5HIAA clearance inhibitor probenecid influenced the drug-induced decrease in 5HIAA levels. Lidocaine and parachloroamphetamine caused substantial release of 5HT. Lidocaine by itself appeared to slightly increase 5HIAA levels. Our results suggest that sodium channel activation strongly inhibits 5HT oxidation in the spinal cord.

Supported by NIH grants NS09743 and NS11255 and the John Sealy Memorial Endowment for Biomedical Research.

88.27

MEASUREMENT OF THE IONTOPHORETIC RELEASE OF MONOAMINES FROM GLASS MICROELECTRODES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC). I.A.Paterson, B.D.Sloley* & A.A.Boulton. Neuropsychiatric Res.Unit,Univ. of Saskatchewan,Saskatoon,Sask,Canada

The iontophoretic release of monoamines from five-barrelled glass microelectrodes was measured in vitro using HPLC with electrochemical detection. This method allowed reproducible measurements of release with currents as low as 3nA, and allowed the simultaneous measurement of the release of upto five amines in different barrels of the electrode. Measurements were made of adrenaline, dopamine, noradrenaline, *p*- and *m*-octopamine, serotonin, *p*- and *m*-synephrine, tryptamine, *p*- and *m*-tyramine.

There was no correlation between the tip size of the electrode, the resistance of the barrel, the spontaneous efflux of the amine, nor the apparent transport number calculated from Faraday's (Hittorf's) law. There was no correlation between transport numbers of different amines within the same electrode, although there was a positive correlation between the spontaneous efflux of different amines within the same electrode.

The relationship between the iontophoretic ejection of an amine and the iontophoretic current was not linear, and the apparent transport number increased with the size of the current. The transport number increased rapidly with currents of 3-20nA and then increased more slowly with currents of 20-200nA. Measurements of non-iontophoretic transport processes could not account for the increase in the transport number in the low current range. These findings show that Faraday's (Hittorf's) law is not an adequate description of iontophoretic release when using smaller iontophoretic currents. The equations of Purves (R.D.Purves, 1981, Microelectrode methods for intracellular recording and iontophoresis, Pub. Academic Press, London) provide a more accurate description of iontophoretic release.

I.A.P. and B.D.S. are Saskatchewan Health Research Board Fellows.

SEROTONIN, HISTAMINE AND OTHER BIOGENIC AMINES III

89.1

AMILORIDE, AN INHIBITOR OF Na-Ca EXCHANGE, INHIBITS SEROTONIN MODULATION OF CONTRACTION OF APYLSIA BUCCAL MUSCLE. L.-X. Liu* & J.L. Ram (SPON: I. Montgomery). Dept. of Physiology, Wayne State Univ., Detroit, MI 48201.

Serotonin (5-HT) potentiates acetyl choline (ACh) elicited contraction of Aplysia buccal muscles. Contraction is also potentiated by zero sodium medium (ONa), an effect attributed to Ca influx via Na-Ca exchange (Ram et al., this volume). To test whether 5-HT might similarly potentiate contraction by activating Na-Ca exchange we used amiloride (AMIL), a Na-Ca exchange inhibitor. Enhancement of ACh contraction by 10^{-8} M 5-HT, measured as % increase in the first contraction after 5-HT compared to the preceding contraction, averaged 160 ± 12% (n=54) in the absence of AMIL. In the presence of AMIL the effect of 5-HT was 0% (no var.)*, .3 mM AMIL (n=4); 50 ± 15%*, .1 mM AMIL (n=10); 60 ± 13%*, .03 mM AMIL (n=13); and 210 ± 60%, .01 AMIL (n=3) (*=p<.005). As percent of vehicle controls, unenhanced ACh contractions were 22%*, .3 mM AMIL; 61%*, .1 mM AMIL; 106%, .03 mM AMIL & 92%, .01 mM AMIL. At ≤.1 mM, AMIL had no effect on ACh depolarization, while .3 mM AMIL reduced its magnitude and speed. Thus, .03 & .1 mM AMIL drastically reduced 5-HT enhancement while having a much smaller effect on contractile and membrane potential responses to ACh. These data are consistent with Na-Ca exchange mediating effects of 5-HT; however, since AMIL has other effects on sodium movements, other interpretations are possible. (supported by MDA).

89.2

SPECIFIC ANTAGONISM AT ENTERIC NEURONAL 5-HT_{1P} RECEPTORS BY BRL 24924: ROLE OF SLOW EPSPS IN ACTIVATION OF GASTRIC INHIBITORY NEURONS. G.M. Mawe, T.A. Branchek, and M.D. Gershon. Dept of Anatomy and Cell Biology, Columbia Univ. P&S, New York, NY 10032

5-HT_3 receptors on enteric neurons mediate a short-lived depolarization (fast response), associated with an increased Na conductance. Fast responses are antagonized by ICS 205-930 and mimicked by 2-CH_3-5-HT. In contrast 5-HT_{1P} receptors on enteric neurons mediate a long-lasting depolarization (slow response), associated with a decreased K conductance. Slow responses are antagonized by dipeptides of 5-HTP and mimicked by hydroxylated indalpines. 5-HT_{1P}, but not 5-HT_3 antagonists, block slow EPSPs for which 5-HT is the transmitter. The 5-HT_{1P}, but not 5-HT_3 receptors, can be labeled by ^{3}H-5-HT. We have now analyzed the effects of BRL 24924 on guinea pig myenteric neurons by using intracellular microelectrodes. Pressure microejection of BRL 24924 onto type II/AH neurons mimicked neither the fast, nor the slow response to 5-HT. On the other hand, BRL 24924 (0.5-1 μM) antagonized slow responses to 5-HT (15/15 cells). Moreover, BRL 24924 also blocked the slow EPSP in myenteric type II/AH neurons that is mediated by the release of endogenous 5-HT. Both of these actions of BRL 24924 were slowly reversible. The action of BRL 24924 was specific, since the slow response of myenteric neurons to substance P, which is similar to that elicited by 5-HT, was not affected by BRL 24924 in 8/8 cells. These results indicate that BRL 24924 is primarily a 5-HT_{1P} antagonist. Unlike other specific 5-HT_{1P} agonists or antagonists, BRL 24924 did not block the binding of ^{3}H-5-HT to 5-HT_{1P} receptors. BRL 24924 (0.5 mg/kg) was found to increase the rate of emptying of a ^{51}Cr-labeled liquid meal from the stomachs of awake freely moving mice. Since BRL 24924 blocks 5-HT-mediated slow EPSPs, these results imply that intrinsic inhibitory neurons of the murine stomach may be tonically activated by serotonergic axons acting through 5-HT_{1P} receptors. Supported by NIH grants NS12969, NS 07062, and NS 22637.

89.3

SEROTONIN EXCITES VISCERAL SENSORY NEURONS BY TWO SEPARATE MECHANISMS. E.P. Christian, G.E. Taylor* and D. Weinreich. Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201

Serotonin (5-HT) is known to stimulate afferent fibers of visceral sensory nerves (Douglas and Ritchie, J. Physiol., 138:31, 1957). One mechanism underlying this excitability increase may be a depolarization of afferent terminals, since 5-HT has been shown to depolarize sensory neurons (Higashi, Nature, 267:448, 1977). Here we report that 5-HT produces an additional excitatory effect by blocking a slow spike afterhyperpolarization (AHP_{slow}) that exists in a subpopulation of sensory neurons in the nodose ganglion (Fowler et al., J. Physiol., 365:59, 1985).

Rabbit and guinea pig nodose ganglia were maintained *in vitro* and neurons recorded with standard intracellular techniques. The depolarizing effect of bath applied 5-HT (0.5-10 μM) was blocked by including 10 μM d-tubocurarine in the superfusate (Higashi and Nishi, J. Physiol., 323:543, 1982). Under these conditions, 5-HT reduced or abolished the AHP_{slow} in a concentration-dependent manner. Blockade of the AHP_{slow} was accompanied by a decrease in the accommodative properties of these neurons, whereas 5-HT did not affect accommodation in neurons not exhibiting an AHP_{slow}. Thus the AHP_{slow} represents a distinct locus where 5-HT can act to increase the excitability of these sensory neurons. (Supported by NIH grant NS 22069).

89.4

ELECTROPHYSIOLOGICAL RESPONSES PRODUCED BY AUTACOIDS ON BRONCHIAL PARASYMPATHETIC NEURONS OF THE GUINEA PIG *IN VITRO*. A.C. Myers and D. Weinreich; Dept. of Pharm. and Exp. Ther., Univ. of Md Sch. of Med, Balt, MD 21201.

Bronchial parasympathetic neurons play an important role in the control of bronchial caliber. Both circulating and locally released neuroactive agents may alter the electrophysiological membrane properties of these neurons. The synaptic and membrane properties of guinea pig bronchial neurons were monitored with standard intracellular microelectrode techniques during superfusion of the compounds listed below (values are x ± SD for 6-30 experiments):

Compound		Δ Em (mV)	Δ Gi (%)	Δ fEPSP(%)
Histamine	10^{-5} M	+4 ± 2	+38 ± 15	no effect
5-HT	10^{-6} M	+3 ± 2	+48 ± 9	+41 ± 19
Epinephrine	10^{-6} M	+4 ± 1	no effect	-48 ± 23
Norepi.	10^{-5} M	+1 ± 3	no effect	-64 ± 20
PGD_2	10^{-6} M	+3 ± 1	-50 ± 10	no effect

Of these compounds only PGD_2 (10^{-6} M) decreased accommodation of phasic firing neurons thus mimicking this change induced by *in vitro* antigen challenge (Undem, et al., FASEB J. 2: A1056, 1988). These data suggest that a variety of endogenous neuroactive compounds may alter the synaptic efficacy of these ganglia. (Supported by NIH grants #NS 22069 & NS 25598).

89.5

INVOLVEMENT OF THE SEROTONERGIC SYSTEM IN DEPRESSION OF THE SPINAL MONOSYNAPTIC REFLEX BY PHENCYCLIDINE IN NEONATAL RATS. Y. Ohno and J.E. Warnick. Dept. of Pharmacol. & Exp. Ther., Univ. of MD Sch. of Medicine, Baltimore, MD 21201.

Phencyclidine (PCP) interacts with several neurotransmitter systems including those for serotonin (5-HT), catecholamines, acetylcholine, σ-opioid and N-methyl-D-aspartate (NMDA) in the central nervous system. This study was undertaken to elucidate the mechanism of PCP action in depressing the spinal monosynaptic reflex (MSR) in neonatal rats. Spinal cords were removed from male Wistar rats (6-9 days old), hemisected and superfused with oxygenated physiological solution *in vitro*. Stimulation of an L_{3-5} dorsal root evoked a MSR in the corresponding ventral root. PCP caused a dose-dependent depression of MSR with 50% inhibition at 8 μM. Neither the NMDA antagonists APV (2-amino-5-phosphonovalerate) and AP7 (2-amino-7-phosphonopentanoate) (100 μM) nor the σ-opioid agonist N-allylnormetazocine (30 μM) affected the MSR. The depressant action of PCP was markedly attenuated by simultaneous exposure to the 5-HT antagonists spiperone (5-HT_1 and 5-HT_2) and ketanserin (5-HT_2), but was unaffected by adrenergic (phentolamine and timolol), dopaminergic (chlorpromazine and pimozide) and cholinergic antagonists (atropine and mecamylamine) which by themselves did not affect the MSR. Our results suggest that the depression of the MSR by PCP is mediated by activation of a spinal serotonergic system and does not involve either σ-opioid or NMDA receptors. (Supported in part by USPHS grant DA-02804.)

89.6

SEROTONIN FACILITATION OF SPINAL MOTONEURON EXCITABILITY IS NOT MIMICKED BY THE $5HT_{1A}$ AGONIST, 8-OH-DPAT. D.A. Jackson and S.R. White. Dept. of VCAPP, Washington State University, Pullman WA 99164-6520

The ventral horn of the rat spinal cord is richly innervated with serotonin (5HT) terminals, and several $5HT_1$ receptor subtypes have been identified in the spinal cord (Huang and Peroutka, Brain Res., 436:173, 1987). Although 5HT is known to facilitate spinal motoneuron excitability in rats, the receptor subtype responsible for this facilitation is not known. However, the $5HT_{1A}$ agonist, 8-OH-DPAT, can mimic some of the spinal components of the "serotonin syndrome," suggesting that $5HT_{1A}$ receptors may mediate motoneuron excitability changes caused by 5HT (Tricklebank et al., Eur. J. Pharmacol., 106:271, 1985). In the present study, the effects of 5HT on glutamate-evoked spinal motoneuron activity were compared to the effects of 8-OH-DPAT in urethane-anesthetized rats. Iontophoretically applied 5HT (20 nA, 30-60 sec) consistently produced a marked facilitation of glutamate-evoked motoneuron firing. This facilitation was sometimes, but not always, preceded by very brief inhibition at current ejection onset. In contrast, 8-OH-DPAT (20-100 nA, 30-60 sec) produced only inhibition of glutamate-evoked firing in these spinal motoneurons. We conclude that facilitation of motoneuron excitability by 5HT is not mediated by $5HT_{1A}$ receptors. Supported by NIH grant NS 24388.

89.7

POST-SPIKE AFTER HYPERPOLARIZING POTENTIALS ARE DECREASED BY SEROTONIN AND TRH IN SPINAL MOTONEURONS OF DECEREBRATE CATS. S.R. White and S.J. Fung. Dept. of VCAPP, Washington State Univ., Pullman WA 99164

Serotonin and thyrotropin releasing hormone (TRH) are colocalized in terminal plexuses in the spinal cord ventral horn and both substances facilitate glutamate-induced motoneuron firing One mechanism by which motoneuron firing rates may be increased is by reducing the afterhyperpolarization (AHP) which follows an action potential. Serotonin has been shown to reduce the AHP in lamprey motoneurons (Van Dongen et al., Brain Res., 306:320, 1986) and in hippocampal pyramidal neurons (Andrade and Nicoll, J. Physiol., 394:99, 1987). We have investigated the effects of iontophoretically applied serotonin (5HT) and TRH on the AHP following action potentials evoked by intracellular current pulses in antidromically identified lumbar motoneurons in decerebrate cats. Both 5HT (25-50 nA, 30-60 sec) and TRH (80-115 nA, 90-120 sec) reduced the amplitude of the AHP. This reduction was more clearly seen in the AHP following the second or third spike of a spike train than following the initial spike. The reduction of the AHP developed slowly following the onset of 5HT or TRH ejection and outlasted the ejection period by more than a minute. In addition to reducing the AHP, 5HT and TRH also produced slow, low amplitude depolarizations of the motoneuron membrane and decreased the threshold for spike initiation by a depolarizing current pulse. Supported by NIH, NS24388

89.8

NOREPINEPHRINE FACILITATES SPINAL MOTONEURON EXCITABILITY RECORDED INTRACELLULARLY IN DECEREBRATE CATS. S.J. Fung, S.R. White, and C.D. Barnes. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164

Previously we reported that locus coeruleus stimulation produces an adrenoceptor-mediated increase in motoneuron excitability in the lumbar spinal cord of the cat (Fung and Barnes, Brain Res., 216:299, 1981). The concept of an excitatory role for norepinephrine (NE) on spinal motoneurons is, however, in conflict with early reports that iontophoretically applied NE hyperpolarized these cells. In the present study we reinvestigated the effect of NE on spinal motoneurons in decerebrate cats, paying particular attention to slowly developing, long-lasting changes in membrane excitability. In 19 antidromically identified hindlimb motoneurons (spike height > 60 mV), iontophoretically applied NE (30-70 nA, 30-60 sec) consistently produced a slow, long-lasting depolarizing potential with an associated increase in cell excitability. NE application was followed by a decrease in the threshold for action potential production by intracellular depolarizing current. NE also facilitated initial segment to soma dendritic transmission in the motoneurons and decreased the afterhyperpolarization which follows action potentials in the cells. None of these NE effects were mimicked by ejection of hydrogen ions from a pH control solution. We conclude that NE facilitates excitability of lumbar spinal motoneurons in cats. Supported by NIH grant NS 24388.

89.9

5-HT AND BIPHASIC CHANGES IN VENTRAL AND DORSAL ROOT MEMBRANE POTENTIALS. A.M. Holohean*, J.V. Vega*, J.C. Hackman and R.A. Davidoff. (SPON: R. Clark). Neurophysiology Lab, VAMC and Dept. of Neurology, Univ. of Miami School of Medicine, Miami, FL 33101.

5-HT receptors are implicated in the modulation of sensory information in the spinal cord, but the nature of the process is unclear. We studied putative 5-HT spinal receptors by means of sucrose gap recordings from ventral (VR) and dorsal (DR) roots of hemisected frog spinal cords superfused with HCO_3^--buffered Ringer's solution (15°C).

5-HT (1nM-1mM), 8OHDPAT (5-HT_{1A} agonist, 1nM-1mM), TFMPP (5-HT_{1B} agonist, 10nM-1mM), and methysergide (mixed agonist/antagonist, 1uM-100mM) produced biphasic effects on the DR and VR membrane potential. Low (nM) concentrations hyperpolarized (0.3-1 mV), while higher concentrations (uM-mM) depolarized (0.2-5 mV) DRs and VRs. 5-HT consistently produced larger VR than DR depolarizations; 8OHDPAT evoked greater DR depolarizations. TTX decreased 5-HT- and abolished TFMPP- and 8OHDPAT-evoked depolarizations. All hyperpolarizations were enhanced in TTX (presumably 2° to reduced depolarizations). The 5-HT_{1A} antagonists spiperone (1-10uM) and spiroxatrine (1-3uM) reduced the 5-HT-hyperpolarizations, while methysergide (1-10uM), mianserin (10uM) and ketanserin (1-10uM) increased hyperpolarizations and reduced depolarizations.

In sum, 5-HT produced biphasic responses in frog motoneurons and afferent terminals with direct 5-HT_{1A}-hyperpolarizations and indirect depolarizations. (Supported by VAMC Funds MRIS #1769 and #3369 and USPHS grant #17577)

89.10

5-HT MODULATION OF REFLEXES AND EXCITATORY AMINO ACID RESPONSES IN FROG SPINAL CORD. R.A. Davidoff, A.M. Holohean*, J.V. Vega* and J.C. Hackman. Dept. of Neurology, Univ. of Miami School of Med. and Neurophysiology Lab, VAMC, Miami, FL 33101.

Because the ability of 5-HT to affect reflexes could result from an effect on synaptic transmission mediated by excitatory amino acids (EAAs), we examined the effects of the amine on the changes in potential produced in ventral (VR) and dorsal (DR) roots by specific EAA receptor agonists and on VR (VRPs) and DR (DRPs) potentials evoked by DR stimuli by means of sucrose gap recordings from DRs and VRs in the isolated, hemisected frog spinal cord superfused with HCO_3^--buffered Ringer's solution.

A low concentration (0.1uM) of 5-HT increased VRPs and DRPs by 18%, but increasing the concentration (100uM) reduced the evoked reflexes by 13%. Application of both concentrations of 5-HT produced variable effects on the potential changes produced by 10 s applications of EC_{50} concentrations of quisqualate (3uM), kainate (10uM), NMDA (30uM), L-glutamate (1mM) and L-aspartate (1mM). In contrast, application of 8OHDPAT (a selective 5-HT_{1A} agonist, 0.01uM) shifted concentration-response curves for quisqualate (1-100uM), NMDA (1-100uM), kainate (10-100uM), and glutamate (100uM-2 mM) to the left and increased the slope of the linear portion of each curve.

These results indicate that 5-HT_{1A} receptors are involved in the spinal effects of 5-HT; these receptors may affect the ability of 5-HT to mediate reflexes and EAA responses. (Supported by VAMC Funds MRIS #1769 and #3369 and USPHS grant #17577)

89.11

SUPRAOPTIC NUCLEUS (SON) NEURONAL RESPONSES TO HISTAMINERGIC INPUTS RECORDED INTRACELLULARLY *IN VITRO*

G. I. Hatton & Q.Z. Yang, Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Horizontal slices of rat ventral hypothalamus containing the pituitary stalk, SON and tuberomammillary (TM) histaminergic neurons were studied. In some slices, one SON and one TM cell were impaled to study synaptic responses. In single impalements of SON cells, responses to micropressure applications of histamine or H_1 and H_2 agonists and antagonists were studied. 3 of 60 SON/TM pairs were found to be synaptically connected. Each of the 3 SON cells was an AVP neuron and was excited synaptically by depolarizing current injected into the TM cell. In spontaneously firing AVP cells, antidromically activated by stalk stimulation, firing frequency and epsps were increased by histamine or H_1 agonist application, effects that were blocked by H_1 antagonists. Putative OX neurons (n=2) were hyperpolarized by histamine. H_2 agonists were without effect. Silent or hyperpolarized SON cells fired single spikes upon stalk stimulation. Histamine application altered this response so that single stimuli resulted in trains of spikes. Thus, AVP-containing neurons are synaptically excited by input from TM cells, probably through activation of H_1 receptors. OX-containing neurons may be inhibited by histamine. Differential release of AVP and OX may be mediated by this pathway. Supported by NIH grant NS16942.

89.12

ELECTROPHYSIOLOGY OF MAGNOCELLULAR (HISTAMINERGIC) TUBEROMAMMILLARY NEURONS (TM) IN SLICES OF RAT HYPOTHALAMUS.

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Histaminergic TM neurons project to many brain areas, including cortex and brain stem, and are apparently involved in control of vasopressin release through modulation of supraoptic neurons to which they have direct projections. Intracellular recordings were made from 25 TM neurons in horizontally cut slices of hypothalamus. For positive identification, about 1/2 of the neurons were also injected with Lucifer Yellow, and some were antidromically activated by electrical stimulation of one target area, the supraoptic nucleus (SON). The following membrane properties were determined (means): E(membrane)= -55mV, AP(amplitude)= -63 mV, R(input)= 181 MΩ, Time constant(membrane)= 5 msec. APs were followed by afterhyperpolarization (100-150 msec dur., 10mV ampl.). TM neurons were characterized by fast Na^+ spikes (1.5 msec dur. at 1/ ampl.) and slow, presumed Ca^{2+} spikes (15-40 mV ampl., 12-400 msec dur.). The fast spikes only were abolished by TTX (10^{-5} M). TM neurons were generally silent in the slices, although some fired spontaneously (2-20 Hz). Depolarizing current injections produced a train of 5-12 APs with progressively increasing interspike intervals. Prolonged current injection did not result in continued firing, suggestive of a Ca^{2+}-activated K^+ conductance as one factor in burst termination. Supported by NIH grant NS16942.

89.13

SEROTONIN (5-HT) AND NOREPINEPHRINE (NE) INDUCE IPSPs IN PYRAMIDAL CELLS OF THE PIRIFORM CORTEX: EVIDENCE FOR A 5-HT_2 AND $alpha_1$ ACTIVATED INTERNEURON. P. W. Sheldon* and G. K. Aghajanian. Departments of Pharmacology and Psychiatry, Yale University, New Haven, CT. 06510

Pyramidal cells of the piriform cortex are known to have a high level of spontaneous IPSPs. Using intracellular recording with KCl-filled electrodes in an in vitro slice preparation of rat piriform cortex, we found that IPSPs could be induced in 20-30% of the pyramidal cells in layer II by bath application of either 5-HT (100μM) or NE (100μM). Induction of IPSPs by 5-HT was blocked by the 5-HT_2 antagonist ritanserin (5μM). Ritanserin also blocked the direct depolarization associated with 5-HT application, but with much reduced potency. The IPSPs induced by application of NE were blocked by the $alpha_1$ antagonist prazosin (2μM).

Because the IPSPs were blocked by both bicuculline and tetrodotoxin, suggesting that they arose from the firing of a GABAergic interneuron, we searched extracellularly for an interneuron which was activated by both 5-HT and NE. We found a group of such presumed interneurons on the border between layers II and III. 5-HT activation of these interneurons was blocked by ritanserin (1μM) ; NE activation was blocked by prazosin (2μM). The activation by 5-HT is potentiated by application of the putative 5-HT_2 agonist 2,5-dimethoxy-4-methylamphetamine (DOM) (1μM).

It appears that piriform cortex possess a GABAergic interneuron which is activated via 5-HT_2 and $alpha_1$ receptors and whose firing produces IPSPs in layer II pyramidal cells. (Supported by MH 17871 and the State of CT.)

89.14

THE EFFECT OF INCREASED CENTRAL SEROTONIN LEVELS ON HIPPOCAMPAL RHYTHMICAL SLOW ACTIVITY AND NEOCORTICAL LOW VOLTAGE FAST ACTIVITY.

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Serotonergic activity was increased in rats by treatment with tranylcypromine and tryptophan; HPLC revealed an 80% increase in the neocortex and an 83% increase in the hippocampus. Both hippocampal rhythmical slow activity (RSA) and neocortical low voltage fast activity (LVFA) were produced. Scopolamine-resistant RSA, thought to be dependent on brain serotonin (Vanderwolf, C.H. & Baker, G.B. Brain Res., 374:342, 1986) maintained its normal relation to behavior, occurring during motor activities such as walking and head movements (Type 1 behaviors). Scopolamine-resistant RSA was not present during classical serotonin syndrome behaviors such as forepaw treading and head weaving. Scopolamine-resistant LVFA, also thought to be dependent on brain serotonin (see above ref.), appeared continuously in the neocortex. No evidence was found to support the view that increased serotonin release promotes quiet sleep or cortical deactivation. The present findings support the view that serotonin is involved in cortical activation.

Supported by grants from NSERC.

89.15

MULTIPLE ACTIONS OF SEROTONIN ON MEMBRANE PROPERTIES OF CEREBELLAR PURKINJE CELLS IN VITRO. H. K. Strahlendorf and J. C. Strahlendorf. Depts. of Physiology and Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

We have reported complex effects of serotonin (5-HT) on the rate and pattern of firing of Purkinje cells (PCs) recorded extracellularly. This study investigates the actions of 5-HT on passive and active membrane properties of PCs. Intracellular recordings were obtained from PCs in sagittal slices of rat cerebellum at 32°-34°C. 5-HT was superfused or applied as a droplet. The resting membrane potential (Vm) of PCs averaged -51mV and the average input resistance (Rm) was 27MΩ. Spontaneous fast and slow spikes were observed. The most consistent effect (75% of PCs) of 100-200μM 5-HT was an increase in Rm that averaged 29%. In 25% of PCs, an average 39% decrease in Rm occurred. 5-HT hyperpolarized 37% of PCs by average of 3mV, depolarized 37% of PCs by an average of 10mV and failed to change Vm in 25% of PCs. Hyperpolarizations were associated with an increase in Rm; depolarizations were accompanied most frequently by an increase in Rm but decreases also were observed. Spiking in response to anodal breaks of hyperpolarizing pulses frequently was enhanced by 5-HT. These preliminary results underscore the complexity of 5-HT actions on PCs and suggest 5-HT may affect a number of ionic conductances in PCs. Supported by NS 19296.

89.16

SEROTONERGIC DORSAL RAPHE NEURONS: ALTERED SPONTANEOUS ACTIVITY AND SENSITIVITY TO 5-MeODMT AFTER LONG-TERM AMPHETAMINE. B.A. Heidenreich* and G.V. Rebec (SPON: G.P. Frommer). Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Amphetamine (AMPH) slows the firing rate of serotonin (5-HT) neurons in the dorsal raphe nucleus. Chronic treatment with a high dose of AMPH attenuates this response (Heidenreich et al., Neuropharmacol., 26:719, 1987). We now have examined the effects of long-term AMPH on the spontaneous activity of 5-HT neurons and on their sensitivity to 5-MeODMT, a 5-HT autoreceptor agonist.

Rats received injections of saline or 10 mg/kg d-AMPH twice daily for 6 days. Recordings of 5-HT cells on the next day revealed no effect of AMPH on spontaneous firing rate, interspike interval (ISI) or variability around the mean ISI. However, ISI variability was inversely related to firing rate in AMPH-treated rats, but not controls. Although both groups had comparable sensitivity to 5-MeODMT, the dose needed to inhibit unit activity correlated significantly with firing rate (r=.98) and ISI (r=-.95) only in the AMPH group. Also, a regular firing pattern was associated with lower effective doses of 5-MeODMT in the saline group. In contrast, regular firing patterns in the AMPH group were associated with higher firing rates and larger effective doses of 5-MeODMT. These results suggest that chronic AMPH enhances the importance of 5-HT autoreceptor control of firing rate relative to other mechanisms.

Supported by PHS Grant DA 02451.

89.17

INHIBITION OF SEROTONERGIC DORSAL RAPHE NEURONS BY p-CHLOROAMPHETAMINE (PCA) AND MDMA ("Ecstacy"): EFFECTS OF A SEROTONIN (5-HT) UPTAKE INHIBITOR AND L-TRYPTOPHAN. J.S. Sprouse and G.K. Aghajanian. Dept. of Psychiatry, Yale Univ. Sch. of Medicine, New Haven, CT 06508.

The mind-altering agent, (±)-3,4-methylenedioxymethamphetamine (MDMA), produces neurochemical effects in rat brain which closely resemble those of the serotonergic neurotoxin, PCA. Like PCA, MDMA causes an immediate and a long-term decrease in brain 5-HT. The initial effects may be due to inhibition of 5-HT synthesis and stimulation of 5-HT release; the later effects may be the result of nerve terminal damage. A comparison of the electrophysiological responses of 5-HT-containing dorsal raphe (DR) neurons to PCA and MDMA has not been examined previously.

Extracellular single unit recordings of DR neurons were made in the in vitro rat brain slice preparation. PCA (10-100 μM) or MDMA (3-30 μM) produced a dose-dependent suppression of DR cell firing. Pretreatment with L-tryptophan (30-100 μM), which stimulates 5-HT synthesis, increased the potency of PCA and MDMA. Pretreatment with the selective 5-HT uptake inhibitor fluoxetine (20 μM), which blocks the acute release and long term depletion of 5-HT by PCA and MDMA, blocked the suppressant effects of PCA and MDMA. These electrophysiological responses, consistent with the neurochemical data, suggest that PCA and MDMA act indirectly on DR neurons through release of endogenous 5-HT. (Supported by MH 17871 and the State of CT)

89.18

SEROTONIN AGONISTS INHIBIT DEPOLARIZING SYNAPTIC POTENTIALS IN THE RAT LOCUS COERULEUS *IN VITRO*. D.H. Bobker* and J.T. Williams. Vollum Institute, Oregon Health Sci. Univ., Portland, OR 97201

Intracellular recordings were made from rat locus coeruleus (LC)neurons *in vitro*. The depolarizing synaptic potential (DSP), elicited by electrical stimulation, was typically 10 - 15 mV in amplitude and is mediated by glutamate and GABA. Superfusion with 5-HT, or the non-specific 5-HT_1 agonist 5-carboxamidotryptamine (5-CT), produced a maximal inhibition of the DSP of 60%. This effect was found to be presynaptic. The EC_{50} for 5-CT was 50 nM, while for 5-HT it was 10 μM. In the presence of cocaine (10 μM), however, the 5-HT concentration-response curve was shifted left and had an EC_{50} of 400 nM. 8-OH-DPAT, the specific 5-HT_{1A} receptor ligand, acted as a partial agonist, producing approximately 60% of the 5-CT response (EC50 = 50 nM). The 5-HT_{1B} selctive ligand, TFMPP, acted as a full agonist (EC50 = 110 nM). None of these agonists had effects on membrane potential or firing rate.

The response to 8-OH-DPAT was antagonized by pretreatment with the 1A specific drug spiroperidol (1μM). The apparent K_D for spiroperidol was 20 nM. At this same concentration, however, there was no effect on the 5-CT induced response. The antagonist LM 21-009 (1B > 1A, 5-fold selectivity) was found to shift the 5-CT response to the right in a parallel manner at doses as low as 100 nM. The apparent K_D was 19 nM. This pharmacologic profile suggests that the presynaptic inhibition of the DSP is mediated by both 1A and 1B receptors. In addition, these results support previous *in vivo* work which demonstrates that 5-HT inhibits sensory evoked LC activity without effecting spontaneous activity.

Supported by USDHHS DA 04523 & NIH HL 07596.

89.19

SEROTONIN SELECTIVELY DECREASES GLUTAMATE-EVOKED EXCITATION OF LOCUS COERULEUS *IN VIVO*. G. Chouvet*, G. Aston-Jones[1], H. Akaoka*, & E. Malekaki*[1] (SPON: F. Strand), INSERM U171, Gif-sur-Yvette, France; [1]Dept. Biol. & Cntr. Neural Sci., New York Univ., NY 10003.

Despite the dense serotonergic input to locus coeruleus (LC), little is known concerning the influence of serotonin (5-HT) on LC physiology. Therefore, we examined the effects of serotonin on spontaneous LC activity and on responses of LC neurons to one of their major afferent transmitters, glutamate (Glu).

Extracellular recordings were obtained from individual LC neurons in 12 halothane-anesthetized rats using a single-barrel micropipette glued onto a 7-barrel iontophoretic micropipette assembly (recording tip extending 12-18 um). Iontophoretic Glu or acetylcholine (ACh) potently activated 91% and 88% of 49 and 25 LC cells, respectively, and NE consistently inhibited LC discharge (95% of 20 cells). However, 5-HT had no consistent effect on LC spontaneous discharge: this agent yielded weak inhibition in 49%, had no effect on 42%, and caused excitation in 9% of LC neurons (N = 49).

Despite weak and variable effects on spontaneous LC discharge, 5-HT consistently suppressed responses of LC neurons to Glu, markedly decreasing Glu excitations (by 60 to 98%) in 83% of 24 cells tested. Such attenuation of Glu-evoked activity by 5-HT was independent of effects of 5-HT on spontaneous activity, occurring in cells whose spontaneous discharge was augmented as well as in those that were inhibited by 5-HT. In addition, this effect of 5-HT was relatively specific to Glu-evoked excitations, as 5-HT did not attenuate (and in some cases enhanced) ACh responses in 10 of 11 neurons. Such effects were also relatively specific for 5-HT: NE, in contrast to 5-HT, suppressed spontaneous more than Glu-evoked activity.

These results indicate a selective filtering or modulatory role for 5-HT in the control of LC activity. As a consequence of 5-HT input, activation of LC neurons by excitatory amino acid afferents would be blunted while tonic, spontaneous discharge and excitation from other inputs (for example, those mediated by ACh) may remain intact. Supported by NINCDS Grant NS24698, ONR Contract N00014-86-K-0493, the Air Force Office of Scientific Research, and INSERM.

90.1

LOCALIZATION OF PHONOLOGICAL PROCESSING IN THE TEMPOROPARIETAL CORTEX BY PET. S.E. Petersen*, P.T. Fox, M.I. Posner and M.E. Raichle, Washington University School of Medicine, St. Louis, MO 63110.

Recent PET blood flow studies have shown activation in left temporoparietal cortex when words are presented auditorily, but not for other types of auditory stimuli, suggesting a role for this area in higher-order auditory, or phonological, processing. This area is not activated during the presentation or repetition of visually-presented words, suggesting that recoding into a sound-based representation is not obligatory during repetition of visual words.

To determine whether temporoparietal cortex is also engaged in phonological processing of visually-presented information, we measured cortical activation during a rhyme monitoring task using averaged PET blood flow subtraction images. Subjects monitored pairs of visually-presented words for rhymes as a phonological task. The control state was simple visual presentation of word pairs. Rhyme monitoring activated left temporoparietal cortex, near the site activated by passive auditory word presentation. This area was not activated during semantic monitoring. These observations strongly implicate the temporoparietal cortex in high-order phonological processing.

90.2

GLUCOSE HYPOMETABOLISM IN STROKE: SPECIFIC OR NONSPECIFIC EFFECT IN APHASIA. E.J. Metter*, W.R.Riege, W.H. Hanson*, C. Jackson*, D. Kempler*, and D. Van Lancker*. NIA, Gerontology Research Center, Baltimore, MD 21224, and VA Med Ctr, Sepulveda, CA 91343.

We examined the distribution of metabolic and structural abnormalities in 45 aphasic subjects studied greater than 1 month post onset in a resting state (eyes and ears unoccluded) with (F18)fluorodeoxyglucose PET, and x-ray CT. Structural damage was most frequent in the parietal and posterior superior temporal areas (67% of subjects). For these same regions, glucose metabolism was abnormal in 82% and 87% of subjects. Aphasia severity had a higher correlation with temporoparietal metabolism than structural measures. Using pathway analysis, regional metabolism had an effect on aphasia which was not accounted for by structural damage. The observations argue that regional hypometabolism in stroke are important in the cause of the aphasia. If metabolic effects were nonspecific (resulting from disruption of projections with no loss of regional function), then the aphasia should show weaker associations with regional metabolism than with structural damage, which was not the case.

90.3

REPRODUCTION OF TEMPORAL INTERVALS BY APHASIC AND NON-APHASIC BRAIN-INJURED PATIENTS. J. Ilmberger, E. Pöppel. Inst. Med. Psychology, Univ. of Munich, Munich, FRG.

Within the continuity of mental processing, successive mental events are usually integrated into coherent patterns forming a gestalt. The temporal limit of this integration process can be measured with different experimental paradigms, for instance temporal reproduction. In reproduction experiments, intervals of approximately 3 seconds are reproduced most accurately; indifference intervals defined in this way are one expression of limits of temporal integration. Indifference intervals possibly refer to language processing, too, as in spontaneous speech pauses are made every two to three seconds. We tested whether disorders of language processing are correlated with disorders of integration time. Time reproduction experiments were performed with aphasic and non-aphasic brain-injured patients and a control-group without brain damage. The results show that aphasic patients have significantly altered auditory indifference intervals. This finding is discussed with respect to possible consequences for language processing.

Supported by DFG Po 121/13-1.

90.4

EFFECTS OF UNILATERAL TEMPORAL LOBECTOMY ON RECOGNITION MEMORY FOR TEXT AND MELODY IN SONGS. S. Samson* and R.J. Zatorre (SPON: C. Anderson). Montreal Neurol. Inst., McGill Univ., Montreal, Canada H3A 2B4.

The effect of left-temporal lobectomy on verbal memory is well-documented, but less is known about the cerebral structures involved in memory for text sung to a melody, or for the melodic component of songs. The performance of patients who had undergone left (N=21) or right (N=20) temporal lobectomy for the relief of intractable seizures was compared with that of 20 normal control subjects. Unfamiliar songs were presented in a recognition paradigm that permitted independent assessment of memory for text or melody. A significant deficit was obtained after left temporal excision in the recognition of both the text and the melody of songs. By contrast, the performance of subjects with right temporal lobectomy did not differ significantly from that of normal control subjects. The results suggest that the left temporal lobe is involved in the recognition of verbal as well as musical components of songs. Preliminary indications are that excision of Heschl's gyri in the left side exacerbates the deficit while hippocampal resection does not.

90.5

PROCEDURAL MEMORY IMPAIRMENT IN SCHIZOPHRENICS. P.B. Vrtunski, D.M. Simpson*, K.M. Weiss* and H.Y. Meltzer. VA Med. Center and CWRU Sch of Med., Brecksville OH 44141.

Groups of normal and schizophrenic subjects were studied in performance with two choice reaction time tasks with visual (pictures) and auditory (spoken words) high (HF) and low (LF) frequency stimuli. The aim was to establish whether the repetition of stimuli facilitates recognition in schizophrenics in the same manner as it does in control subjects and whether the repetition effect is independent of the stimulus material used. Stimuli were presented once or three times. The principal findings were that a) with both stimulus conditions schizophrenic subjects were significanly impaired; b) The stimulus repetition effect was not observed with pictorial stimuli, but was observed with word stimuli (LF words always having longer latencies); and c) There were significant group differences in latency reduction due to word repetition: whereas the controls showed large decrement in reaction time mostly at second stimulus presentation, response latency of the schizophrenic subjects followed this pattern only with LF words. With HF words no latency reduction was seen until the third presentation. The conclusion reached was that in procedural memory, as seen with high frequency words, stimulus encoding in schizophrenics at first presentation may be only partially executed.

90.6

NORMAL MCCOLLOUGH EFFECT IN ALZHEIMER'S DISEASE AND GLOBAL AMNESIA. R.L.Savoy* and J.D.E.Gabrieli (SPON: S. Kosslyn). Rowland Institute for Science, Cambridge, MA 02142 and Dept. of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139.

The McCollough Effect (ME), a long-lasting pattern-contingent color aftereffect in normal human vision, has often been offered as a candidate for a simple form of human learning and memory. Independent of the as yet unknown mechanisms underlying the ME, it seemed worthwhile to examine the effect in persons with known deficits in memory.

After 5 minutes of staring at a bright ME adapting pattern, normal subjects have measurable color aftereffects at least 1 hour later. We induced such effects in 7 subjects: 2 patients with mild Alzheimer's Disease, H.M. (a patient with global amnesia due to bilateral medial temporal lobectomy who has been studied for the 35 years since his operation), and 4 age-matched control subjects. H.M. and the Alzheimer's patients showed MEs of strength and duration comparable to those of the control subjects.

These results demonstrate an intact visual aftereffect in H.M. and in patients with Alzheimer's disease. Further, the results extend the domain of intact aftereffects in amnesia to a particularly long-lasting effect.

Supported by grants MH24433, AG06605, P50OAG5134, RR00088, and the Rowland Institute.

90.7

EFFECTS OF OPERANTLY CONDITIONING CORTICAL SOMATOSENSORY EVOKED POTENTIAL (SEP) AMPLITUDE ON THE BLINK REFLEX. R. Dowman. Department of Surgery, University of Alberta, Edmonton AB., Canada T6G 2B7.

Stimulation of the median nerve evokes cortical potentials (SEP) and a blink reflex. The present work investigated the effects of operantly conditioned changes in SEP component P100 amplitude on the blink reflex in humans.

SEPs were evoked in 8 subjects by stimulation of the median nerve and recorded from over the contralateral somatosensory (SI) and frontal cortices, each referenced to the contralateral earlobe. The blink reflex was recorded from above and below the contralateral eye. The SEP conditioning procedure was comprised of baseline, SEP training, and reversal training modes. During the SEP training mode subjects were rewarded (money) when SI cortex P100 amplitude was larger than (uptrain) or smaller than (downtrain) its baseline mode mean. During the reversal training mode the reward contingency was reversed: uptrainers became downtrainers and vice versa. Each training mode lasted 5-10 sessions (1 session/day).

Four of the 8 subjects successfully changed SI cortex P100 amplitude in the direction of training. There were no changes in frontal cortex P100 amplitude. The blink reflex in these subjects was larger during uptraining than downtraining. The small blink reflex relative to P100 and the absence of change in frontal cortex P100 during conditioning suggests that the SI cortex P100 component is not generated by muscle potentials associated with the blink reflex. These results also suggest that P100 conditioning alters descending cortical modulation of the brainstem blink reflex pathways.

Supported by Alberta Heritage Foundation for Medical Research and Alberta Mental Health Advisory Council.

90.8

EFFECTS OF FOCAL FOREBRAIN INJURY ON FEATURE-BASED VISUAL SEARCH. R. B. Bolster and P. Birk, Dept. of Psychiatry, Fac. of Med., Univ. of Manitoba. Winnipeg, Man. R3E OW3

Visual selective attention in neurosurgical patients was assessed using a computer-based search task modelled after Treisman (1977). Patients with focal forebrain damage were compared to age-matched nonpatient controls on the ability to locate and identify colored-form targets in the presence of nontarget distractor items. The target was a green square, presented for 50 msec amid a 3 x 3 array of colored forms. Two array types were used, a "feature disjunction" array, with red square distractors, and a "feature conjunction" array with red diamond, red square, and green diamond distractors. Patients with dorsolateral frontal damage were selectively impaired on conjunctive arrays, making more errors to distractors sharing one feature with the target. Patients with orbital frontal injury were unimpaired. Patients with right, but not left, temporal lobectomy showed a pattern similar to the dorsolateral frontal group. Parietal patients were also impaired in comparison to normals, but errors were related to the position of the target rather than the features of the distractor items.

90.9

DYSCALCULIA IN PATIENTS WITH VERTIGO. J. Risey* & W. Briner Dpt of Otolaryngology, Tulane Univ. School of Medicine, New Orleans, La. 70112.

Vestibular disturbances have been associated with learning disabilities and other CNS disorders but not with specific disorders of cognition. One author (JR) has noted discrete and nearly identical errors in counting (dyscalculia) by approximately 20% of patients seen for ENG Tests. Patients skipped and displaced decades when asked to count backwards by twos.

8 patients with vertigo and dyscalculia were given the math subtests of the WAIS, counting tasks, visual error recognition tasks, ENG and audiometric exams including Neuroaudiological Tests. This same workup was given to 16 patients without dyscalculia (8 with and 8 without vertigo); all groups were matched for educational level and conventional audiometric results. The patients with dyscalculia exhibited poorer performance than patients from the other groups on the arithmetic and digit span portions of the WAIS and on the Neuroaudiological Tests.

This phenomenon appears not to be an isolated arithmetic anomaly but a dysfunction reflected in other arithmetic abilities. It appears to involve more than just the auditory/speech function; it also involves visual recognition of numerical sequences. The results of the Neuroaudiological tests suggest that the area of dysfunction is not the primary auditory cortex but may be the result of auditory association area dysfunction. Dyscalculia appears to be a common event among adults complaining of vertigo.

90.10

IMPAIRED PROBLEM SOLVING IN PARKINSON'S DISEASE (PD): INFLUENCE OF SET-SHIFTING DEFICIT. A. Cronin-Golomb, S. Corkin, and J.H. Growdon. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, and Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

PD often is associated with specific cognitive deficits in the absence of dementia, including the inability to suppress inappropriate learned responses. We assessed problem solving ability in 9 nondemented, never medicated subjects with PD ($\bar{X}$ age 63.8, education 14.9 years) and 7 matched healthy control subjects (HCS). The 10 problems required subjects to deduce a 'poisoned food' after study of lists of 3-food meals and their outcomes. Information provided by a single outcome was positive (the diner 'died') or negative ('lived'). All information (meals, outcomes, subject's deduction after each meal) was available at all times. PD subjects' performance was worse than HCS' (PD: $\bar{X}$ 7.4, SD 1.3; HCS: $\bar{X}$ 9.0, SD 1.0; $p<.05$). Most PD errors occurred on negative meals immediately following correct assessment of positive meals. Problems consisting exclusively of negative meals were performed correctly. The results suggest that subjects with PD can develop a learning set within a problem, even after a single trial, and are unable to suppress it on subsequent trials when that set leads to incorrect responses. This dysfunction must be considered when assessing problem solving and other multicomponential cognitive abilities in PD.

90.11

EFFECT OF TESTOSTERONE OR NANDROLONE ON SELF-ESTIMATES OF PERFORMANCE. A.C. Zold*, C.J. Hannan, Jr., K.E. Friedl* and S.R. Plymate* (SPON: R. Aronstam). Clinical Investigation Dept. and Psychology Service, Madigan Army Medical Ctr, Tacoma, WA 98431.

In order to quantify psychiatric changes reported with anabolic steroid use, a battery of psychological tests were administered to male subjects before and after a six week regimen (dosed/wk) of testosterone enanthate (Te) or Nandrolone decanoate (Nan). The standard psychomotor test of peg board rate was supplemented with a measure of the subjects ability to estimate their own performance. At trial 1 subjects were informed of the normal range of response for the task before giving their self-estimate. At trial 2 the subject knew his time for completion of trial 1 before giving his self-estimate. Results in the table are mean changes in seconds ± SE.

Estimate of Peg Board	Hand*	100mg Te n=6	300mg Te n=5	100ng Nan n=6	300ng Nan n=7
Δ Trial 1	D	1.8±5.3	-4.2±4.3	-6.7±3.2	1.9±3.2
(post-pre)	N	7.8±2.2†	2.2±2.9	10.5±8.2	16.4±6.6†
Δ Trial 2	D	3.3±3.5	2.8±2.2	-0.3±2.9	2.9±2.4
(post-pre)	N	4.0±2.4	-0.6±3.4	4.3±3.5	7.0±3.1

* D = dominant, N = nondominant † $p<0.05$ by Wilcoxon

Changes in self-estimates of performance indicate an increase or more optimistic prediction in two of the treatment groups after steroid administration.

90.12

FACES AS RELEASERS OF CONTAGIOUS YAWNING: AN APPROACH TO THE NEURAL BASIS OF FACE PERCEPTION. R. R. Provine. Dept. of Psychology, Univ. of Maryland Baltimore County, Baltimore, MD 21228.

The contagiousness of yawns is a non-invasive means of assaying the yawn-evoking potency of various facial features. This is an investigation of face perception and a search for the ethological "releasing stimulus" that triggers the "fixed action pattern" of yawning. The yawn-evoking potency of variations on a 5 min. series of 30 videotaped repetitions of a yawning face were compared with each other and a series of 30 smiles. The yawn variations were: 1) axial orientation, 0°, 90°, 180°, 270°; 2) still image; 3) high contrast; 4) no mouth; 5) no eyes; 6) mouth only; 7) eyes only; and 8) no facial features.

Animate video images of yawning faces in all axial orientations evoked yawns in more subjects than featureless or smiling faces, and no single feature such as the gaping mouth was necessary to evoke yawns. The yawn recognition process was maximally activated by the overall configuration of the yawning face regardless of its axial orientation. A similar lack of feature and axial specificity in many face-specific neurons in the brains of monkeys suggests a related stimulus analysis. These results about yawning and face-specific neurons join those from prosopagnosia (face non-recognition) in brain damaged humans and perhaps facial "imitation" in human neonates, which suggest that special neural mechanisms detect and process information about faces.

90.13

FACIAL EXPRESSION AND THE NEOCORTEX. B. Kolb and L. Taylor*, Univ. of Lethbridge, Lethbridge, Canada, T1K 3M4, and Montreal Neurol. Inst., Montreal, Canada, H3A 2B4

This study examined the relative roles of the left and right frontal and temporal lobes in the production and perception of facial expression. Subjects (Ss) were asked to produce an expression appropriate for a faceless character in real life cartoon drawings, and to mimic the emotional expression portrayed in a series of photos. Subsequently Ss chose the "best match" expression for the faceless character from a series of 6 key photos of people depicting different basic expressions, and also matched the series of photos with the 6 keys. The left frontal-lobe group produced significantly less intense expression on both production tests than any other group. Right temporal Ss performed poorly on both perception tests whereas the left-temporal Ss were normal on the face-matching task. Frontal lobe Ss were also impaired at both tests. Thus, (1) left frontal lobe lesions impair both the production and perception of facial expression, (2) right temporal and right frontal lesions impair the perception of facial expression, and (3) left temporal lesions only affect the perception of emotional expression in context.

90.14

KNOWING WITHOUT LEARNING? SEMANTIC CATEGORIZATION BY THE DISCONNECTED RIGHT HEMISPHERE OF INFORMATION KNOWN ONLY BY THE LEFT HEMISPHERE. J. Sergent, Montreal Neurological Institute, McGill University, Montreal (Quebec) H3A 2B4, Canada.

One of the most influential findings in the study of commissurotomized patients concerns the capacity of the right hemisphere to subserve verbal functions such as language comprehension and lexical decision. This finding is of critical importance for the formulation of models of hemispheric specialization, reading, and recovery from aphasia and suggest a specific contribution of the right hemisphere to verbal processes. However, recent evidence of subcortically mediated interhemispheric transfer in commissurotomized patients makes it conceivable that a right-hemisphere correct lexical decision does not indicate that the required information is represented in this hemisphere. Such a possibility was examined in two commissurotomized patients (L.B. & N.G.), using familiar and unfamiliar faces as stimuli. Two preliminary experiments aimed at establishing the nature of the information that can be transferred between the hemispheres, and showed that information about facial identity was not exchanged while information about sex, age, emotion and professional category, was. In the third experiment, the professional category of each unfamiliar face was initially taught to the patients by presenting the faces in the right visual field, such that only the left hemisphere learned semantic information about the faces. A reaction-time categorization task was then conducted on these faces appearing either in the right or the left visual field. Responses were faster and more accurate to right than to left presentations, but they were nonetheless significantly better than chance in the left visual field, even though the right hemisphere had not learned the appropriate association. The results suggest that right-hemisphere responses may not necessarily reflect knowledge and competence of this hemisphere and offer an alternative account of the reported right-hemisphere capacity to perform verbal semantic categorizations and lexical decisions.

90.15

EVIDENCE FOR MISDIRECTED REACHING TO 'PLACES' BUT NOT 'TARGETS' IN PATIENTS WITH RIGHT-HEMISPHERE LESIONS. A. D. Milner* and M. A. Goodale. University of Western Ontario, London, Ontario, Canada. N6A 5C2.

Patients with lesions of the right hemisphere were tested on two visually guided reaching tasks in which the kinematics of their limb movements were recorded optoelectronically (WATSMART). In the first task (Targets), they were asked to reach out and put the index finger of their right hand as quickly and as accurately as possible on a small target that was presented randomly in one of five locations between 20 cm left and right of their body axis. In the second task (Places), two targets were presented 18 cm apart and the subjects were asked to put their index finger on the point midway between the two targets. The spatial locations of the required endpoints in the two tasks were identical. Despite the fact that the patients showed little clinical evidence of visual neglect, they showed a consistent rightward bias in the terminal position of their finger in the Places task as compared to the Targets task. This bias was not present in control subjects. However, the initial heading directions of the reaching movements made by the patients were equivalent in the two tasks and tended to deviate to the right side as compared to reaches made by control subjects. These results suggest that 'recovery' from visual neglect may, in fact, reflect the use of compensatory adjustments to the trajectory of reaching movements as they unfold and that these adjustments are more easily made for reaches to discrete targets than for reaches to places defined by more distal cues.

90.16

HAND PREFERENCE SHIFTS AND SEX DIFFERENCES IN THROWING AND INTERCEPTING. N. V. Watson and D. Kimura. University of Western Ontario, London, Canada N6A 5C2.

In humans, the organization of movements of both upper limbs relies significantly on a left-hemisphere "praxis" system. To the extent that right hand preference reflects this left-hemisphere dependence, a task that maximizes spatial complexity of a target (presumably engaging right hemisphere systems), and minimizes motor programming demands, might yield a diminished disparity between the two hands.

Left and right hand abilities were measured independently for two motor tasks in 48 normal right-handed adults: 1) throwing a dart at a stationary target, 2) using the open hand to block a projectile at varying trajectories and velocities. ANOVA yielded a significant task by hand interaction (F = 17.5, $p < .001$). Left hand performance didn't differ from the right hand for the intercepting task (t = .81, p = .42), but was significantly worse for throwing (t = 5.93, $p < .001$). A sex difference favouring males was evident for both tasks ($p < .001$ for each), which was not reducible to differences in physique or athleticism.

Thus, right-handedness was abolished in the spatially demanding interception task but not in the motorically demanding throwing task. This suggests that hand preference in humans is dynamic, varying with task demands and with the degree of left/right hemisphere processing involved in a given task.

90.17

HUMAN OLFACTORY DISCRIMINATION AFTER FOCAL CEREBRAL EXCISION: MONORHINAL DEFICITS? R.J. Zatorre and M. Jones-Gotman* (SPON: H. Jasper). Montreal Neurol. Inst., Montreal, Canada H3A 2B4.

Olfactory discrimination was studied in 96 patients with unilateral cerebral excision and in 20 normal control subjects. Patients were grouped according to side and site of lesion in the temporal (N=62), frontal (N=26), or parietal (N=8) lobe. Detection thresholds for n-butyl alcohol did not differ across subject groups or across nostrils (Jones-Gotman & Zatorre, Neuropsychologia 26:387-400, 1988), ruling out primary olfactory sensory loss. The discrimination task involved monorhinal presentation of unfamiliar odorants in pairs, which subjects judged as same or different in quality. The results showed: (1) severe impairment after frontal-lobe lesions, with a generally greater deficit on the nostril ipsilateral to the lesion. We interpret this as reflecting the importance of orbitofrontal cortex in olfactory processing. (2) A significant but milder deficit, confined to the ipsilateral nostril, was obtained after temporal-lobe lesions. (3) Normal discrimination was observed after parietal-lobe lesions. (4) Normal subjects performed significantly better with the right nostril, raising the question of hemispheric asymmetry for odor processing.

90.18

COLOR VISION DEFICITS IN ALZHEIMER'S DISEASE (AD). J. Cohen*, A. Cronin-Golomb, J.H. Growdon, and S. Corkin (SPON: B. Vermeire). Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114, and Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

We administered the City University Colour Vision Test to 36 AD patients and 11 age-matched healthy control subjects (HCS). The test consists of 10 plates, each with a target circle of a particular hue surrounded by four circles each of a different hue. Subjects point to the outer circle closest in hue to the target. The AD group made more errors overall than did the HCS group ($p<.05$), and made more tritan (blue-yellow) than protan (red) ($p<.05$) or deutan (green) ($p<.05$) errors. We observed further that a number of AD patients showed marked difficulty in discriminating between blue and green items on the Stroop Test, which requires naming of red, green, and blue stimuli. Of 67 AD patients, 15% made blue-green errors, compared with 6% of the 36 age-matched HCS.

Further studies will be necessary to identify the lesion(s) accounting for color deficits. Damage to extrastriate cortex (area 18) has been associated with dyschromatopsia in humans and in monkeys; this region is often affected in AD. Alternatively, impaired color vision could result from lesions of the retina or optic nerve, which also may be damaged in AD. Our behavioral findings prompt caution in interpreting results from the Stroop Test and other cognitive measures that rely upon preserved color vision.

90.19

ATTENTION ENHANCES TACTILE PATTERN IDENTIFICATION.
K. C. Whang*, H. Burton, G. L. Shulman. Depts. of Anat. and Neurobiol., Neurol. and Neurol. Surg., Washington Univ. Sch. of Med., St. Louis, MO 63110.

Discriminations between patterns of vibration demonstrate attentional effects in touch. Normal human subjects detected patterns of amplitude changes in 250 Hz vibrations applied to the index and middle fingertips of both hands. They were required to determine in which of two choice intervals a target pattern occurred.

During each interval, each finger received two pulses of increased or decreased amplitude from a fixed base level. Within an interval, the 200-ms. pulses could begin at any of four start times spaced apart by 600 ms. each. The target pattern, presented to one of the four fingers during one of the two choice intervals, was an increase followed in time by a decrease. The other pulse patterns presented were all distractors. A tactile cue in each interval indicated the finger at which a target could appear with 80% validity.

Subjects performed significantly better when the target appeared at the cued finger. The results show that spatial attention can improve the accuracy with which tactile temporal patterns can be identified. In other tasks where the target was a single pulse instead of a pattern, no improvements in performance were found with cueing. The single-pulse targets were defined by their amplitudes and durations, and were more difficult to detect than the pulses that constituted the pattern elements.

A spatial pattern task analagous to the temporal pattern task is being investigated. Subjects are cued to the time at which the target pattern, a simultaneous increase to the left of a decrease, is likely to occur.

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90.20

INFLUENCE OF SENSORY AND MOTOR ATTENTION ON PRECORTICAL RESPONSES TO VISUAL STIMULATION IN HUMANS. R. G. Eason, Marta T. Oakley, and J. M. Harding.* UNC-Greensboro; Los Alamos Nat. Lab.; & Marian Area Couns. Ctr., Inc., Ohio.

We have been investigating the hypothesis that when one selectively attends to a location in space where a task relevant stimulus is expected to appear (relevant location), subcortical neural pathways activated by stimuli appearing at that location are biased by centrifugal influences of cortical origin such that information is preferentially transmitted over them (NS Abstr's., 1985, 1986, 1987).

Using the paradigm of Eason, Harter, & White (Phy. & Beh., 1969), we have new data indicating that: (1) the ERG b-wave is significantly enhanced, as noted in earlier studies, when sufficiently salient evoking stimuli are presented at the relevant (attended) location compared to when that same stimulus is irrelevant; (2) frontally and parietally recorded short-latency VER-deflections (30-40 msec onset) evoked by stimuli too weak to elicit a measurable ERG tend to be more negative for relevant than for irrelevant locations; and (3) the magnitude and polarity of the relevancy effect is greater and more negative when subjects are set to make a finger lift or saccade response to target stimuli than when silently counting the stimuli.

These studies provide further evidence that spatial selective attention can alter sensory transmission precortically at a number of subcortical levels, and that such alteration is dependent on the saliency (size) of the stimulus, and the type of response the subject is set to make.

BEHAVIORAL PHARMACOLOGY: PSYCHOSTIMULANTS

91.1

EFFECT OF CAFFEINE ON SOCIAL BEHAVIOR, EXPLORATION AND MOTOR ACTIVITY: INTERACTION WITH ETHANOL. L.A. Hilakivi, M.J. Durcan and R.G. Lister. Lab. Clin. Stud., NIAAA, DICBR, 9000 Rockville Pike, Bethesda, MD 20892, USA.

The present study examined caffeine's effects in NIH Swiss mice in a test of social behavior and in a holeboard test of exploration and locomotor activity. The interaction of caffeine with ethanol was also investigated. In experiment 1, singly-housed mice were injected i.p. with 0, 15, 30 or 60 mg/kg caffeine and put in pairs into a familiar arena for 7.5 min during which their social behavior was studied. Moreover, group-housed mice were individually tested for 8 min in a holeboard apparatus, with which they were unfamiliar, following similar treatments. Caffeine did not significantly affect the time spent in social interaction (sniffing, following). Motor activity in the social behavior test was increased by 60 mg/kg caffeine ($p<.05$). Caffeine had a biphasic effect on the time spent in aggressive behavior (tail rattling, biting, fighting): 15 mg/kg significantly increased aggression ($p<.02$) and 60 mg/kg decreased it ($p<.03$). The time spent in avoidance-irritability behavior (withdrawing, shrieking, startling) was dose-dependently reduced ($p<.001$). In the holeboard, a dose-related increase in locomotor activity by caffeine was seen ($p<.05$), but there were no significant changes in the time spent head-dipping. In experiment 2, caffeine was given in combination with 0 or 2.0 g/kg ethanol prior to the social behavior and holeboard tests. 30 mg/kg caffeine partly reversed the ethanol-induced reduction in social interaction ($p<.05$), and 60 mg/kg caffeine reversed the ethanol induced increase in locomotor activity, both in the social behavior test ($p<.01$) and in the holeboard test ($p<.01$). Since only the highest dose of caffeine reversed the locomotor stimulant effect of ethanol, this antagonism may be mediated by a non-adenosinergic mechanism.

91.2

AN ANALYSIS OF THE STIMULANT AND HALLUCINOGENIC PROPERTIES OF (+) OR (-) 3,4-METHYLENEDIOXYAMPHETAMINE (MDA). J. Broadbent and J. B. Appel. *Behavioral Pharmacology Lab., Dept. Psychology, University of South Carolina, Columbia, SC 29208 USA.*

Drug discrimination studies (in animals) suggest that one reason for the continuing abuse of MDA may be the similarity of the subjective effects of the (+) and (-) isomers of this amphetamine derivative to those of stimulant and hallucinogenic compounds, respectively. This possibility was examined further in two groups of rats trained to discriminate (+) or (-) MDA (1.25 mg/kg) from saline (N=16/group). The notion that the different isomers of MDA have different stimulus properties was supported to some extent by findings that (+) MDA partially generalizes to both *d*-amphetamine and cocaine while (-) MDA may generalize to mescaline and *d*-LSD. However, the two enantiomers were also found to cross generalize; this suggests that the stimulus properties of MDA must be more complex than previously indicated and that further analysis of the substitution of hallucinogens for (+) MDA and stimulants for (-) MDA is needed.

91.3

DEVELOPMENTAL CHANGES IN OPIATE ANALGESIA IN DEER MICE: SEX AND POPULATION DIFFERENCES. D. G. Innes and M. Kavaliers (SPON: F. S. Tepperman). Div. Oral Biology, Univ. Western Ontario, London, Ontario, Canada.

There is evidence for developmental changes in nociception and opiate-induced analgesia. It has also been shown that there are sex and population differences in nociceptive sensitivity and analgesia. Whether or not sex and population differences exist during the development of opiate analgesia is, however, not clear. Accordingly, in the present study we describe developmental changes in nociception and opiate-induced analgesia in two different populations of male and female deer mice: Peromyscus maniculatus artemisiae from a mainland region and P. m. angustus from small coastal islands. Throughout the course of development (10- 30 days of age) males displayed significantly greater nociceptive responses and levels of mu (morphine) and kappa (U-50,488) opiate-induced analgesia than females. In both sexes there were similar significant differences between the rates of development of mu and kappa opiate analgesia. In addition, throughout the course of development the male and female insular mice displayed significantly greater morphine (mu) and significantly lower U-50,488 (kappa) induced analgesia than the mainland animals. In all cases, maximum sex differences in analgesia were evident at weaning (18- 20 days of age) before sexual maturity was attained.

91.4

ANALGESIC SUBSTITUTED AMPHETAMINES. J. M. Beaton, F. Benington*, R. D. Morin*, M. A. Khaled* and J. A. Monti. Neuropsychiatry Research Program and Department of Psychiatry, University Station, Birmingham, Alabama 35294, U.S.A.

We have previously shown that two amphetamine analogues, the alpha-methyl and alpha-ethyl derivatives of methylenedioxymethamphetamine, have potent analgesic properties. We believe that the analgesic action of these agents is due in part to their substituents in the para position. Therefore, a series of amphetamine analogues, substituted in the para position were synthesized and tested for their ability to induce analgesia in mice. The compounds tested were 4-hydroxy-amphetamine (4-HO-AMP), 4-methoxy-AMP (4-MeO-AMP), 4-HO-2,6-dimethyl-AMP, 4-MeO-2,6-dimethyl-AMP and the N-methyl derivatives of four compounds. Analgesia was measured with a tail-flick apparatus. Dose response curves were obtained using groups of five mice for each dose. The doses ranged from 0.75 to 24 μ mol/kg. The animals were tested at 30 min intervals, beginning 30 min after intraperitoneal administration of the compounds in a volume of 0.1 ml/10g. Testing continued until each animal's response time had returned to preinjection control levels. All compounds showed analgesia. In general, the N-methylated compounds were less potent than the nonmethylated equivalent compounds. It is hypothesized that the mode of action of the analgesia is via the mu opiate receptor.
(Supported in part by the Alabama Consumer Research Fund).

91.5

EFFECTS OF INTRACEREBROVENTRICULAR (ICV) INJECTIONS OF MORPHINE IN THE HAMSTER. P. Schnur, C. Archuletta* and R. Martinez*. Department of Psychology, University of Southern Colorado, Pueblo, CO 81001.

Subcutaneous (SC) injections of morphine in hamsters produce hyperactivity at low doses and hypoactivity at high doses. Across a range of doses, hypoactivity is followed by recovery and then by hyperactivity. Both effects are naloxone reversible. The present work studied the effects of ICV administration of morphine on locomotor activity in the hamster. In Experiment 1, six doses of morphine were tested (0, 0.5, 2.5, 5, 25 & 50 ug) in a 3 hr test session. Results indicated that the three lowest doses of morphine elicited transient hyperactivity. The higher doses elicited dose-related hypoactivity but no hyperactivity. In Experiment 2, ICV morphine (0, 30 ug) and naloxone (0,1,3,10,30 ug) were administered. Morphine produced hypoactivity but no hyperactivity. Naloxone antagonized morphine-elicited hypoactivity in a dose-dependent manner. Low doses of naloxone (1 & 3 ug) blocked morphine-elicited hypoactivity, though incompletely. High doses of naloxone (10 & 30 ug) not only blocked morphine-elicited hypoactivity but produced hyperactivity in its place. Results are compared to those following SC injections of morphine and naloxone.

Supported by NIH/MBRS Grant #RR-08197-07

91.6

AMPHETAMINE INJECTED REPEATEDLY INTO THE VTA OR THE N. ACCUMBENS DOES NOT PRODUCE ENVIRONMENT-SPECIFIC SENSITIZATION OR CONDITIONING OF ITS LOCOMOTOR ACTIVATING EFFECTS. P. Vezina and J. Stewart. C.S.B.N., Dept. of Psychology, Concordia University, Montreal, Canada, H3G 1M8.

Recently, it was shown that repeated injections of amphetamine into the ventral tegmental area (VTA) but not the n. accumbens (NAC) produce a sensitized locomotor response to a subsequent systemic injection of amphetamine or morphine (Kalivas and Weber, Neurosci. Abst.,13: 600, 1987). In the present experiments, the role of amphetamine action in these two sites in the development of environment-specific sensitization and conditioning of the locomotor activating effects of amphetamine was investigated. During training, groups of rats received bilateral injections of amphetamine (2.5 µg/0.5µl saline/side) either into the VTA or the NAC prior to being placed in activity boxes and saline (0.5 µl/side) in their home cages (COND, conditioning groups), saline in the activity boxes and amphetamine in their home cages (PSEUDO, pseudoconditioning groups), or saline in both environments (Control groups). On a subsequent test with morphine (1.0 mg/kg, i.p.), only rats that had received intra-VTA amphetamine showed activity levels that were higher than those of Control animals, replicating the above findings. Further, this sensitized response was evident in both the intra-VTA COND and PSEUDO groups, suggesting that amphetamine action in the VTA is not sufficient to produce environment-specific sensitization. On a separate test with saline, no evidence for conditioned activity was found in either the intra-VTA or the intra-NAC groups. These findings, together with others demonstrating that conditioned activity is produced by intra-VTA morphine, suggest that the action of released DA at other mesocorticolimbic DA neuron terminals may be necessary to produce conditioning.

91.7

EXTINCTION TRAINING DEMONSTRATES THAT DIFFERENT MECHANISMS UNDERLIE THE STIMULUS CONTROL OF THE EXPRESSION OF ENVIRONMENT-SPECIFIC BEHAVIORAL SENSITIZATION PRODUCED BY MORPHINE OR AMPHETAMINE. J. Stewart and P. Vezina. Center for Studies in Behavioral Neurobiology, Dept. of Psychology, Concordia University, Montreal, Canada, H3G 1M8.

The effect of extinction training on previously established environment-specific sensitization of the locomotor activating effects of either d-amphetamine sulfate (1.0 mg/kg) or morphine sulfate (10.0 mk/kg) was compared in two experiments designed to investigate the relation between sensitization and conditioning. During training, groups of animals were administered drug, i.p., prior to being placed in activity boxes, and saline in their home cages (COND, conditioning, groups), drug in their home cages, and saline in the activity boxes (PSEUDO, pseudoconditioning groups), or saline in both environments (Control groups). Evidence for conditioning and environment-specific sensitization was found in both experiments in tests given following training during which all animals were administered saline or drug, respectively. By the last trial of extinction training, during which all animals had received only saline injections and were repeatedly tested in the activity boxes, no behavioral differences between groups were evident. In a final test for environment-specific sensitization given after extinction, it was found that, in morphine-treated animals, behavioral sensitization was still evident in the COND group, but that the PSEUDO group was not different from the Control group, a finding that suggests that the drug reinstated the excitatory control of sensitization by the environmental stimuli. In amphetamine-treated animals, however, behavioral sensitization was evident in both COND and PSEUDO groups following extinction, suggesting that, prior to extinction, the expression of sensitization in the PSEUDO group was under inhibitory control.

91.8

ASSESSING COMPLEXITY OF RAT LOCOMOTOR BEHAVIOR USING INVARIANT MEASURES FROM ERGODIC THEORY. M.P. Paulus*, M.A. Geyer, L.H. Gold and A.J. Mandell. Laboratory of Biological Dynamics & Theoretical Medicine. UCSD Sch. of Med., La Jolla, CA 92093.

The study of complex systems suggests that the understanding of complicated behavior may be facilitated by calculating measures that characterize the amount of information produced per time unit. Ergodic theory provides measures that are invariant under coordinate transformations, e.g. metric and topological entropy, fractal dimensions, and characteristic exponents. In an experimental setting they can be estimated using symbolic dynamics, which transforms a trajectory of a discrete time series into symbol sequences through a "meaningful" partition that can be fixed by the experimenter or generated dynamically according to the result of the recorded trajectory using a "kd-tree". The dynamics are characterized by a shift operating on the sequences, which can be represented as a topological Markov chain. The (largest) Perron Frobenius eigenvalue, spectral decomposition, and the number of periodic orbits approximated by an appropriate zeta function yield information about the underlying dynamical process. Groups of animals that were treated with stimulant drugs (Amphetamine, MDMA, Apomorphine, Scopolamine) and exhibited comparable increases in locomotor activity (photobeam breaks) were analyzed using an individual dynamical partition of the 4-dimensional x-y-t-behavior space. The resultant symbol sequences were analyzed to obtain their mutual information content, the metric and topological entropy. The results indicate that ergodic measures reveal differences in the nature of the hyperactivity elicited by the various drugs studied and emphasizes the value of these techniques to characterize trajectories generated by complex time series.

91.9

MULTIVARIATE BEHAVIORAL ANALYSIS OF AMPHETAMINE (AMPH) CONDITIONED LOCOMOTION. L.H. Gold, G.F. Koob and M.A. Geyer. Research Institute of Scripps Clinic and UCSD Dept. of Psychiatry, La Jolla, CA 92037.

Conditioned locomotion in rats was examined with a computerized Behavioral Pattern Monitor (BPM), a system which provides detailed information regarding amount and qualitative patterning of locomotor activity and investigatory responses. Each BPM chamber consists of a 30.5 x 61 cm black Plexiglas holeboard with a 4 x 8 array of infrared beams to define the X-Y position of the rat and 3 floor holes, 7 wall holes and rearing touchplate to record various facets of exploratory behavior.

Rats were divided into 2 groups and injected SC with saline or 0.75 mg/kg AMPH immediately prior to placement in the BPM chambers for a 2h experimental session. A small dish of peanut butter was placed underneath each chamber to enhance the saliency of the testing environment. Following each conditioning session, rats were returned to their home cages and injected with the alternate solution. Conditioning training lasted 5 days and on the 6th day all rats were injected with saline prior to placement in the BPM. Rats who had been previously injected with AMPH in the BPM exhibited significant increases in horizontal locomotion, rearing and investigatory holepokes on the test day. X-Y plots and statistical measures of the rats' locomotor paths were used to further characterize the nature of the unconditioned (UCR) and conditioned (CR) responses. Such descriptive measures of spatial and temporal patterning of behavior may help to determine whether the increase in motor activity measured on the test day is a CR which resembles the UCR or rather a generalized excitement reflective of cued anticipation of a reinforcing drug.

91.10

PLACE CONDITIONING WITH d-AMPHETAMINE: EVIDENCE OF AN AVERSION. A-M. Wall, R.E. Hinson and A. Streather. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C2.

In Experiment 1 a dose-response study of place conditioning was conducted. Male, Sprague-Dawley rats receiving one of 0.0, 0.05, 0.1, 0.5, 2.0, 5.0, 7.5, or 10.0 mg/kg of d-amphetamine underwent ten four-day cycles of standard place conditioning. On alternate days, each rat was injected with its designated dose of d-amphetamine while confined to its originally non-preferred end of a three compartment, straight alley-box. On intervening days each rat was injected with saline while confined to its originally preferred compartment. Following each four-day cycle, a choice test was administered in which each rat was allowed 20 minutes access to the entire chamber. Contrary to previous research, no evidence of a preference was found. In fact, doses of amphetamine $\geq$ 0.5 mg/kg induced a significant avoidance of the end in which amphetamine had been administered. In Experiment 2, animals received one of 0.0, 0.5, 2.0 or 5.0 mg/kg of amphetamine and underwent place conditioning procedures identical to animals in Experiment 1. In contrast to Experiment 1, animals were given a single choice test following ten four-day place conditioning cycles. All groups that received amphetamine exhibited a conditioned place avoidance. The observed amphetamine-induced place avoidances may have been due to inhibitory conditioning.

91.11

THE EFFECTS OF ANTIHISTAMINES ON AMPHETAMINE-INDUCED STEREOTYPY. F.Jordan-Palylyk and Ann Robertson. Dept. of Psychology, Univ. of Alta., Edmonton, AB T6G 2M7

It has been suggested that histamine interacts with dopamine (DA) in controlling some behaviors. For example, the H_1 antagonist diphenhydramine reversed haloperidol-induced suppression of self-stimulation (Carey,R.,Pharm.Bio.Behav., 17:851,1982). The present study investigated the DA-histaminic interaction more directly by studying the effects of 2 H_1 antagonists, diphenhydramine HCl (DP) and tripelennamine HCl (TP) on amphetamine-induced (A-I) behaviors in 32 Sprague-Dawley male rats. Different groups of rats received DP (0.0, 1.6, 4.0 or 10.0 mg/kg, IP) or TP (0.0, 5.0 or 10.0 mg/kg IP). All rats were then injected with amphetamine (0.0, 0.5, 1.0, 2.5 and 5.0 mg/kg IP). Behavioral observations were taken for 1 hour. At 2.5 mg/kg amphetamine, stereotyped sniffing and head movements were increased 59% by 1.6 mg/kg DP, 112% by 4.0 mg/kg, and 160% by 10.0 mg/kg. Concomitantly, A-I rearing was dose-dependently decreased. DP alone did not alter behavior, nor did it alter other A-I behaviors. TP had no significant effect on any A-I behaviors. DP's facilitation of A-I stereotypy can be interpreted as evidence for a DA-histaminic interaction;however, the differential actions of DP and TP suggest that DP's effect on A-I stereotypy is not necessarily due to its antihistaminic properties. DP's actions may be due to moderate anticholinergic activity (Kubo et al,Jpn.J.Pharm., 43:277,1987),although DP does not alter scopolamine's behavioral effects (Sansone et al,Psychopharm.,93:155,1987).

SOMATOSENSORY CORTEX I

92.1

LOCAL CONNECTIONS IN MOUSE BARREL CORTEX. K.L. Bernardo*, J.S. McCasland, T.A. Woolsey and R.N. Strominger*. Dept. Neurosurg., Div. Exp. Neurol. & Neurosurg. and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

We targeted focal extracellular HRP injections into barrel cortex, in vitro, in order to demonstrate the distribution patterns of labeled cells projecting to the labeled site. The distribution of label in the tangentially sectioned hemispheres, with respect to the cortical barrel field, was mapped in one or two ways: 1). A Grinnell image processor with a video camera, connected to a Zeiss computer microscope equipped with a motorized stage, was used to reconstruct regional stain densities from tissue sections. 2). The x,y and z coordinates of individual cells in each section were determined with an Eutectics system.

Results have been analyzed for hemipheres in which the labeling site was confined to supragranular layers (i.e., layers II,III) within barrel cortex. The majority of labeled cells in all cortical layers are related to barrels in the same row as that which was injected. As compared to layer IV, labeled cells tend to be distributed more widely within the supra and infragranular layers. These trends hold true for injections confined to individual barrels, as well as for injections placed between barrels. We conclude that interbarrel connections are most numerous within the same barrel row. This "row tendency" is consistent with and a basis for functional organization of rodent somatosensory cortex.

92.2

BASIC RULES GOVERNING THE SYNAPTIC ORGANIZATION OF THE CEREBRAL CORTEX. E. L. White, A. Keller and E. El-Hanany*. Dept. of Morphology, Ben Gurion Univ. Sch. Med., Beer Sheva, Israel.

Investigations on the quantitative aspects of synapses between identified pre- and postsynaptic elements in mouse SmI cortex has provided the basis for the formulation of a set of rules governing cortical synaptic relationships: Rule 1: Every neuron within the target region of a projection receives input from the projection. Corollary to Rule 1: Axon terminals from any extrinsic or intrinsic source synapse onto every morphological or physiological neuronal type within their terminal projection field. In practice this means that a pathway will form synapses with every element in its target region capable of forming the type of synapse normally made by the pathway (i.e., asymmetrical or symmetrical). Rule 2: Different dendrites of a single neuron form similar synaptic patterns, i.e., the numbers, types, proportions and spatial distribution of synapses is similar, provided the dendrites are exposed to similar synaptic inputs. Corollary to Rule 2: Axonal pathways form similar synaptic patterns onto all the dendrites of a single neuron provided the dendrites occur within the target region of the axonal pathway. Rule 3: Neuronal types receive characteristic patterns of synaptic connections; the actual numbers, proportions and spatial distribution of the synapses formed by each neuronal type occur within a range of values. Corollary to Rule 3: Different extrinsic and intrinsic synaptic pathways form specific proportions of their synapses with different postsynaptic elements (spines vs. dendrites, one cell type vs. another). Comparisons with data from other studies suggest these rules can be used to predict synaptic relations in all areas of the cortex and in all species. Supported by NIH grant NS20149-05.

92.3

SOMATOTOPIC ORGANIZATION AND THALAMIC CONNECTIONS OF THE SECOND SOMATOSENSORY AREA, S-II, IN SQUIRREL MONKEYS. C.G. Cusick and M.M. Manning. Dept. of Anatomy, Tulane Univ., New Orleans, LA 70112.

The location and somatotopic organization of the second somatosensory area was studied using microelectrode mapping techniques in five squirrel monkeys anesthetized with ketamine hydrochloride. S-II was found on the upper bank of the lateral sulcus, and responded predominantly to low threshold stimuli on the contralateral body surface. Receptive fields were typically larger than in area 3b. For example, receptive fields on the hand sometimes included large areas of the palm and the entire glabrous surface of two or more fingers. Within the hand area in S-II, the palm was represented mainly caudally and the digits rostrally. Ulnar surfaces tended to be more deeply placed in the sulcus than radial surfaces. The representation of the teeth and tongue in S-II was rostral to that of the hand. The foot area in S-II adjoined the hand caudally, and was more deeply placed in the sulcus. A representation of the trunk and limbs was found deep to the hand and foot regions.

WGA-HRP was injected into physiologically identified hand and mouth representations in S-II. Labeled cells and terminations were identified in the ventroposterior inferior nucleus, and less dense in the ventroposterior nucleus proper, posterior nucleus, and anterior pulvinar. Supported by NIH Grant DE07695.

92.4

SPATIAL ORGANIZATION OF CORTICOTHALAMIC CELLS IN THE RAT SmI VIBRISSA/BARREL CORTEX. J. Chmielowska*, G.E. Carvell* and D.J. Simons. Depts. of Physiology and Physical Therapy, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Horseradish peroxidase (HRP) was used to examine the distribution of corticothalamic projection cells in the whisker representation of the SmI cortex. Under physiological control using double-barreled pipettes, HRP was iontophoretically deposited in the vibrissa area of the thalamic ventrobasal complex (VB) of normal adult rats. Labeling in the cortex had a columnar appearance. Dense patches of anterogradely labeled axons formed a barrel-like pattern in lower layer III and layer IV; supragranular labeling was densest and extended most superficially, to laminae I/II, over the center of the barrel. Retrogradely labeled neurons were observed in lower layer V and layer VI. Their density/area was $\sim$1.5X greater in the regions deep to the barrels than in the regions below the interbarrel septa. Extensive axonal ramifications were not observed in upper layer V, suggesting that if axon collaterals of corticothalamic cells were labeled, these terminated locally and/or superficially in the thalamic recipient zone. The vibrissal column thus appears to contain a core zone that is intimately associated with the thalamic barreloids. Supported by NIH NS 19950.

92.5

VELOCITY-DEPENDENT RESPONSE PROPERTIES OF NEURONS IN LAYERS II-IV OF THE CAT PRIMARY SOMATOSENSORY (SI) CORTEX. J.D. Greenspan. Dept. of Physiology and Dental Research Center, Univ. of North Carolina, Chapel Hill, NC 27514.

Many SI cortical neurons are responsive to surface-parallel movements across the skin's surface. Some of these movement sensitive units generate greater responses with certain parameters of stimulation than with others. In this study, single-unit recordings were made in the cat SI cortex, while cutaneous, brushing stimuli of various velocities and directions were presented.

Units recorded in layer IV gave regular, excitatory responses to brushing stimuli, while units recorded in layers II-III gave simple excitatory responses, excitatory responses followed by post-stimulus inhibition, or inhibitory responses. Units could be classified as rapid brush, slow brush, or very slow brush, based on the stimulus velocity that produced the greatest response. Rapid brush units, commonly encountered in layer IV, gave greater responses to stimuli ≥10cm/s, and little or no response to stimuli ≤1cm/s. Slow brush units, commonly encountered in layers II-III, gave reliable responses to stimuli 0.5-1.0cm/s, greater responses at 5-10cm/s, and little or no response to stimuli 50-100cm/s. Very slow brush units did not respond to stimuli ≥10cm/s, and gave greater responses to stimuli at 1cm/s than at 5cm/s. (Supported by NIH grant NS-20159).

92.6

PROJECTIONS FROM SIV AND ADJACENT CORTEX TO PRIMARY SENSORY AREAS IN THE CAT. D. Minciacchi*, A. Granato*, A. Antonini*, G. Tassinari* and L. Zanolli*. (SPON: European Brain and Behavior Society). Inst. of Neurology and Anatomy, Catholic Univ., Rome and Inst. of Physiology, Univ. of Verona, Italy.

We have previously described the lack of topographical organization in the visual and somatosensory representations of the cat's anterior ectosylvian sulcal cortex (AES) (Minciacchi, D. et al., Brain Res. 410: 21, 1987). The present study was aimed at analyzing the anatomical organization of AES efferents to primary sensory cortices. A multiple retrograde tracing technique was here employed. Four different fluorescent tracers were injected under electrophysiological control in four cortical regions: the face (SIfa) and forepaw (SIfp) representation fields of the first somatic sensory area, the vertical meridian (V1vm) and periphery (V1pe) representations of the first visual area.

SI projecting cells were observed throughout AES: SIfa neurons were more numerous in the rostral part of AES and SIfp neurons were preferentially located in its caudal part. In the rostral part of AES SIfa cells were segregated in the dorsal bank of the sulcus while SIfp cells were placed in the ventral bank. In the caudal part of AES labeled cells were observed almost exclusively in the dorsal bank of the sulcus in which SIfa and SIfp neurons were located respectively in its outer and inner part. Neurons projecting simultaneously to SIfa and SIfp by means of axonal branches were extremely rare. Scattered neurons were labeled from the V1 injections thus confirming the weakness of AES projections to V1.

The present evidence of a clear topography in the AES projections to SI is in contrast with the lack of topographical organization of both somatosensory and visual representations in this area. These data suggest that AES is a high order associative area in which the processing of multisensory informations requires a strict topographic interrelation with SI.

92.7

SII IN NEW WORLD MONKEYS DEPENDS UPON INPUTS FROM SI FOR ITS ACTIVATION. P.E. Garraghty, T.P. Pons, and J.H. Kaas. Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240.

Cortical somatosensory areas SI and SII do not have the same relationship to each other and the thalamus in all mammals. Most notably, these two cortical areas are independently activated by thalamic inputs in cats (Burton et al., Neurosci. Abstr., 13:75, 1987), but are arranged hierarchically with SII depending on SI for its activation in Old World monkeys (Pons et al., Science, 237:417, 1987). This difference in dependence on thalamic or cortical inputs of a presumably homologous area led us to study the relationship of SI and SII in two species of New World monkeys, marmosets and owl monkeys. During our experiments, we first mapped SII cortex, and then demarcated the extent of the representation of a particular body part (e.g., the hand) in area 3b and adjacent parts of areas 3a, 1, and 2), which we then lesioned by aspiration. We then immediately remapped SII. Since we had recorded the locations of recording sites from our first map on a photograph of the brain, we were able to relatively precisely reposition the electrode to sample the same cortex. We found that the part of SII which had initially been responsive to stimulation of the body surface whose representation was lesioned in SI was now silent. This effect was not due to a general depression of cortex since parts of SII cortex adjacent to the silent zone could be activated, as could parts of SI cortex within 100 μm of the lesion.(Supported by NS16446.)

92.8

THALAMUS-EVOKED SYNAPTIC RESPONSES OF INHIBITORY INTERNEURONS OF MOUSE SMI (BARREL) CORTEX IN VITRO A.Agmon and B.W.Connors, Neurology Dept., Stanford Univ. Sch. of Med., Stanford, CA 94305 and Brown Univ., Providence, RI 02912.

Using our previously described thalamocortical slice preparation (Soc. Neurosc. Abstr. 13:247, 1987) we have extended our survey of intrinsic firing properties and VB-evoked synaptic responses in mouse barrel cortex neurons. As described, responsive regular-spiking (pyramidal) neurons were encountered in all cellular layers and were usually strongly inhibited at a disynaptic latency. In about half of these cells the IPSP was preceded by a monosynaptic EPSP which was seldom strong enough to fire the cell. In contrast, fast-spiking neurons were encountered mainly in layers IV and the V/VI border, which are known to be the thalamocortical afferents' termination layers. Consistent with their position, they were ususally strongly excited at a monosynaptic latency and would fire upon VB stimulation when their membrane potential was held close to threshold. Fast-spiking neurons are believed to be GABAergic interneurons. We conclude that inhibitory interneurons are major recipients of thalamocortical input and serve an important role in sensory information processing in the neocortex.

This work was supported by NIMH training grant MH17047, NIH grant NS12151 and the Klingenstein Foundation.

92.9

CALLOSAL CONNECTIONS IN THE FORELIMB ZONE OF SII AND SIV IN CATS. P. Barbaresi, S. Bernardi, T. Manzoni. Institute. of Human Physiology, University of Ancona, Ancona (Italy).

HRP and microelectrode recording techniques were used to study in the cat the callosal connections in the forelimb zones of SII and SIV. Tracer was injected in electrophysiologically identified sites of SII or SIV body map. Subsequently, receptive fields of neurons in the homologous area of the contralateral side were mapped with microelectrodes. The topographical distribution of HRP labelled callosal neurons in relation to the body representation was studied on planar cortical maps reconstructed with a computer from TMB-processed serial sections cut from the anterior ectosylvian gyrus. Callosal neurons were present in the digit, hand and arm zones of SII but they were unevenly distributed: each zone contained randomly distributed sub-zones in which callosal neurons were either numerous or rare. In callosal and relatively acallosal sub-zones receptive fields were similar. In SIV callosal neurons were distributed homogeneously throughout the forelimb zone. Labelled neurons of heterotopical callosal afferents to SII and SIV were in SI and in a cortical region lying in the lower and upper banks of the anterior suprasylvian sulcus.

92.10

INTRACORTICAL MICROSTIMULATION IN SOMATOSENSORY CORTEX IN ADULT RATS AND OWL MONKEYS RESULTS IN A LARGE EXPANSION OF THE CORTICAL ZONE OF REPRESENTATION OF A SPECIFIC CORTICAL RECEPTIVE FIELD. G.H. RECANZONE* and M.M. MERZENICH, Coleman Laboratory, Departments of Physiology and Otolaryngology, Univ. of CA, San Francisco, S.F., CA 94143

Intracortical microstimulation (ICMS) has been commonly used in experiments defining movement representations in motor cortex. It has recently been demonstrated that ICMS can itself alter movement representations in the motor cortex of adult rats (Nudo, R.J. and Merzenich, M.M., *Soc. Nsci. Abstr.*, 1987; also see Brown and Sherrington, *Proc. Roy Soc. B* 85, 1912). In the present study, we investigated the consequences of localized ICMS within the body surface representation of the somatosensory koniocortical field in rats (SM1) and owl monkeys (area 3b).

Primary somatosensory cortex was exposed in Nembutal-anesthetized animals. Multi-unit cutaneous receptive fields were carefully defined with fine-tipped glass probes or von Frey hairs using glass micropipettes introduced at multiple sites in very fine grain within a limited sector of this cortical field. A stimulating microelectrode was then placed in the center of this cortical zone to a depth of 700-850μm. Charge-balanced monophasic pulses were delivered for up to 6 hrs. (3-7μA, 200 μsec pulses, 300pps bursts for 40 msec delivered 1/sec). At variable post-stimulation times, receptive fields were defined throughout the same cortical zone, and in many cases at the identical cortical sites.

Among the results were the following: 1) The specific receptive field defined at the stimulation site came to be represented at many penetration sites up to more than 600μm away from the stimulation location. 2) This expansion progressed throughout the course of stimulation. 3) Driven activity at the stimulation site was reduced during the stimulation period, but recovered following the stimulation period.

These results support the hypothesis that input coincidence plays an important role in cortical "functional minicolumn" formation and demonstrate that cortical neurons can select a new subset of driving inputs over a short time course.

Supported by NIH Grants NS-10414 and GM-7449, the Coleman Fund, and HRI.

92.11

VASCULAR PATTERNS IN MOUSE AND RAT BARREL CORTEX. T.A. Woolsey, J. Christensen*, N. Hunt*, R. Kohn*, J. Matter* and R. Strominger* (SPON: W. Landau). Division of Experimental Neurology & Neurosurgery and McDonnell Center for Studies of Higher Brain Function and A.D.R.C., Washington University School of Medicine, St. Louis, MO 63110.

The angioarchitecture of the brain is as distinctive as its neuronal architecture. There are brain region specific patterns of blood vessels. Patel (Brain Res., **289**:65-70, 1983) showed a relationship between capillary density and the whisker barrels in the rat by careful reconstruction from semithin sections of rat cerebral cortex. We have extended these observations with a variety of vascular and neuronal marking techniques. All approaches show patterns of capillary networks which are specific for individual barrels in layer IV of the rodent somatosensory cortex. This relationship is being quantified and a number of different images will be shown. The barrel specific capillary networks suggest a closer relationship between the development of functional neuronal groups and their vascular supply than previously suspected. The somatosensory cortex of the rodent offers an especially favorable site to study the relationship between the dynamic properties of functional groups of neurons and their blood supply. (Supported by NIA AG05681-03, McDonnell Center for Studies of Higher Brain Function and the Illinois-Eastern Iowa District of the Kiwanis.)

92.12

METABOLIC PLASTICITY FOLLOWING NEONATAL CORTICAL BARREL-FIELD INFARCTION. W.D. Dietrich, O. Alonso, R. Busto*, B.D. Watson*, and M.D. Ginsberg. Cerebral Vascular Disease Research Center, Department of Neurology, University of Miami, School of Medicine, Miami, FL, 33101.

Abnormal metabolic patterns of cortical field activation induced by whisker stimulation are observed in adult rats after chronic cortical barrel-field (BF) infarction (Dietrich et al, Soc. Neurosci. Abstr., 12:1168, 1986). To characterize the metabolic consequences of brain infarction in the immature brain, 20-day old male Wistar rats (n=7) underwent photochemically-induced BF infarction of the right SmI cortex. 60 days later, patterns of activation-induced increased glucose utilization were assessed with quanitative ^{14}C-2-deoxyglucose autoradiography. Unilateral whisker stimulation activated contralateral cortical regions anterior and lateral to the BF infarct. Lateral to the infarct, a single region of stimulation-induced increased glucose utilization (162% of control) was apparent, which extended more laterally than the normal SmII BF region. Subcortically, depressed rates of glucose utilization were documented within several thalamic nuclei underlying the infarcted zone. A decreased capacity to activate the contralateral ventrobasal thalamus in response to whisker stimulation was demonstrated. Ipsilateral to whisker stimulation, abnormal patch-like aggregates of increased glucose utilization (161% of control) within somatosensory cortex were detected. Thalamic nuclei (Posterior, Lateroposterior) also demonstrated abnormal metabolic responses to whisker stimulation (159% of control). Although similar patterns of stimulation-induced increased glucose utilization are observed following either neonatal or adult BF infarction, abnormal patterns are more pronounced following neonatal BF infarction. Supported in part by NIH grant NS05820; WDD is an Established Investigator of the AHA.

92.13

BARREL-FIELD CIRCUIT ACTIVATION IN THE NEONATAL RAT. O. Alonso, W.D. Dietrich, R. Busto*, and M.D. Ginsberg. Cerebral Vascular Disease Research Center, Department of Neurology, University of Miami, School of Medicine, Miami, FL, 33101.

In adult rats, unilateral whisker stimulation combined with ^{14}C-2-deoxyglucose autoradiography results in the metabolic activation of the major relay stations of the whisker-barrel circuit. This study was designed to document the metabolic activation patterns in rats ranging from 20-23 days of age. Male Wistar rats were briefly anesthetized with halothane and restrained in a plaster body cast. In 7 rats, all the large whiskers on one side of the face were stroked 2-3 times/sec with a brush for 5 min before and 40 min following a pluse injection of 2DG. In nonactivated rats (n=8), local cerebral metabolic rate of glucose (lCMRglu) was significantly depressed in most grey matter regions compared to values obtained in nonactivated adults (4 months). Unilateral whisker stimulation resulted in highly-focal regions of increased glucose utilization occupying the BF regions of contralateral SmI and SmII. Percentage difference between activated and nonactivated BFs was 124% in neonates compared to 36% in young adults. In addition to these focal alterations in lCMRglu, widespread elevations in lCMRglu were detected in brain regions outside the BF circuit. For example, within the ipsilateral frontal cortex, whisker stimulation increased lCMRglu by 42% over nonactivated values. In contrast, widespread metabolic consequences were not detected in activated young adults. These metabolic consequences to the physiological activation of this well-defined somatosensory circuit in the neonatal rat are consistent with a lack of inhibition at this stage of neuronal development. Supported in part by NIH grant NS05820; WDD is an Established Investigator of the AHA.

92.14

THE DEVELOPMENT AND TOPOGRAPHIC ORGANIZATION OF THE SOMATOSENSORY CORTICES OF THE NEONATAL PIG. R.H. Ray and S.L. Craner.* Dept. of Physiology, East Carolina University, Greenville, N.C. 27858

The perinatal ontogenesis of electrical activity and the topographic organization of the somatosensory cortices was determined in domestic pigs ranging in age from 7 days preterm to 60 days postnatal. Somatosensory evoked potentials (SEPs) and single and multiunit activity were used to determine 1) the details of the topographical representation in primary (SI) and secondary (SII) somatosensory areas and 2) whether SI and SII show evidence of differing rates of functional maturation. Standard histological techniques were used to examine the cytoarchitectural development of the somatosensory cortices in animals across all age groups. Complete topographical representations of SI and SII were present in all animals studied, including piglets 7 days preterm. The body map in SI was oriented similarly to that of other mammals, however, multiple representations of the rostrum, face and mouth were present including a separate ipsilateral face representation. The pig SII cortex had a "strip-like" orientation of the limb representations and an inverted trunk orientation. Multiple face regions were also found in SII. Response properties, including waveforms, latencies, and frequency-following capacities of SEPs, suggested that functionally the pig SI matures more rapidly than SII. Cytoarchitectural studies supported this conclusion.

92.15

CELLULAR AND MOLECULAR SUBSTRATES FOR CRITICAL PERIOD-DEPENDENT ALTERATIONS IN SOMATOTOPY. N. G. F. Cooper and D. A. Steindler. Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis, TN 38163.

The somatosensory barrel field cortex of mice contains a distinctive neuronal pattern that can be observed in Nissl-stained sections. The cell aggregates of layer IV neurons are the cortical representations of vibrissae present in the face, and the pattern of cell aggregates mirrors the pattern of vibrissae. Pertubation of vibrissae and their underlying nerve fibers in early postnatal animals leads to the development of altered patterns of Nissl-stained cell aggregates seen in the cortex. On the other hand, pertubation of vibrissae at later times does not lead to such changes. Presumably the substrates, necessary for the neuronal rearrangements are only present in the first postnatal week. In this study, the middle rows of vibrissae were cauterized in anesthetized neonatal animals. The animals were anesthetized several days later and cardiac perfused with aldehyde fixative. Sections of the cerebral cortex were cut on a vibratome and washed with a tris-buffered saline (TBS) and incubated in the peroxidase conjugated lectin, peanut agglutinin, which binds to the galactosyl carbohydrate residues of glycoconjugates. After incubation in the lectin-conjugate, sections were washed in TBS and reacted with hydrogen peroxide in the presence of the chromogen, diaminobenzidine. The distribution of peroxidase reaction product in sections was observed with a light microscope and compared to control sections of somatosensory cortex from animals that had no cautery treatment. The data demonstrates that cauterization of vibrissae leads to altered lectin- binding patterns in the cortex. Specifically, carbohydrate-rich boundaries between individual barrels in rows that represent the cauterized vibrissae were missing. It is suggested that the lectin-positive boundary regions contain molecules that help move the neurons into their characteristic patterns. Interactions between afferents and glial-rich boundaries (Cooper and Steindler,Brain Res., 380:341,1986) are responsible for shaping neuronal cytoarchitecture.The transient boundary regions it is argued, form the basis of the critical period. Support-NIH:NEI EY02708 (NGFC).

93.1

MALE AND FEMALE RATS ARE AFFECTED DIFFERENTLY BY NEONATAL STRESS: AUTONOMIC AND IMMUNE SYSTEMS RESPONSES. L.L. Ross, C.E. Taylor*, A. Seggos* and B. Weston*. Departments of Anatomy and Microbiology and Immunology, Med. College of Pennsylvania, Philadelphia, PA 19129.

We have shown that the adrenomedullary-sympathetic nervous system is permanently altered by stress administered during the neonatal period and that this alteration results in a modified response to stress administered to the adult. Further, males and female rats are affected differently by neonatal stress. In the present study, we expand on these findings and include data on the influences of neonatal stress on the immune system.

Beginning on P1 rat pups (Sprague-Dawley, VAF) were subjected to two forms of stress for 1 hour daily for 10 days: immobilization and maternal separation. At 40 or 60 days of age, representative groups of animals were immunized with a polysaccharide antigen and then immobilization stressed 5 days later. Others were stressed without prior immunization. These animals and appropriate controls were sacrificed and assayed for catecholamine content of adrenal glands and plasma and for the numbers of antibody-producing plaque forming cells specific for the antigen administered.

For adrenal and plasma catecholamines, the neonatally-stressed females showed a heightened response compared to the males, the latter being more like the control, unstressed animal. While neonatally stressed animals of both sexes showed a suppression of the immune response, the males had a greater degree of suppression.

Supported by NIH grants MH38365 and HD21633.

93.2

LYMPHOID TISSUE MONOAMINE CONTENT AND IMMUNE RESPONSIVENESS IN CHICKS TREATED IN OVO WITH 6-HYDROXYDOPAMINE. H.M. Brown-Borg and F.W. Edens*. Dept. of Poultry Science, North Carolina State University, Raleigh, NC 27695.

An existing hypothesis suggests that the sympathetic nervous system influences the immune response. Studies were conducted to determine the effects of in ovo general sympathectomy on catecholamine content of lymphoid tissues in chicks. Anti-sheep red blood cell (SRBC) antibody production and delayed-type hypersensitivity response to phytohemagglutinin (PHA) were measured concurrently. Eggs were injected with either 100 mg/kg 6-hydroxydopamine (6-OHDA) or water (CON) at d18 incubation. At 3.5 weeks of age, chicks were challenged intravenously with SRBC and bled on days 0,2,4,6,8 and 10 post-challenge. Bursae and spleens were removed on the same days and norepinephrine (NE), epinephrine (E), dopamine (DA) and serotonin (5HT) were determined using HPLC-EC. A intradermal injection of PHA (100ug) in the wing web was given to 10 birds in each group and the site of injection was measured at 0, 12 and 24 hours post injection. Antibody titers were significantly increased in the 6-OHDA birds on days 6,8 and 10 when compared to controls ($P<.05$). Splenic NE and DA increased with increasing antibody titers. Splenic DA was significantly increased in the 6-OHDA birds on day 6 which coincided with the peak in antibody titers ($P<.05$). No significant differences were found in cell-mediated immunity. These data indicate that the catecholamines are involved in the immune response in birds.

93.3

PRESENCE OF BETA-ADRENERGIC RECEPTORS ON DEVELOPING SPLEEN CELLS AND ALTERATIONS IN IMMUNOLOGIC REACTIVITY FOLLOWING NEONATAL SYMPATHETIC DENERVATION K.D. Ackerman, S.Y. Felten, D.L. Felten, and S. Livnat Depts. of Neurobiol. & Anat., Psychiatry, and Microbiol. & Immunol. Univ. of Rochester, Sch. of Med., Rochester, NY 14642

Previous studies from our laboratory have shown that noradrenergic sympathetic nerves are present within specific compartments of the spleen at the earliest stages of postnatal development. Norepinephrine produced by these nerves may play a functional role in the development of both lymphoid and non-lymphoid cells, provided that these cells have adrenergic receptors and can respond to adrenergic signals. In this study, neonatal rat spleens were examined for the presence of beta-adrenergic receptors and changes in immunologic reactivity following neonatal sympathectomy.

The presence of beta-adrenergic receptors was measured on spleen cells from 1 to 90 days of age using the ligand iodocyanopindolol. Scatchard analysis revealed a Kd of 8-13 pM in all age groups, consistent with a high affinity beta receptor. Bmax was 400-500 sites/cell from 1-7 days, rising to 1000-1200 sites/cell at 21 days, and then declining to 600-700 sites/cell at 90 days.

Spleen cells from 10 day old rats, sympathectomized with 6-OHDA at birth, showed decreased spontaneous proliferation, reduced ConA-induced suppression of the background proliferation, and diminished NK cell activity. In contrast, by 41 days of age, spleen cells showed increased NK cell activity but control levels of spontaneous and ConA-induced proliferation. These findings suggest that at the earliest stages of postnatal development, splenocytes possess beta-adrenergic receptors and demonstrate altered responsiveness following chemical sympathectomy.

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93.4

PATTERN AND TIME COURSE OF RE-INNERVATION OF NORADRENERGIC FIBERS INTO THE SPLEEN FOLLOWING DENERVATION WITH 6-HYDROXYDOPAMINE. D. Lorton, D.L. Bellinger, D. Hewitt, S.Y. Felten, and D.L. Felten. Dept. of Neurobiol. & Anat., Univ. of Rochester, Sch. of Med., Rochester, NY.

The time course and pattern of reinnervation of noradrenergic (NE) nerve fibers into the spleen following chemical sympathectomy with acute treatment of 6-hydroxydopamine (6-OHDA) was examined in young adult male Fischer 344 rats. On 1, 3, 5, 10, 21, and 56 days after 6-OHDA treatment, NE nerve fibers in the spleen were examined with glyoxylic acid fluorescence histochemistry. 6-OHDA treatment resulted in almost complete NE denervation, sparing only a small number of fibers at the hilar region at day 1 after treatment. Sprouting of fibers into the spleen was apparent by day 5 and continued to increase between 10 and 56 days after treatment. NE reinnervation first appeared at the hilar region by day 5, largely associated with the vasculature. The density of NE fibers increased in a radial fashion from the hilus with increasing post-lesion times. At 10 and 21 days after treatment, NE fibers coursed along the blood vessels in the hilar region, continued into the spleen with the vasculature, and extended into the parenchyma of the white pulp in regions near the hilus. Regions distal to the hilus, remained denervated. By 56 days post-treatment, NE fibers had reinnervated a vast portion of the spleen; however, sprouting NE fibers did not extend to regions extremely distal to the hilar region. Regions of splenic white pulp near the hilus appeared to be hyperinnervated. In all 6-OHDA treated spleens, the compartmentation of sprouting NE fibers was similar to that in saline injected controls, but the density differed according to the distance from the hilus. Supported by grants N0014-84-K-0488 from ONR, RO1 MH42076 from NIMH, RO1 NS25223 and T32 MH18260 from NIH.

93.5

NORADRENERGIC SYMPATHETIC NERVE FIBERS ARE ASSOCIATED WITH IMMUNE COMPARTMENTS BUT NOT SMOOTH MUSCLE DURING EARLY NEONATAL DEVELOPMENT OF THE RAT SPLEEN. D.L. Felten, K.D. Ackerman, S.Y. Felten, J.A. Olschowka, and S. Livnat. Depts. of Neurobiology & Anatomy, Psychiatry, and Microbiology and Immunology, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

In the adult rat spleen, sympathetic noradrenergic (NE) nerve fibers form neuroeffector junctions with both smooth muscle cells and lymphocytes. We investigated whether the development of innervation in these different compartments occurred in parallel or followed separate time courses. Light and EM immunocytochemical observations of the spleen at days 1 and 3 revealed tyrosine hydroxylase-positive (TH+) nerve fibers in the periarteriolar lymphatic sheath (PALS) in a periarteriolar location, with lymphocytes present between these fibers and the central artery, and macrophages present distally. The TH+ fibers did not contact central arteriolar smooth muscle cells; only later, at day 7, were vascular plexuses of TH+ nerve fibers routinely present. We also found ß-adrenergic receptors on splenocytes at 1 day of age. We suggest that NE nerves communicate with cells of the immune system as their principle targets during early neonatal development of the spleen, and may influence development of the compartmentation of the splenic white pulp and subsequent immune competence.

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93.6

GUANETHIDINE SYMPATHECTOMY ENHANCES T-CELL RESPONSE IN NEONATAL BUT NOT ADULT RATS. T.T. Nguyen*, D.K. Nelson*, C.J. Krco* and V.L.W. Go* (SPON: B. Guthrie). Mayo Clinic, Rochester, MN 55905.

Acute chemical sympathectomy (SX) by 6-hydroxydopamine (6-OHDA) and surgical SX have both been shown to enhance T-cell function in the neonatal animal, but not in the adult. We examined the effect of chronic chemical SX using guanethidine, which selectively destroys peripheral sympathetic neurons. Methods: Adult (n=14) and neonatal (n=8) rats were injected (5 wks ip, and 3 wks sc, respectively) with either guanethidine (40 mg/kg/d) or vehicle and sacrificed 1 wk after cessation of treatment. Degree of SX was determined by dopamine-B-hydroxylase (DBH) assay of peripheral nerve. T-cell mitogen-induced lymphocyte (mesenteric lymph nodes) proliferation was measured by ^{3}H-thymidine incorporation in the presence of Concanavalin A (ConA). Results: Guanethidine-treated animals showed 90% depletion of DBH. Treated neonatal rats showed a significant increase in T-cell activation over controls (48 and 64% with 2.5 and 1.25 µg/ml ConA, respectively; $p<0.002$). However, treated adult rats showed no significant difference ($p=.292$) in their T-cell response to ConA over controls. Conclusion: Guanethidine SX enhanced T-cell mitogen response in the neonate, but not in the adult. This differential effect on the immature and mature immune systems is similar to that seen following surgical and 6-OHDA SX. These data support the importance of neural input to the developing immune system.

93.7

INTERACTION OF SECOND MESSENGER SYSTEMS LINKED TO THE ß-ADRENERGIC AND T CELL RECEPTORS ON HUMAN T LYMPHOCYTES. S.L. Carlson and T.L. Roszman* Dept. Microbiology and Immunology, Univ. Kentucky College of Medicine, Lexington, KY 40536

The sympathetic nervous system innervates lymphoid tissues, with fibers extending into the parenchyma, particularly into T cell zones. The neurotransmitter norepinephrine (NE), which is released from these fiber terminals, interacts with lymphocytes through binding to the ß-adrenergic receptor (ß-AR) expressed on the lymphocyte surface. Many studies have shown that NE or ß-adrenergic agonists are capable of modulating lymphocyte function. In particular, ß-adrenergic agonists have been shown to inhibit T cell activation stimulated through the T cell receptor (TCR). The mechanism responsible for such effects of ß-adrenergic activation is not known. It is important, therefore, to examine the transmembrane signalling induced by stimulation of the ß-AR in T cells, particularly in relation to intracellular signals necessary for T cell activation.

We are examining the interactions at the second messenger system level, between the ß-AR and the TCR. The ß-AR is linked to the cAMP system, and the TCR to the phosphotidylinositol (PI) system. Stimulation of human T cells with 10^{-5}M isoproterenol, a ß-adrenergic agonist, causes a rapid increase in cAMP, which begins to decline by 10-15 minutes. Stimulation of the TCR with a monoclonal antibody to the TCR (anti-T3 mAb) alone has no effect on cAMP. Simultaneous stimulation with isoproterenol and anti-T3 mAb causes a potentiation of the cAMP response. This suggests that a component of the PI system has a potentiating effect on the ß-AR-linked cAMP system. In addition, we are examining the effect of ß-AR stimulation on the TCR-linked activation of the PI cycle through measuring IP3 production, Ca2+ fluxes, and PKC activity. Understanding how the second messenger systems linked to the ß-AR and the TCR interact and modulate one another will aid in understanding the physiological role of the sympathetic nervous system in modulation of T cell activation in response to antigens.

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93.8

HIGH AFFINITY DOPAMINE BINDING TO MOUSE THYMOCYTES AND MYTILUS EDULIS (BIVALVIA) HEMOCYTES: HEMOCYTE MONOAMINERGIC INTERACTION. G. B. Stefano, X. Zhao*[1], D. Bailey*, M. Metlay* and M.K. Leung. Center for the Study of Aging, SUNY/ Old Westbury, Old Westbury, N.Y. 11568.

The present report demonstrates that both mouse thymocytes and Mytilus edulis hemocytes contain a novel type of DA receptor. Scatchard analysis of these data revealed a single class of high-affinity binding sites with an affinity constant (K_d) of 6.6 nM, and a binding site density (B_{max}) of 141 pmol/g protein in mice and a K_d of 7.6 nM and a B_{max} of 66 pmol/g protein for the hemocytes. In older Mytilus the K_d was the same and the B_{max} was reduced (48 pmol/g protein; P>0.05; not evident for the mice cells). Displacing specifically bound DA was investigated and the catecholamines (NE > E) and the DA agonist epinine were the most potent and apomorphine and butaclamol were of moderate potency. This indicates that DA binding site is different from D_1 and D_2 sites. The hemocytes appear to be of two types, cells with a granular and agranular cytplasm. Other studies reveal that a group of granulocytes contain serotonin. The study suggests that vascular and neural interactions also exist in invertebrates indicating further their establishment early in metazoan evolution. Grants NIH-MBRS 08180 & ADAMHA-MARC 17138

93.9

THE ORIGIN OF ACETYLCHOLINESTERASE-POSITIVE NERVE FIBERS IN THE SPLEEN OF YOUNG ADULT RATS. D.L. Bellinger, S.Y. Felten, and D.L. Felten. Dept. of Neurobiol. & Anat., Univ. of Roch., Sch. Med., Rochester, N.Y.

The source of acetylcholinesterase-positive (AChE+) nerve fibers in the spleen of young adult male Sprague-Dawley rats was examined using a modified thiocholine method for specific AChE with nickel-3,3'-diaminobenzidine enhancement following surgical and 6-hydroxydopamine (6-OHDA) denervation procedures. AChE+ staining in all control groups was present in several populations of lymphoid cells, including small follicular cells, small grainy lymphocyte-appearing cells in the marginal zone, and large macrophage-like cells with AChE+ granules, throughout the spleen. AChE+ neural-like profiles were present along the vasculature, in the trabeculae, and to a lesser extent, in the white pulp, near the capsule, and in the follicles. Complete superior mesenteric-celiac ganglionectomy resulted in a total loss of noradrenergic (NE) as well as AChE+ fibers, and an expected loss of AChE staining in all lymphoid cells. No loss of AChE+ fibers was seen following bilateral subdiaphragmatic vagotomy. Treatment with 6-OHDA resulted in almost complete NE denervation, and loss of a majority of AChE+ fibers in all compartments 1 day after treatment, without apparent loss of AChE staining in lymphoid cells. This was followed by a progressive increase in AChE staining in neural profiles, as NE fibers subsequently reinnervated the spleen between 10 and 56 days following 6-OHDA treatment. The distribution of AChE+ neural profiles closely overlaps the distribution of NE fiber in the spleen, suggesting co-localization; however, some AChE profiles appear to be present in non-NE nerve fibers as well. Supported by grants N00014-84-K-0488 from ONR, RO1 NS25223 from NIH, RO1 MH42076 and T32 MH18822 from NIMH.

93.10

NOVEL MUSCARINIC RECEPTOR ON B CELL LINE UP REGULATED BY GLUTAMINE. T. Radojcic and K. Bulloch(T. Melnechuk, spon.) Neuroimmune Physiology Lab, Helicon Foundation/ Depts. Psychiatry and Pediatrics, Univ. of Cal San Diego, CA 92093

Recent studies indicate that cholinergic agonists directly influence T cell function and development. Other reports suggest that humoral immunity may also be effected by cholinergic mechanisms but do not indicate at what level cholinergic transmission exerts its effect. In this study we report the presence of a novel muscarinic receptor on a cloned murine B cell line L10 A2J that is up regulated by glutamine. In radioligand binding assays, these cells specifically bind ^{3}H-QNB at two affinities (Kd 1-2nM, 1mM). The specific binding could be displaced by 70-100uM atropine. Similar affinities are observed on lymphocytes. The number of muscarinic receptors of L10.2J was up-regulated by l-glutamine. Cells grown in glutamine depleted culture media did not bind QNB, whereas the number of receptors doubles when the concentration of glutamine is raised from 10-50mM. Supported by:grant #N00014-85K-0528 ONR and a grant from the Joan B. Kroc Foundation of Psychoneuroimmunology

93.11

FUNCTIONAL LOCALIZATION OF MAST CELLS IN DIFFERENT BRAIN AREAS. A.D. Konstantinidou*, N. Flaris*, M. Lambracht-Hall*, and T.C. Theoharides. Dept. of Pharmacology, Tufts University School of Medicine, Boston, MA 02111.

Mast cells have been identified in the brain histologically only. We examined their differential distribution in the brain using their secretory characteristics. We used a custom-made perfusion system with four wells so that we could simultaneously study the following areas labelled with ^{3}H-serotonin: thalamus, hypothalamus, cortex and cerebellum. Mast-cell release was induced by the classic mast cell secretagogue, compound 48/80, and the perfusate was assayed for ^{3}H-serotonin, histamine, β-hexosaminidase (a secretory granule enzyme) and LDH. There was no substantial release of LDH in any of the areas tested. The prevalence of mast cells in different areas was estimated from the extent of mediator release as follows: hypothalamus >> thalamus > cortex. There was insignificant release from cerebellum (without meninges), in accordance with previous anatomic studies, thus permitting us to use it as a negative control. This is the first study demonstrating the differential distribution of brain mast cells based on functional criteria. Release of important mediators from brain mast cells could influence neuronal secretion with possible effects on behavioral and neuroimmune disorders.

94.1

ANTIBODIES TO THE m1 MUSCARINIC ACETYLCHOLINE RECEPTOR. S.J. Wall, G.R. Luthin, and B.B. Wolfe. Dept. of Pharmacology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

Cloning and sequence analyses of rat muscarinic cholinergic receptor (MAChR) genes have demonstrated the existence of at least four distinct receptor subtypes. Rabbit polyclonal antibodies (m1 Abs) were raised against a synthetic 17 amino acid peptide conjugated to KLH. The peptide sequence corresponded to the C-terminal of the rat m1 MAChR. Initial screening with a solid phase radioimmunoassay indicated that the antisera had a high titer directed against the m1 peptide. The m1 MAChR is recognized with high affinity by pirenzepine (PZ), an antagonist possessing differential selectivity for MAChR subtypes. Uniform labelling of MAChR can be observed with the nonselective antagonist QNB. The m1 Abs were able to precipitate successfully QNB and PZ binding activities solubilized from several brain regions, but not from heart. Precipitation of binding activity was reversed in the presence of m1 peptide but not in the presence of C-terminal peptides of the m3 or m4 MAChRs. The m1 Abs precipitated QNB binding from Chinese hamster ovary (CHO) cells transfected with cDNA for the m1 MAChR, but not from CHO cells containing cDNA for the m2 MAChR. Together, these data indicate that the m1 Abs exhibit selectivity for the m1 MAChR subtype and may be suitable for immunoaffinity enrichment and tissue level quantitation of m1 MAChRs. (Supported by NS23006, GM31155, and MH14654).

94.2

PROPERTIES OF M1 AND M2 MUSCARINIC ACETYLCHOLINE RECEPTORS IN AFFINITY PURIFIED PREPARATIONS. Ramon Diaz-Arrastia and Stanley H. Appel, Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.

To investigate the molecular basis of muscarinic acaetylcholine receptor (MAchR) subtypes, the properties of receptors from porcine heart and forebrain were studied in native membranes, after detergent solubilization and affinity purification, and after reconstitution with purified G proteins. Affinity chromatography was carried out on ABT-agarose (JBC 260:7927, 1985). The purification procedure yielded highly enriched MAchR, approximately 15-20% pure, and resolved from endogenous G proteins. Membrane bound receptors from the two tissues differed in kinetic rates of binding to classical antagonists, equilibrium binding affinities for pirenzipine, and equilibrium binding affinities for carbachol. Crude soluble receptors differed only in kinetic rates of binding to classsical antagonists--affinities for pirenzipine and carbachol were indistinguishable. Affinity purified receptors were identical in all parameters tested. Affinity purified receptors were reconstituted with purified Gi. MAchRs purified from both tissue sources interacted with Gi in identical fashion as determined by the ability of muscarinic agonists to stimulate the GTPase activity. This research was supported by grants from the John A. Hartford Foundation and the Helen C. and Robert J. Kleberg Foundation.

94.3

SELECTIVE ANTAGONISTS BLOCK ^{3}H-PROPYLBENZILYLCHOLINE MUSTARD BINDING TO MUSCARINIC RECEPTIVE PROTEINS OF DIFFERENT MOLECULAR WEIGHT. S. McLeskey*, W. Wojcik. FGIN, Georgetown Univ., Washington D.C. 20007.

Four distinct muscarinic receptive proteins, m-1 through m-4 have been reported. The m-3 receptive protein has a higher MW (90 KD) than the m-1, m-3 and m-4 proteins which have a MW of about 66 KD. ^{3}H-propylbenzilylcholine mustard (^{3}H-PBCM) can affinity label these muscarinic receptive proteins and, after SDS-PAGE with subsequent autoradiography, two bands (90 KD, 66 KD) of labeled proteins can be seen. In studies of selective muscarinic antagonists, pirenzipine and (-)QNX were found to be slightly selective in blocking the muscarinic mediated stimulation of phosphotidylinositol hydrolysis (M-PI) over the muscarinic mediated inhibition of adenylate cyclase (M-AC). Methoctramine was 600 times more selective at the M-AC than at the M-PI. In displacement studies of these three antagonists against ^{3}H-PCBM, both pirenzipine and (-)QNX completely prevented ^{3}H-PCBM binding to the 90 KD protein (m-3?) with a slight loss in binding to the 66 KD band of protein (m-1, m-4 ?). Methoctramine selectively blocked the ^{3}H-PCBM binding to the lowest MW portion of the 66 KD band (m-2?).

94.4

SHIFT OF THE PREDOMINANT Mr FORM OF THE AVIAN RETINA mAChR IN VITRO. **A.F. Skorupa*, J.S. Aguilar*, M.I. Fonseca*, and W.L. Klein (SPON: D. Gibbs). Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.**

Muscarinic acetylcholine receptors (mAChR) exist in developing chick retina in two molecular weight (Mr) forms (86KD and 72KD), whose relative proportions shift during synaptogenesis in vivo (Large et al., PNAS, 82: 8785, 1985). We have used aggregate and monolayer cultures to study a possible role for cell-cell interactions in the shift. Embryonic day 9 (E9) retinas were grown in high density monolayer cultures (10^{6} cells/cm^{2}) on poly-L-lysine-coated dishes for 2 to 8 days (C8). mAChR Mr profiles were obtained from 3H PrBCM-labeled broken membranes and intact cells using SDS-urea-PAGE. The predominant mAChR Mr form changed from 86 to 72KD between E9C2 and E9C8. Membrane mAChR Mr profiles from E9C8 low density retina cultures (10^{5} cells/cm^{2}) are less shifted than those from high density cultures, suggesting a role for cell-cell interactions. Cells grown in aggregates have similar membrane mAChR Mr profiles to high density monolayers. Receptor activity is not involved in the shift, as growing aggregates with 0.1 uM atropine does not influence the predominant Mr form.

94.5

IDENTIFICATION OF MUSCARINIC ACETYCHOLINE RECEPTOR KINASE (mAChRK) IN THE RAT BRAIN SYNAPTIC MEMBRANE, A.K. Ho*, Y-J Zhang*, Q-L Ling* and R. Duffield* (Spon: J. Stewart), Dept. Basic Science, U. of Ill. Coll. of Medicine-Peoria.

Phosphorylation has been suggested as a possible mechanism for receptor desensitization. Purified muscarinic acetylcholine receptor (mAChR) can be phosphorylated by cAMP-dependent protein kinase (cAMP-PK) (Ho et al. BBRC, 137, 142, 911, 1987) and by protein kinase C (Haga et al. TIPS Supp. 1988). We present here preliminary findings to demonstrate the possible existence of an agonist activated mAChR kinase which was co-purified with the mAChRs from rat brain synaptic membranes by ABT-affinity and hydroxyapatite chromatographies. Purified mAChR was phosphorylated by this crude kinase in the presence of [γ-^{32}p]-ATP and Mg^{2+}. The enzyme activity was increased by one to four-fold in the presence of either carbachol (0.1 to 1mM) or acetylcholine (ACh 50-100 μM). This effect was blocked by atropine. SDS-PAGE radioautography showed the presence of the heavily labeled band indicating the ^{32}P-mAChR ($\simeq$70 Kd) and two other labeled bands of M.W. 62 and 58 Kd respectively. Since phosphorylation of mAChR occurred in the absence of cAMP-PK, cAMP, or Ca^{2+} phospholipid and in the presence of EGTA and was activtated by either carbachol or ACh, we tentatively conclude that a mAChR kinase may be present in the synaptic membrane. [Supported in part by grants from AFAR, BRSG and American Heart Association, Illinois Affiliated and 1RO1NS25273 from NIH.]

94.6

MOLECULAR COMPONENTS OF A MUSCARINIC RECEPTOR-G PROTEIN COMPLEX ISOLATED FROM RAT HEART. G.R. Luthin, D.R. Manning*, V. Kamath*, L. Schretzman*, and B.B. Wolfe. Dept. of Pharmacology, Univ. of PA. Sch. Med., Philadelphia, PA 19104-6084.

High-affinity agonist binding to muscarinic acetylcholine receptors (MAChRs) is believed to reflect an interaction of the agonist-occupied receptor with a guanine nucleotide-binding (G) protein. We have solubilized and isolated MAChR-G protein complexes from rat heart and identified the molecular components of the complex. Heart membranes were incubated with ^{3}H-oxotremorine-M (oxo-M) or ^{3}H-QNB, then solubilized in digitonin/cholate. Receptors were enriched by chromatography over wheat germ agglutinin (WGA)-agarose, then were immunoprecipitated using a monoclonal antibody to the cardiac MAChR (Luetje et al., Biochemistry 26:6892, 1987). Immune pellets were dissolved and subjected to SDS-PAGE and Western blotting, using antibodies of general specificity for G_{α} and G_{β} subunits. In the presence of oxo-M, a 39 kD protein was identified in the Western blots. Levels of the protein were much reduced or absent when QNB-labelled receptors were used, or when a control ascites was used in the immunoprecipitation. Using antibodies of more defined specificity for G_{α} subunits, we have tentatively identified the 39 kD protein as the α-subunit of G_{o}. These data suggest that oxo-M promotes a physical interaction of the cardiac MAChR with the α-subunit of G_{o}. (Supported by NS23006, GM31155, and CA 39712).

94.7

LOW-K_M GTPASE AS A MEASURE OF TOTAL MUSCARINIC RECEPTOR ACTIVITY IN BRAIN AREAS. S. Ghodsi-Hovsepian*, W.S. Messer, Jr., Z. Mazloum*, B. Shankar* and W. Hoss (Spon: C.L. Hinman) Dept. of Medicinal and Biological Chemistry, Univ. of Toledo, College of Pharmacy, Toledo, OH 43606

A family of structurally similar receptors mediates a number of muscarinic responses in the CNS. G-proteins couple the various receptors to their effector systems in brain. The pharmacology of muscarinic responses has been examined by the action of subtype-selective agonists and antagonists on biochemical and electrophysiologic responses. These studies do not however, address the fraction of total muscarinic receptors in a given brain region that may be associated with a particular response. Receptor-mediated low-K_m GTPase stimulated by full agonists such as acetylcholine or carbachol is a measure of the total muscarinic activity for a particular brain region. Previous studies (Ghodsi-Hovsepian et al., *Soc. Neurosci. Abstr.* 13:1374, 1987) indicate differential coupling between muscarinic receptors and G-proteins comparing different brain regions.

Oxotremorine-M is a full agonist for some responses, e.g., phosphoinositide (PI) turnover, whereas oxotremorine is a weak partial agonist for PI turnover but is effective for inhibition of acetylcholine release at presynaptic receptors. We have compared the ability of oxotremorine-M and oxotremorine to stimulate low-K_m GTPase in the rat cortex and hippocampus. Both agonists stimulated low-K_m GTPase activity in cortex and hippocampus with EC_{50} values in the low-to-submicromolar range, generally more potent than carbachol. Oxotremorine produced a significant but partial response in both areas. Oxotremorine-M produced a full response in the hippocampus, but only a partial response in the cortex. The data indicate that a significant fraction of receptors in both cortex and hippocampus as well as other areas (e.g., pons-medulla) are responsive to oxotremorine and therefore, not associated with PI turnover. These data are consistent with the finding (Dutar and Nicoll *Soc. Neurosci. Abstr.*. 13:152, 1987) that three of four electrophysiologically measured responses in the hippocampus can be elicited by oxotremorine, whereas both carbachol and oxotremorine-M activate all four responses. Supported by NS23929 and DA04068

94.8

SELECTIVE INACTIVATION OF G PROTEINS ASSOCIATED WITH MUSCARINIC RECEPTORS BY HEAT. R.L. Dennison, W. Boswell*, N. Harmer*, E. Wright*, T.K. Narayanan, and R.S. Aronstam Dept. Pharmacol. & Toxicol., Medical College of Georgia, Augusta, GA 30912.

The influence of heat on muscarinic receptors-G protein interactions was investigated in neural membranes from rat brainstem. A suspension of membranes was immersed in a water bath at 50°C for 1-30 min. This treatment lowered the proportion of receptors which displayed high affinity agonist binding from 64 to 21% without affecting the number or affinity of antagonist binding sites. The half time of this effect was less than 5 min. To test the possibility that this change reflected a specific inactivation of receptor-associated G proteins (receptors coupled to G proteins display high affinity agonist binding while uncoupled receptors display low affinity binding), we determined the influence of heat on two G protein functions, guanine nucleotide binding and GTPase activity. Both the binding of [^{35}S]GTP and the hydrolysis of alpha-[^{32}P]GTP were inhibited by exposure to heat with a half time of 2-3 min. These findings suggest that exposure of neural membranes to low heat for a short period of time selectively inactivates receptor-associated G proteins without affecting the receptors. This treatment may be useful in removing functional G proteins in reconstitution procedures. (Supported by GM-37948, AA-07698 and the Georgia Heart Association).

94.9

GLANDULAR M_2 MUSCARINIC RECEPTORS MEDIATE INHIBITION OF CYCLIC AMP FORMATION IN MOUSE NEUROBLASTOMA CELLS. C.L. Amrhein*, W. Surichamorn and E.E. El-Fakahany. (SPON: O.H. Choi). Dept. of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

The effects of M_2-selective muscarinic receptor antagonists on receptor-mediated inhibition of cyclic AMP formation (an M_2 response as defined by pirenzepine) were investigated in mouse neuroblastoma cells (clone N1E-115). This response was measured using 25 μM forskolin in the absence or in presence of the muscarinic agonist carbamylcholine. For comparison, we also determined the potency of these antagonists in inhibiting muscarinic receptor-mediated increases in cyclic GMP synthesis and phosphoinositide (PI) hydrolysis, responses which are triggered by M_1 receptors. AF-DX 116, a cardioselective M_2 muscarinic antagonist, was less potent at blocking muscarinic receptor-mediated inhibition of cyclic AMP formation than the cyclic GMP or PI responses. In contrast, the glandular M_2-selective antagonist, hexahydro-sila-difenidol, exhibited the reverse selectivity, being > 100 fold more potent than AF-DX 116 in blocking the cyclic AMP response. According to the known rank order of potency of these two antagonists at the M_1 and the two M_2 receptor subtypes, these data provide evidence that inhibition of cyclic AMP levels in this neuronal clone is mediated by the glandular M_2 muscarinic receptors. (Supported in part by NIH grants NS-24158, AG-07118 and AG-00344)

94.10

PREFERENTIAL COUPLING OF SUBTYPES OF MUSCARINIC RECEPTORS TO SPECIFIC SECOND MESSENGER SYSTEMS: THE m1 RECEPTOR PREFERS PHOSPHOINOSITIDE BREAKDOWN WHILE THE m2 RECEPTOR PREFERS INHIBITION OF ADENYLATE CYCLASE. Min Li* and Barry B. Wolfe. U. PA Sch. Med., Phila. PA 19104. Spon: (P. Salzman)

Recently, several genes have been cloned that code for proteins having the properties of muscarinic cholinergic receptors. Chinese hamster ovary (CHO) cells normally not expressing muscarinic receptors were transfected with a plasmid containing DNA coding for either m1 (CHO-m1) or m2 (CHO-m2) receptors (Peralta et al., EMBO J. 6: 3923, 1987). These cells (a gift to us from Dr. D.J. Capon, Genentech Inc.) were used to examine the relationship between two specific, defined, receptor subtypes and two second messenger systems, inhibition of adenylate cyclase activity and stimulation of phosphoinositide breakdown. CHO cells expressing m2 receptors had a much higher (10 x) density of (^{3}H)-QNB binding sites than cells expressing m1 receptors. Never the less, using equal numbers of cells per assay tube, cells expressing m1 receptors demonstrated a 10-fold increase in inositol phosphate production in response to 1 mM carbachol while cells expressing m2 receptors had less than a 2-fold response. On the other hand, in agreement with Ashkenazi et al. (Science 238: 672, 1987), carbachol strongly inhibited adenylate cyclase activity in cells expressing m2 receptors but not in cells expressing m1 receptors. Thus, these receptor subtypes demonstrate a preferance for coupling to a given second messenger system. This preferance does not seem to be absolute but rather depends on the density of receptors per cell. Supported by GM31155.

94.11

INHIBITION OF MUSCARINIC RECEPTOR STIMULATED PHOSPHOINOSITIDE TURNOVER IN SK-N-SH NEUROBLASTOMA CELLS BY AGENTS REPORTED TO INCREASE INTRACELLULAR cAMP. M. Akil*, S.K. Fisher. Neuroscience Laboratories, University of Michigan, Ann Arbor, MI 48104.

The effects of agents known to increase intracellular cAMP on muscarinic receptor stimulated phosphoinositide turnover (PPI) in the human neuroblastoma cell line SK-N-SH was examined. Intact SK-N-SH cells were first prelabeled with [^{3}H]Inositol for 45 min at 37°C and then exposed to carbachol for 45 min in the presence or absence of the agents of interest. Release of total inositol phosphates (IP), phospholipid labeling and cAMP concentrations, were determined. Forskolin inhibited the PPI response to carbachol significantly in a dose and time dependent fashion (50% at $5x10^{-5}$ M and 40 min), without influencing basal IP release. cAMP was increased 200-300% under these conditions. The effects of dibutyryl cAMP, theophylline, and isobutyl methylxanthine on stimulated IP release and cAMP were similar to those of forskolin. Chronic (24 hr) treatement of cells with cholera toxin also inhibited the PPI response, but in the absence of detectable increase in cAMP concentration. Prostaglandin E1, on the other hand, increased cAMP concentrations dramatically (2500-3000%) in the presence of a diesterase inhibitor, but did not have a significant effect on stimulated IP release. These results point to the complexity of interaction between these two second messenger systems and suggest, furthermore, that their interrelationship is unlikely to be a simple quantitative one. (Supported by NIMH grant MH 42652).

94.12

DESENSITIZATION OF MUSCARINIC INCREASES IN NEURONAL FIRING RATES MAY BE RELATED TO INOSITOL LIPID HYDROLYSIS.

N.J. Pontzer* and F.T. Crews. Dept of Pharmacology, Univ. of Florida College of Medicine, Gainesville, FL 32610.

We have studied a series of muscarinic agonists which have varying intrinsic activity for stimulation of phosphoinositide (PI) hydrolysis in hippocampal slices. The ability to stimulate PI hydrolysis was compared to the stimulation of extracelluler firing rates in CA1 hippocampal neurons. All of the muscarinic agonists studied produced large increases in firing rate in contrast to their differences in intrinsic activity at stimulation of PI hydrolysis. At higher concentration firing rates decreased for all drugs except oxotremorine, the weakest partial agonist at stimulation of PI hydrolysis we studied. A comparison of the two dose response curves suggested that the decreases in firing rate occurred at a threshold for PI stimulation. Oxotremorine did not produce desensitization and not reach the PI threshold. Full PI agonists caused a pronouced concentration dependent decrease in firing. Partial agonists, which only approached the PI threshold, slowly desensitized. Carbachol stimulated PI hydrolysis and firing rate desensitization are reduced in the presence of oxotremorine. These studies suggest that threshold levels of PI hydrolysis decrease muscarinic stimulated firing rates.

To further test this hypothesis lithium was used to deplete neuronal PI stores reducing the ability of agonists to stimulate PI hydrolysis. Lithium pretreatment appeared to increase firing rates and reduce desensitization of firing rates by high concentrations of carbachol. (Supported by NIH grant #AG06660.)

94.13

MUSCARINIC STIMULATION OF PI TURNOVER AND NOREPHINEPHRINE RELEASE IN PC12 CELLS OCCUR VIA INDEPENDENT MECHANISMS. A. Takashima* and J. G. Kenimer. Lab. of Cellular Physiology, CBER, FDA, Bethesda, MD 20892.

PC12 cells, preloaded with ^{3}H-NE or ^{3}H-myo-inositol, were used to quantitate the effects of methacholine on PI turnover and NE release. Methacholine stimulation resulted in a biphasic increase in IP_3 levels and in NE release. In both cases there was a rapid increase for the first 3 min to levels which remained stationary, or slightly increased, over the next 12 min. IP_2 and IP levels were each shown to increase over the entire 15 min test period. Pertussis toxin pretreatment of the cells resulted in parallel dose dependent inhibitions of both PI turnover and NE release suggesting that both events are dependent upon G-protein participation. These results are consistant with previous suggestions that muscarinic-stimulated PI turnover and NE release are coupled events. To further investigate this hypothesis we performed release and PI turnover experiments under conditions designed to discriminate between these events. Under Ca^{2+}-free conditions methacholine-stimulated release was inhibited but PI turnover was unaffected. In contrast, pretreatment with phorbol dibutyrate abolished methacholine-stimulated PI turnover without inhibiting stimulation of NE release. In addition, treatment of cells with AlF_4^- or vanadate was shown to stimulate PI turnover without significantly affecting the release of NE. These results demonstrate that methacholine-stimulated NE release can occur under conditions in which PI turnover is not stimulated and that, conversely, stimulation of PI turnover can occur without release of NE. We interpret these results to indicate that these two muscarinic-dependent events are stimulated via independent mechanisms. We are currently investigating whether different muscarinic receptor subtypes and/or different pertussis toxin sensitive G-proteins are involved.

94.14

AF-DX 116 (MUSCARINIC M2) BINDING SITES IN RAT BRAIN: PRE-SYNAPTIC MODULATION OF ACETYLCHOLINE RELEASE. P.A. Lapchak, D.M. Araujo, R. Quirion and B. Collier. Depts. Pharmacol.& Psychiat., McGill University, Montreal, Canada, H3G 1Y6.

The present study characterized the binding of the muscarinic antagonist (ant) [3H]AF-DX 116 to muscarinic receptors in rat brain, and tested the effect of AF-DX 116 on acetylcholine (ACh) release from rat brain slices.

The results show that [3H]AF-DX 116 binds saturably and specifically to two apparently distinct classes of sites in rat forebrain homogenates (kd= 2.63 nM & 63.3 nM). [3H]AF-DX 116 also bound specifically to homogenates from cortex, striatum and hippocampus. The ligand selectivity pattern of these sites indicated that AF-DX 116, atropine, and (-)QNB potently competed for [3H]AF-DX 116 binding sites, whereas pirenzepine (M1 ant) was a poor competitor. AF-DX 116 (10 uM) increased K+ evoked (25 mM) ACh release from cortex (43.6%), striatum (41.0%), and hippocampus (58.0 %). Atropine and (-)QNB (non-selective ant, 10 uM) also increased the K+ (25 mM) evoked release of ACh from the three brain areas (34-54%). This effect was not shared by pirenzepine (M1 ant) or 4-DAMP (M3 ant). In summary, [3H]AF-DX 116 specifically binds to two populations of muscarinic receptors in rat brain; some of these binding sites are located presynaptically on cholinergic terminals where they mediate positive feedback modulation of ACh release when activated. (Supported by MRC, Canada and FCAR and FRSQ, Quebec

94.15

REGULATION OF ACETYLCHOLINE RELEASE BY M_2 AUTORECEPTORS T. Scranton* and W. Hoss (Spon: A. Iannone) Department of Medicinal and Biological Chemistry University of Toledo College of Pharmacy Toledo, Ohio 43606

Both biochemical and behavioral data have suggested the existence of presynaptic muscarinic receptors that regulate acetylcholine release in the CNS. In order to characterize these receptors in a direct manner, the effects of muscarinic ligands on acetylcholine release from cortical nerve endings were investigated. Synaptosomes prepared from rat cerebral cortex were preloaded with [^{3}H]-choline and release of [^{3}H]-acetylcholine was stimulated by incubation at 37° in the presence of 25 mM K^+ and 10μM hemicholinium-3 for 5 min.

The muscarinic agonist oxotremorine inhibited acetylcholine release in a dose-dependent manner, reaching a maximal value of 30% at 10μM. The nonselective antagonist atropine as well as the M_2-selective antagonists gallamine and AFDX-116 restored release to the control level in a dose-dependent manner. The M_1-selective antagonist pirenzepine was, however, ineffective. None of the antagonists affected unstimulated release.

These data indicate that presynaptic autoreceptors of the M_2 subtype inhibit the release of acetylcholine from nerve endings in the cerebral cortex of the rat brain.

Supported by NS 23929 and DA 04068.

94.16

STIMULATION OF CENTRAL MUSCARINIC RECEPTORS: PROSTAGLANDIN SYNTHESIS, A REQUIREMENT FOR PHARMACOLOGICAL ACTION. J.J. Buccafusco, V. Magri, D.P. Daly, R.G. Owen and D.K. Parker (SPON: W.J. Jackson). Dept. Pharmacology and Toxicology, Medical College of Georgia and Vet. Admin. Med. Ctr., Augusta, Georgia 30912.

Stimulation of central muscarinic receptors can result in profound increases in arterial blood pressure (BP) in several species, including human. Our earlier studies indicated that the vasoconstrictor response to acetylcholine in the isolated rabbit lung, in situ, was mediated through the release of prostaglandins (PG) (Catravas, et al., J Pharmac Exp Ther, 231:236, 1985). The purpose of this study was to determine whether the pressor response to intracerebroventricular (icv) injection of carbachol (C) in conscious rats was dependent upon brain PG synthesis. Icv injection of 5μg of C produced an immediate increase in BP which became maximal (41mmHg) by 5-10 min after injection and remained elevated for more than 30 min. Pretreatment with either 0.3 or 3μg of indomethacin (IN) 15 min earlier resulted in a dose-dependent inhibition of the C-induced pressor response. The 3μg dose completely eliminated the response. In contrast, the pressor response (up to 36mmHg) to icv injection of nicotine was not altered by IN pre-treatment. Thus, stimulation of muscarinic, but not nicotinic, receptors involved in central BP regulation requires PG synthesis for the expression of its pharmacological response. Supptd: HL30046 and Vet Admin Med Ctr.

CHARACTERIZATION OF NICOTINIC RECEPTORS

95.1

ACETYLCHOLINE RECEPTOR (AChR) IN A MUTANT MUSCLE CELL LINE ACCUMULATES IN THE ENDOPLASMIC RETICULUM. Y. Gu*, C. Murphy-Erdosh* and Z. W. Hall. Dept. of Physiology, Univ. of California, San Francisco, CA 94143-0444.

Using a replica technique, we have previously isolated a variant of the C2 mouse muscle cell line, T^-, whose myotubes make a normal amount of the AChR, but express a reduced amount on the surface (20-25% of wild-type). The remainder is accumulated in an internal pool that is 3-5 times normal levels. Pulse-chase experiments and the kinetics of transfer of internal AChR to the surface after inhibition of protein synthesis suggest that internal T^- AChR consists of two independent populations: ca. 25% is transported to the surface with normal kinetics; and the remainder is not transported to the surface and turns over slowly.

SDS-PAGE and Western blotting of the AChR subunits in T^- myotubes shows that alpha and beta subunits are normal, but that gamma and delta subunits have faster than normal mobilities. Treatment with endoglycosidases H and F shows that gamma and delta subunits of T^- AChR contain only high mannose sugars rather than the normal mixture of high-mannose and complex sugars. Subcellular fractionation of T^- variants demonstrates that internal AChR is not accumulated in the Golgi apparatus, but most likely remains in the endoplasmic reticulum. Our results thus suggest that the primary defect in the T^- variant is either a defect in N-linked glycosylation or a defect in transport.

95.2

CALCITONIN GENE-RELATED PEPTIDE REGULATES PHOSPHORYLATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IN RAT MYOTUBES K. Miles, P. Greengard and R. Huganir (SPON:S.Halpain) The Rockefeller University, New York, N.Y. 10021

The nicotinic acetylcholine receptor (nAcChR) is a ligand activated ion channel which mediates synaptic transmission at the neuromuscular junction. The Torpedo nAcChR has been shown to be a substrate for protein kinase C, cAMP-dependent protein kinase and a tyrosine specific protein kinase. Forskolin and cAMP analogues each increased the phosphorylation of the nAcChR in intact cells suggesting that cAMP-dependent protein kinase regulates phosphorylation of the nAcChR. Phosphorylation of the nAcChR by cAMP-dependent protein kinase has been shown to increase the rate of receptor desensitization. Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide localized presynaptically at the neuromuscular junction which has been shown to increase the synthesis of nAcChR. We report that CGRP increases phosphorylation of the nAcChR in rat myotubes. After exposure to 10^{-7}M CGRP, phosphorylation of the nAcChR δ subunit reached maximal levels within 5-10 minutes whereas phosphorylation of the α-subunit reached maximal levels after 20 minutes. β subunit phosphorylation decreased after CGRP treatment. The time course and specificity of nAcChR phosphorylation induced by CGRP is comparable to that obtained with forskolin. CGRP may be a physiological regulator of nAcChR desensitization at the neuromuscular junction.

95.3

A NON-AGONIST ACTIVATOR OF THE NICOTINIC ACETYCHOLINE RECEPTOR. G. Escalona de Motta, J.A. Prieto*, J.A. Lasalde*, R.M. Hann*, P.A. Ferchmin, and V.A. Eterović. Dept. Biochemistry, Univ. Central del Caribe, Inst. Neurobiology and College of Pharmacy, Univ. of Puerto Rico Med. Sci. Campus, and Dept. Chemistry, Univ. of Puerto Rico at Río Piedras, Puerto Rico, USA

N-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES) was dissolved in acetonitrile with triethylamine and reacted with acetic anhydride. The resulting monoacetyl-TES (MAT) was not a cholinergic agonist, yet it increased the amplitude of miniature end-plate potentials in frog sartorius muscle, without affecting the time-course of miniature end-plate currents. MAT (10^{-7} to 10^{-5} M) also increased the amplitude of ACh-induced tension in frog rectus abdominus muscle. MAT did not appear to alter receptor desensitization produced by repetitive applications of ACh. In other experiments performed on purified receptor from electric organ of Torpedo californica, MAT did not affect the equilibrium binding of [^{3}H]ACh or receptor affinity state transitions induced by preincubation with carbamoylcholine. Therefore, either MAT acts directly at the level of the ion channel without affecting the binding parameters or this compound affects muscle but not electric organ.

(Supported by NINDS-N507464, NIH-MBRS RR08102 and NIH-RCMI RR08102)

95.4

MODULATION OF THE ACTIVITY OF THE NICOTINIC ACETYLCHOLINE RECEPTOR BY A SUBSTANCE ISOLATED FROM *TORPEDO* ELECTROPLAQUE.. B.A. Coleman, G.A. Weiland, & R.E. Oswald. Dept. of Pharmacology, Cornell University, Ithaca, NY 14852.

A substance isolated from *Torpedo* electroplaque was found to modulate the activity of the nAChR. Extracted tissue was applied to C18 columns, washed and eluted with acetonitrile. Eluents were lyophilized and resuspended in water. These eluents inhibited agonist-potentiation of PCP binding to the high affinity site for noncompetitive blockers on the nAChR of *Torpedo* receptor-rich membranes, but had no effect on PCP binding in the absence of agonist. The eluents also inhibited α-bungarotoxin (αBgt) binding to the membranes. Control eluents (treated and extracted columns without tissue) showed little or no effect. By measuring the effect of test eluents on the time-dependence of the inhibition of the initial rate of [^{125}I]αBgt binding by carbamylcholine the eluent appeared to increase the rate of desensitization. No increase in the extent of desensitization at equilibrium was observed at the concentrations tested. Furthermore, test eluents inhibited carbamylcholine stimulated ^{22}Na flux into both reconstituted, purified nAChR vesicles and PC12 cells.

The inhibitory activity was acid and heat stable and was dialyzable through 6,000-8,000 MW dialysis tubing. High concentrations of test eluent inhibited acetylcholinesterase activity, though it is not yet known if the test substance is hydrolyzed by the enzyme. Block of PCP binding and competition for αBgt binding is not affected by 3 mM EDTA suggesting that the substance is not a divalent cation. These studies suggest that a substance(s) may be present in the *Torpedo* electroplaque which is capable of interacting with a binding site(s) on the nAChR. Similar results have been reported for substance P (J. Neurochem. 49:253; Molec. Pharmacol. 32:625). We are currently examining the possibility that this inhibitory activity might be substance P or a structurally-related tachykinin.

95.5

LOW CONCENTRATIONS OF TETRAHYDROAMINOACRIDINE AND PHYSOSTIGMINE BLOCK SODIUM FLUX IN PC12 CELLS. M.T. Edge, G.E. Kemp, and R.J. Bradley, Neuropsychiatry Research Program, University Alabama at Birmingham, Birmingham, AL 35294.

We have studied the effects of two anticholinesterases used in the treatment of senile dementia of the Alzheimer type (SDAT) on the carbachol-induced sodium flux in PC12 cells. This line of cells from a rat pheochromocytoma expresses a ganglionic receptor which exhibits a cholinergic pharmacology. These cells are then particularly well suited for studying, *in vitro*, the drugs used in the treatment of this disease which results in a diminished cholinergic activity in the brains of its victims. Cells were plated onto poly-L-lysine coated dishes and then preincubated with concentrations of physostigmine or 1,2,3,4-tetrahydro-9-aminoacridine (THA) for up to 30 minutes at 37°C in a controlled atmosphere. Sodium flux was initiated by the addition of 600μM carbachol in the presence of 0.7μCi $^{22}Na^+$ for 30 seconds either with or without the anticholinesterase being present. Significant reductions in flux were observed after preincubation with either anticholinesterase. Some reduction in flux was observed with co-introduction of the anticholinesterases and carbachol but without prior incubation. Physostigmine and THA produced similar reductions in flux at high concentrations with > 80% reduction at 50μM physostigmine or THA. Neither anticholinesterase at concentrations of up to 5mM produced appreciable flux in the absence of carbachol. We hypothesize that the reductions in flux observed here are due to desensitizing effects of the anticholinesterases. Although the concentrations of the anticholinesterases used in these experiments were low, it is unclear if they are physiologically relevant. The pharmacological implications of these observations are uncertain at this time as they pertain to the palliative effects such drugs exert in the treatment of SDAT.

Supported in part by MH39115.

95.6

INTERACTIONS OF ALLOSTERIC ANTAGONISTS WITH NICOTINIC ACETYLCHOLINE RECOGNITION SITES IN BRAIN. H. Takayama*, M.D. Majewska and E.D. London (SPON: B. Gold). Neuropharmacol. Lab., NIDA ARC, Baltimore, MD 21224.

Noncompetitive blockers (NCBs) of the nicotinic acetylcholine receptor (NR) interact primarily with the associated cationic channel. We investigated how these and other potential NCBs or effectors of NR interact with nicotinic binding sites in rat forebrain membranes using [^{3}H]-methylcarbamylcholine (MCC), a NR ligand. MCC bound with high affinity to NR receptors (Kd = 1.1 nM, Bmax = 27 fmol/mg protein). Among NR agonists, the order for inhibition of MCC binding was as follows: l-nicotine >> d-nicotine > carbamylcholine >> acetylcholine. Atropine was inactive up to millimolar concentrations. Several NCBs which block the NR-operated cationic channel also affected MCC binding. Local anesthetics, phencyclidine, mecamylamine and hexamethonium inhibited MCC binding at micromolar concentrations. Among NCBs, chlorpromazine (CPZ) had a unique effect (biphasic): CPZ potentiated MCC binding (50 - 80%) below concentrations of 100 μM, and reduced binding at higher concentrations. Enhancement of binding reflected both an increase of affinity and the density of sites, and the decrease was due to apparently competitive displacement of MCC binding. The anesthetic steroid, alphaxalone, also affected MCC binding in a biphasic manner, similar to CPZ. The data suggest that NCBs represent a heterogeneous group of substances which interact with NR by various mechanisms.

95.7

MODULATION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR FUNCTION BY THE THYMIC PEPTIDE FRAGMENT THYMOPENTIN. R. Afar*, J. M. Trifaro* and M. Quik. Dept. of Pharmacology, McGill Univ., Montreal, Que. and Univ. of Ottawa, Ottawa, Ont., CAN.

Thymopoietin affects α-bungarotoxin binding in Torpedo electric organ and impairs function at the neuromuscular nicotinic acetylcholine receptor. Using the biologically active 5 amino acid thymopoietin fragment, thymopentin (TP-5), we sought to assess whether this peptide could also alter nicotinic activity in neuronal cells. To determine this, the effect of TP-5 was studied on the release of ^{3}H-noradrenaline (^{3}H-NA) from bovine adrenal chromaffin cells in culture. TP-5 (10^{-5}M to $3x10^{-4}$M) resulted in a concentration dependent decrease in acetylcholine stimulated ^{3}H-NA release, while basal release was not affected. This decline in stimulated release was optimal after a 20 min exposure of the cells in culture to TP-5. TP-5 (10^{-5}M) decreased overall acetylcholine stimulated ^{3}H-NA release by 22% ($p<0.05$). This decrease in release was more pronounced for the second and third stimulation periods with acetylcholine than for the first. TP-5 inhibited nicotine (3μM) and acetylcholine ($3x10^{-5}$M), but not high potassium (56mM), evoked amine release. Thus, TP-5 appears to selectively inhibit nicotinic sensitivity in adrenal chromaffin cells. These findings indicate that TP-5 may modulate neuronal nicotinic receptor function.

95.8

KAPPA-BUNGAROTOXIN ANTAGONIZES THE ACTIONS OF NICOTINE IN PURKINJE CELLS OF THE RAT CEREBELLUM. **R. de la Garza, Y. Chiappinelli*, B.J. Hoffer, and R. Freedman. Univ. of Colo., Health Sci. Ctr., Dept. of Pharmacol. C236, 4200 E. 9th St., Denver, CO 80262 and Denver VAMC. * St. Louis Univ., St. Louis, MO.**

Recent studies from our laboratory have shown the heterogenous electrophysiological actions of nicotine in the rat cerebellum. The specific NMJ blocker α-Bungarotoxin (α-BTX), antagonized the actions of nicotine on cerebellar interneurons but not on Purkinje cells. To further explore the selectivity of nicotine actions on these cell populations, the snake toxin k-Bungarotoxin (k-BTX), which is selective for ganglionic nicotine receptors, was tested.

The inhibitory actions of nicotine, applied by micro-pressure or microiontophoretic ejection, on Purkinje cells were blocked by K-BTX. The extent of blockade, however, differed depending on the mode of nicotine application. k-BTX produced a small, but significant, 39% reduction in the actions of pressure ejected nicotine (p. 0.01), but a larger 80% reduction in the actions of iontophoretic nicotine (p. 0.001). The present data support the hypothesis of multiple nicotinic sites of action in mammalian brain. The data also suggests that snake toxins may be used to characterize putative functional nicotine receptors.

95.9

PROPERTIES OF NEURONAL NICOTINIC RECEPTORS FROM CHICK RETINA. R.H. Loring[1], E.Aizenman[2], S.A. Lipton[2] and R.E. Zigmond[1]
Depts. of Pharmacology[1] and Neurology[2], Harvard Med. School, and Div. of Neuroscience[2], Children's Hospital, Boston MA 02115

Neuronal bungarotoxin (NBT, 100 nM; also known as bungarotoxin 3.1, toxin F or kappa-bungarotoxin) antagonizes a nicotinically mediated depolarization recorded from intact chick retina, while the neuromuscular blocker α-bungarotoxin (BGT, 10 μM) does not. Receptor function in retina is blocked by treatment with the reducing agent, dithiothreitol, and function is restored by oxidizing with dithiobisnitrobenzoic acid, as has been shown for nicotinic receptor function in muscle and autonomic ganglia. In the presence of 1 μM BGT, ^{125}I-NBT binds to homogenates of chick retina with a k_d of 3 nM and this binding is displaceable by nicotinic drugs. However, unlike immunopurified nicotinic receptors from chick brain (e.g. Whiting & Lindstrom, 1986 J. Neurosci. 6:3061), which have high affinity for nicotine (k_d=3 nM), ^{125}I-NBT binding in chick retina is displaced with low affinity by nicotine (k_i=400 nM). Chick retina does have high affinity ^{3}H-nicotine binding (k_d<20 nM), but this binding is not displaced by 1 μM NBT. Low affinity ^{3}H-nicotine binding is partially displaced by NBT. Thus, the class of functional nicotinic receptors recognized by NBT in chick retina appears to have low affinity for nicotinic agonists. Detergent solubilization by cholate, CHAPS, CHAPSO or Triton X-100 causes alteration of NBT binding sites such that nicotinic ligands no longer displace specific ^{125}I-NBT binding to solubilized sites detected on DEAE-cellulose columns. Since these same detergents readily solubilize muscle nicotinic receptors without pharmacological impairment, neuronal nicotinic receptors from chick retina appear to be more easily denaturable. Supported in part by NS22472, NS12651, EY05477 and a Fight for Sight award.

95.10

ISOLATION OF A cDNA CLONE CODING FOR A NEW NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR a-SUBUNIT. D. McKinnon*, J. Boulter*, E. Wada*, S. Heinemann and J. Patrick. (SPON: G. Lemke). Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92138.

A family of neuronal nicotinic acetylcholine receptor subunit genes has been described in rat and chicken which now includes the genes alpha2, alpha3, alpha4 and beta2. The initial basis for classification of these receptor subunits as either alpha or beta subunits was the presence or absence of two cysteine residues at positions corresponding to positions 192 and 193 of the *Torpedo* electric organ and skeletal muscle a subunit. This classification scheme has been given considerable validity by the demonstration that each of the alpha subunits can form a functional nicotinic receptor when expressed in *Xenopus* oocytes in combination with the beta2 subunit (Boulter et al., 1987, P.N.A.S. 84:7763; Wada et al., 1988, Science 240:330). We have recently cloned another member of the neuronal nicotinic AChR gene family from rat hippocampus and PC12 cDNA libraries. The deduced amino acid sequence of this newly identified subunit has two cysteine residues at positions corresponding to amino acids 192 and 193 of the skeletal muscle a subunit. For this reason the new subunit has provisionally been named alpha5.

Using *in situ* hybridization, the presence of mRNA coding for the alpha5 gene has been demonstrated in the cingulate cortex, endopiriform nucleus, subiculum, layer IV of the isocortex, substantia nigra pars compacta and interpeduncular nucleus of rat brain. The pattern of expression of alpha5 RNA is different from that of the previously identified neuronal nicotinic AChR subunit RNAs. We have also shown that the alpha5 subunit is expressed in both PC12 cells and adrenal chromaffin cells.

While the alpha5 subunit has significant amino acid sequence homology with other neuronal alpha subunits, it is not yet clear whether it is a functional homologue.

95.11

IDENTIFICATION OF A cDNA ENCODING A NEW SUBUNIT OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

E.S. Deneris*, J. Boulter*, J. Patrick, and S. Heinemann. Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138

We identified a family of genes encoding nicotinic acetylcholine receptors (nAchR) expressed in the brain. To determine the extent of neuronal nAchR diversity, brain cDNA libraries were screened at low stringency with probes made from previously characterized cDNAs. A radiolabelled cDNA encoding the alpha3 subunit of neuronal nAchRs was used to screen a cDNA library prepared from mRNA isolated from the diencephalon of the rat. Screening a half million plaques resulted in the isolation of a clone containing an insert of 2.2kb that encodes a 53kd protein similar to but different than previously reported nAchR subunits. The encoded protein has the hallmarks of neurotransmitter-gated ion-channel superfamily subunits including two cysteine residues homologous to cysteines 128 and 142 of the Torpedo alpha subunit. Absent from the deduced primary structure are cysteine residues corresponding to cysteines 192 and 193 of the Torpedo alpha subunit. The greater amino acid sequence identity to the neuronal nAchR subunits relative to the $GABA_A$ or glycine subunits leads us to place this subunit in the neuronal nAchR subunit family. Thus, in view of the absence of two adjacent cysteine residues, we have named the encoded protein neuronal non-alpha2 (non-alpha1 corresponds to beta2). Hybridization in situ has shown that the non-alpha2 gene is expressed in the central nervous system. However, unlike the beta2 gene, mRNA hybridizing to the non-alpha2 probe is found in only a few regions. Strong signals are found in the medial habenula, the substantia nigra pars compacta, and reticular thalamic nucleus.

95.12

STRUCTURAL DETERMINANTS OF FUNCTIONAL DIFFERENCES BETWEEN SUBTYPES OF NICOTINIC ACETYLCHOLINE RECEPTORS. J.G. Connolly*, J. Boulter*, S.W. Rogers*, A. O'Shea*, J. Patrick and S. Heinemann. (SPON: P.D. Gardner). Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138.

We have isolated cDNAs coding for subunits of mouse muscle, (alpha1, beta1, gamma and delta), and rat brain (alpha2, alpha3, alpha4 and beta2) nicotinic acetylcholine receptors (nAchRs). Functional receptors were expressed following injecting combinations of RNAs derived from these clones into Xenopus oocytes, and their electrophysiological responses to a variety of pharmacological reagents were tested. Preliminary data suggest that below 0.01 mM agonist, equal concentrations of acetylcholine and nicotine give approximately equal responses for the alpha2+beta2 and alpha4+beta2 receptors. However, for the alpha3+beta2 and mouse muscle receptors, more than a ten-fold higher concentration of nicotine is required to reproduce the response to acetylcholine. The neuronal receptors were not blocked by alpha-bungarotoxin, but were ,with the exception of the alpha2+beta2 combination, blocked by 3.1 toxin. Thus these homologous receptors are pharmacologically distinct. It is now important to localise the structural bases of these and other receptor properties. We have begun this work by functionally expressing chimaeric receptors in which the extracellular domains of the alpha2 and alpha4 subunits have been reciprocally exchanged.

95.13

CLONING OF GENOMIC SEQUENCES ENCODING SNAKE ACETYLCHOLINE RECEPTOR. D. Neumann*, D. Barchan*, M. Horowitz*, M. Souroujon*, E. Kochva* and S. Fuchs. Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel, and Dept. of Zoology, Tel Aviv University, Tel Aviv, Israel.

The acetylcholine receptor (AChR) at the neuromuscular junction of Elapid snakes binds cholinergic ligands but unlike other muscle AChRs, does not bind α-bungarotoxin (α-BTX). Administration of α-BTX in concentrations several orders of magnitude higher than its LD_{50} for other species did not result in snake death. To characterize snake AChR α-subunit, DNA and RNA were isolated from Natrix tesselata. In Southern blots of snake DNA digested with several restriction enzymes we detected DNA fragments which reacted with a ^{32}P-labeled cDNA probe of the mouse AChR α-subunit. A genomic library of DNA fragments in the range of 4-6 Kb, obtained by EcoRI digestion, was constructed. The library was probed with the mouse AChR α-subunit cDNA and a positive clone containing an insert of 4.3 Kb was isolated and characterized. Partial sequence analysis of this clone revealed a fragment of 530 bp, obtained by PstI digestion, which contains a stretch of 185 bp homologous to the mouse α-subunit cDNA. This stretch corresponds to bases 448-633 of the mouse AChR α-subunit cDNA and codes for amino acid residues 96-157. Using the 530 bp fragment as a probe, a single snake AChR mRNA of approximately 3.7 Kb was detected on Northern blots.

95.14

MAPPING OF MAIN IMMUNOGENIC REGION ONTO THE NICOTINIC ACETYLCHOLINE RECEPTOR SEQUENCE. W. H. M. L. Luyten and J. Lindstrom. Molecular Neurobiol. and Receptor Biol. Labs, Salk Institute, San Diego CA92138.

The nicotinic acetylcholine receptor (nAchR) at the neuromuscular junction is the target of an auto-immune response in the human disease myasthenia gravis. The majority of antibodies in myasthenia patients are directed against a single set of closely-linked conformationally-sensitive epitopes, named main immunogenic region (MIR). The location of the MIR on the primary structure of the nAchR (recently obtained for several species using recombinant DNA techniques) is important for understanding the pathogenesis of myasthenia gravis and for developing a treatment. Ratnam et al. (Biochemistry 25, 2621-2632) found that only three out of 50 MIR monoclonal antibodies (mabs) (at 10-100 nM) bind to *Torpedo* α-subunit (~2 μg) on blots. Barkas et al. (Science 235, 77-80) reported many more MIR mabs binding (at 50-200 nM) to a series of mouse nAchR α-subunit fragments (10-40 μg) expressed from the corresponding cDNA clone as fusion proteins in bacteria; they concluded that the MIR mapped to two distinct epitopes within α6-85. Under similar conditions, however, we could demonstrate binding of MIR mabs to a *Torpedo* α87-208 fragment and even to abundant bacterial proteins, suggesting at least some aspecific binding. Using a number of control and anti-nAchR mabs with known epitopes, we established that more specific immunoreaction was obtained at lower mab (20 nM) and antigen concentrations, and by using 5% nonfat dried milk rather than gelatin or tween as quenching agent. Under these conditions only two MIR mabs (198, 210) reacted specifically with *Torpedo* α-subunit fragments expressed in bacteria. These mabs probably recognise the same epitope since they compete with one another for binding to *Torpedo* α-subunit on blots, but mab210 has the higher affinity on blots (K_D ~5 nM); its epitope lies within α46-92.

95.15

ANTIBODIES AGAINST AN α-BUNGAROTOXIN-BINDING PEPTIDE OF THE α-SUBUNIT OF THE ACETYLCHOLINE RECEPTOR. D. L. Donnelly-Roberts* and T.L. Lentz, Department of Cell Biology, Yale University Sch. Medicine, New Haven, CT 06510.

A 32 amino acid synthetic peptide (32 mer) comprising residues 173-204 of the α-subunit of the Torpedo acetylcholine receptor (AChR) was shown previously to bind α-bungarotoxin (α-Btx) with the same affinity as isolated α-subunit (PNAS 82:8790,1985). Polyclonal and monoclonal antibodies were raised against the 32 mer and tested for their ability to inhibit binding of α-Btx to the 32 mer. This region of the receptor appears to be relatively nonimmunogenic and thus far has yielded only IgMs. The polyclonal antibodies inhibited 42-54% of toxin binding; the most effective monoclonal antibody, 37%; and pooled monoclonal antibodies, 48%. To map the epitopes of the antibodies, their reactivity against shorter peptides comprising portions of the 32 mer was tested. The antibodies that were most effective in inhibiting binding of toxin were directed against residues 185-191. These antibodies also reacted with native receptor, either in membranes or affinity purified and detergent solubilized. The failure of any of the antibodies to completely inhibit toxin binding indicates toxin-binding determinants are not restricted to a single antibody epitope on the 32 mer. Efforts are now underway to obtain high affinity antibodies (IgGs) for testing in other functional assays. Support by NSF grant BNS 85 06404 and NIH grant NS 21896.

95.16

A PROPOSED MODEL FOR INTERACTIONS AT THE AGONIST-BINDING SITE OF LIGAND-GATED RECEPTOR ION-CHANNELS V.B.Cockcroft*, G.G.Lunt, E.A.Barnard*†, D.J.Osguthorpe* Depts. of Biochemistry and Chemistry, University of Bath, Bath, BA2 7AY, U.K. †MRC Unit of Molecular Neurobiology, Hills Road, Cambridge, CB2 2QH

Ligand-gated receptor ion-channels are a super family of proteins in the nervous system of vertebrates and invertebrates. Members of the family include receptors for acetylcholine, GABA, glycine and glutamate. In the nAChR of Torpedo electroplax the region 128-142 of the αsubunit has been implicated in the binding of agonists. This region contains a disulphide bridge and is highly conserved in all members of the super-family. Previous models proposed that the region forms a β-hairpin loop with a type VIa turn. Using computer modelling, we present a general model for ligand recognition in which a formal positive charge on a sp^3 nitrogen atom of the ligand results in an electrostatic interaction with the invariant aspartate residue close to the proposed turn. Specificity is determined by the type of residue on the opposite strand that faces the aspartate and by the residue preceding the cis-proline of the turn. In particular we account for the differences in the stereoselectivity and the potency of a range of compounds that recognise the nicotinic acetylcholine receptor. (We are grateful to Shell Research Ltd and the SERC for their generous support.)

LEARNING AND MEMORY: HIPPOCAMPUS

96.1

BEHAVIORAL EFFECTS OF HIPPOCAMPAL AND DIENCEPHALIC LESIONS IN RHESUS MONKEYS. H. Mahut, M. Moss and W. Grisham*, Psychol. Dept., Northeastern Univ., Boston, MA 02155

Selective ablations of the hippocampal formation in monkeys are necessary and sufficient to impair memory and object-reward association learning (Mahut et al., Neuropsychologia, 19:201,1981; Mahut et al., J. Neurosci. 2: 1214, 1982). But ablations of dorsal-medial thalamus also impair memory in monkeys (Aggleton and Mishkin, Neurosci. Abstr. 7: 1981). In comparing the two post-operative profiles in the same project we found that 1. Monkeys in both operated groups were severely and equally impaired in object recognition (DNMS with 10-130 sec delays and lists of 3-10 items). However, 2. Only hippocampectomized monkeys were impaired in learning object-reward associations (concurrent discrimination between members of 8 pairs of objects). This can be accounted for by the greater sensitivity to inter-item interference on the list version of the DNMS task shown by hippocampectomized monkeys. Finally, 3. Monkeys in both operated groups showed equally rapid rates of forgetting at 10 min, 1 and 24 hr intervals.

These findings suggest that the hippocampal formation and the dorsal medial thalamus share important aspects of mnemonic functions. They also suggest that the precise nature of the distinctions between bitemporal and diencephalic amnesias remains to be specified.

96.2

THE HIPPOCAMPUS AND PLACE MEMORY IN RHESUS MONKEYS. S.J. Angeli*, E.A. Murray and M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

An earlier study (Parkinson et al., J. Neurosci., in press) found that hippocampectomized (but not amygdalectomized) monkeys failed each of two trial types of a spatial memory task: (1) "object-place" trials, requiring one-trial memory for the association of an object and its location; and (2) "place" trials, a version of spatial delayed response requiring one-trial memory for location only. To determine whether the monkeys in that experiment failed the "place" trials because of interference from a strategy needed to remember the more difficult "object-place" trials, we trained naive monkeys on "place" trials only. First the monkey was required to displace two sample objects, each covering an unbaited well on a 3-well test tray; six seconds later the monkey was given a choice between one of the samples and a duplicate, one covering the well it covered previously (now baited, i.e. correct place) and the other covering the previously unused well (unbaited, i.e. incorrect place). Preoperatively, the monkeys scored 85% correct responses, whereas following bilateral hippocampectomy they achieved only 60% correct. The results indicate that a) impairment on the "place" trials in the earlier study was not due to interference from the "object-place" trials, and b) performance on this version of spatial delayed response, unlike performance on the classical version, depends critically on the hippocampus, despite the use of very short intratrial delays.

96.3

GABA-ERGIC NEURONS IN THE RAT HIPPOCAMPAL FORMATION: ULTRASTRUCTURAL CHARACTERIZATION AND SYNAPTIC RELATIONS WITH CATECHOLAMINERGIC TERMINALS. *C.E. Bacon*, C. Abate and T.A. Milner (SPON: D.A. Fischman).* Div. of Neurobiology and Molecular Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

The ultrastructural morphology of neurons containing γ-aminobutyric acid (GABA) and their relation to catecholaminergic terminals exhibiting immunoreactivity for the catecholamine synthesizing enzyme tyrosine hydroxylase (TH) were examined in the rat hippocampal formation (HF) using dual immunoautoradiographic and peroxidase anti-peroxidase labeling methods to simultaneously localize antisera from two different species. By light microscopy, GABA-ergic perikarya and processes co-distributed most noticeably with TH-containing processes in the hilus of the dentate gyrus and in strata lucidum, radiatum and lacunosum-moleculare of the CA3 region of the hippocampus. Thus, these regions were examined further by electron microscopy. GABA-like immunoreactivity (LI) was detected in neuronal perikarya, dendrites and axon terminals. The GABA-containing perikarya were large, ovoid (20x40μm) and contained abundant cytoplasm and an indented nucleus with one nucleolus. Synaptic junctions on perikarya and dendrites with GABA-LI were both symmetric and asymmetric with 63% (55 out of 88) of the pre-synaptic terminals unlabeled. The remaining terminals were immunoreactive for GABA (11%) or TH (26%). Terminals with GABA-LI (0.5-1.6μm) contained numerous small clear and 1-3 large dense-core vesicles. Of the synapses formed by GABA-ergic terminals, 29% (42 out of 143) were with unlabeled perikarya and dendrites; whereas 9% were with perikarya and dendrites containing GABA-LI. Additionally, 23% of the GABA-ergic terminals were in direct apposition with TH-labeled terminals and 14% contacted the same perikarya or dendrite as a TH-containing terminal. The remaining 25% of the terminals with GABA-LI were without any apparent synaptic relations. The results suggest that GABA-ergic neurons can be modulated by both GABA and catecholamines. Moreover, GABA-ergic terminals either alone or in conjunction with catecholaminergic terminals can regulate pyramidal cells and granule cells as well as interneurons in the HF. (Supported by NIH grant MH42834.)

96.4

EFFECT OF FIMBRIA/FORNIX, HIPPOCAMPAL, AND HIPPOCAMPAL/AMYGDALA LESIONS ON PERFORMANCE OF 8-PAIR CONCURRENT OBJECT DISCRIMINATION, OBJECT REVERSAL, AND T-MAZE ALTERNATION IN RATS. C.G. Wible and D.S. Olton. Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Experiments with rats and monkeys lead to conflicting conclusions about the relative contribution of the fimbria/fornix, hippocampus, and amygdala to the memory deficit in amnesia. However, the two species have usually been tested on different tasks and after different types of lesions. The present experiment tested rats in two tasks that have been used previously with monkeys (an 8-pair concurrent object discrimination and an object reversal) and a task that is commonly used to test rats (the T-maze alternation). Lesions of the fimbria/fornix, hippocampus alone, and the hippocampus combined with the amygdala differentially impaired choice accuracy. This experiment will contribute to a resolution of the apparent conflicts between conclusions based on data from rat models and monkey models of amnesia.

96.5

HIPPOCAMPAL MOSSY FIBRE DISTRIBUTION IN RELATION TO TESTS OF SPATIAL MEMORY IN MICE. J. M. Lassalle*. Lab. d'Ethologie, U. de Tours, France, B. Bulman-Fleming and D. Wahlsten. Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Inbred BALB/cWah and C57BL/6J mice and their reciprocal F_1 hybrids were derived from grafted ovaries and then fostered to surrogate mothers to assess effects of maternal environment. After 8 weeks of age, each mouse was tested for water escape learning, memory for spatial arrangement of objects in an open field, and learning of an 8-arm Olton radial maze. At 100 days of age, mice were perfused with sodium sulfide and horizontal sections were stained using the Timm's method to reveal mossy fibres. Measures of hippocampus included a) percent of the area of CA3 and CA4 occupied by the intra- and infrapyramidal mossy fibres, and b) percent of CA3 and CA4 occupied by pyramidal cells below the anomalous intrapyramidal mossy fibre termination typically seen in BALB/c. Both measures of hippocampus showed intermediate inheritance, whereas measures of learning and memory showed either complete dominance or overdominance. Neither measure correlated with behavioral differences within a strain. No maternal environment effects on behavior or hippocampus were detected.

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96.6

HIPPOCAMPAL AND MEDIODORSAL THALAMIC INTERACTION IN MEMORY. K. A. Stokes* and P. J. Best, Dept. Psych., Univ. of Virginia, Charlottesville, VA 22901.

Separate memory systems may exist in the medial and basolateral limbic systems. Both hippocampal (HIPP) and mediodorsal thalamic (MD) lesions impair radial maze performance in rats. If these structures operate as two independent systems, then damage to both should impair performance additively. Rats with MD & HIPP damage showed no improvement on a radial maze task, whereas animals with MD lesions alone showed some improvement over later trials. On two additional tasks requiring an explicit discrimination of extramaze cues, combined lesion of MD & HIPP impaired performance more than MD lesion alone, but not in a directly additive manner.

Although a mass action hypothesis may obtain, we believe that additional hippocampal damage may enhance response perseveration (which may in turn increase errors); however, the hippocampus does not appear to contribute a totally independent mnemonic capacity which is dissociable from the contribution of MD in these tasks. MD and the hippocampus may be part of two interdependent systems, such that lesion of either impairs performance in mnemonic tasks, but for somewhat different reasons.

Supported by NSF grant BNS-8119020 to PJB
KAS is currently at the University of Western Ontario
PJB is currently at the University of New Orleans

96.7

HIPPOCAMPAL ANATOMY AND BEHAVIORAL COVARIATION IN CHIMERIC MICE. I. M. Bär*, W. E. Crusio*, and H. Schwegler* (SPON: A. M. Lefcourt). Institute of Human Genetics, University of Heidelberg, Federal Republic of Germany.

We investigated covariation between behavior and hippocampus anatomy in a population of 35 aggregation chimeras between the inbred mouse strains C57Bl/6J and BALB/ cJ. Factor analysis showed a positive association between learning and activity in a water-maze and the size of the intra- and infrapyramidal mossy fiber (iip-MF) terminal field. A similar relationship of the iip-MF with measures of activity, but not with other exploratory variables, was found in an open-field. These results are partly at variance with results obtained earlier using inbred and hybrid mice [Crusio, W. E., and Schwegler, H., *Behav. Brain Res.* **26**, 153 (1987)]. It is hypothesized that the different results are caused by the albino allele carried by BALB/c mice.

We also examined the hippocampal lamination patterns in the chimeric animals. Adjacent to patterns intermediate between those seen in the donor strains, several MF-distribution patterns not occuring in the inbred strains were observed.

96.8

Hippocampal Inactivation Effects on spatial memory. Edwin J. Barea, Michael R. Hickey* and Douglas C. Smith, Dept. of Psychology, Southern Illinois Univ., Carbondale, IL 62901.

Though it is widely held that the hippocampus (HPC) is involved in memory, the specific role of this structure is still unknown. Olton et al.(1979) proposed that the HPC is involved in "working memory" (WM, information useful only for a given trial), but not "reference memory" (RM, information constant across trials), whereas Rawlins proposed that the HPC is involved in processing all types of mnemonic information whenever the stimuli to be associated are temporally discontiguous (Rawlins, 1985).

We now report the effects of reversible inactivation of the HPC vs the amygdala (AMY) on a working/reference memory task in a 12 arm-radial maze. Long Evans rats learned to retrieve food from 8 WM arms and not to visit 4 unbaited RM arms (Olton & Papas, 1979). Following criterion attainment, Ss were implanted with bilateral cannulae in the HPC (n=11) or the AMY (n=10) and then retrained to a criteria of 7 out of 8 correct for 10 consecutive days. The following day, they received bilateral infusions of 1 µl lidocaine, a Na+ channel blocker. The results expressed as mean number of WM and RM errors pre-, during, and post-lidocaine were: HPC-WM, 0.25, 3.75, and 0.42: HPC-RM=0.42, 1.0, and 0.17. For the AMY-WM mean errors were 0.09, 0.36, and 1.0, while AMY-RM=0.36, 0.45, and 0.55. These findings indicate the HPC but not the AMY must be functional for WM. Animals are now in testing on a delayed nonmatching to sample task and these results will also be presented.

96.9

EFFECTS OF HIPPOCAMPAL LESIONS ON ACQUISITION AND EXTINCTION OF TRACE EYE-BLINK CONDITIONING IN RABBIT. J.R.Moyer,Jr., R.A.Deyo* and J.F.Disterhoft, Dept. of Cell Biology and Anatomy, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Reports of single and multiple neuron activity have demonstrated hippocampal plasticity during eye-blink conditioning in rabbits (Berger & Thompson,PNAS, 75: 1572-1576, 1978). Biophysical studies of hippocampal slices have correlated postsynaptic reductions of a Ca^{++}-mediated potassium current with acquisition of the CR (Disterhoft et.al., PNAS, 83: 2733-2737, 1986). Hippocampal lesions do not impair acquisition of a short-delay eyeblink task in rabbits, but they do affect extinction of delay conditioning (Akase and Disterhoft, 1987). Conflicting reports exist regarding the effects of hippocampal lesions on trace conditioning (in which the CS and UCS are discontiguous).

Subjects were 13 young adult male albino rabbits. Aspiration lesions removed either a) the hippocampus plus overlying neocortex (N=4) or b) overlying neocortex only (N=5). There were 4 sham-operated controls. Rabbits were habituated and then trained using a trace conditioning paradigm employing: a 100ms tone CS, a 300ms trace period (neither CS nor UCS) and a 150ms corneal air puff UCS. All rabbits were trained to a behavioral criterion of 2 consecutive sessions of at least 80% CRs. 24 h later, 3 extinction sessions (CS but no UCS) were begun. Analysis of the last 2 days of training indicated no differences between the groups in trials to criterion, %CRs, CR or UCR amplitudes. During extinction, however, hippocampal lesioned rabbits showed significantly more responses ($F(2,10)=4.12$, $p<.05$) and larger response amplitudes ($F(2,10)=3.09$, $p<.029$). Lesioned rabbits also showed no change in mean response latency while the other groups progressively increased in latency ($F(2,10)=3.99$, $p<.05$), so that by the 3rd day, the response latency for both control groups was nearly twice that of the lesioned group. None of these differences between the groups were seen during acquisition, so they must be related to the extinction process. These results suggest that the hippocampus may be involved in an inhibitory modulation of brainstem/cerebellar circuitry during extinction of trace conditioning. Further studies are being conducted to test acquisition and retention of more difficult conditioning tasks. These should provide additional information as to the role of hippocampal plasticity in associative learning.

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96.10

ELECTROPHYSIOLOGY OF CORTICAL-HIPPOCAMPAL CONNECTIONS. A. M. Borroni, R. L. Berry, and T. J. Teyler. Dept. of Neurobiology, NE Ohio Univ. College of Medicine, Rootstown, Ohio 44272.

A recent theory of hippocampal function, the "Memory Indexing Theory" (Teyler & DiScenna, 1985, Neurosci. Biobehav. Rev.,9, 377-89), proposes that the hippocampus indexes cortical sites associated with a given experience and, by later reactivating these sites, allows recall of the experience. To test this theory, the present study investigates the electrophysiology of cortical-hippocampal connections.

Adult male Long Evans rats anesthetized with urethane were placed in a stereotaxic apparatus and the cerebral cortex exposed. The cortex was electrically stimulated at 1 mm intervals and field potentials recorded in the dentate hilus, yielding electrophysiological maps of the cortical projection to the hippocampus. Responses in the septal hippocampus were maximal when anteromedial cortex was stimulated. Current source density analysis of these responses revealed current sinks (50 msec latency) located in the dendritic fields of the dentate gyrus granule cells, suggesting a multisynaptic pathway through the entorhinal cortex. Data obtained from different hippocampal locations will be presented. Supported by ONR grant #86K0664.

96.11

FIMBRIA-FORNIX LESIONS IN RATS IMPAIR CHOICE ACCURACY IN AN OBJECT DELAYED MATCH-TO-SAMPLE DISCRIMINATION. A.L. Markowska* and D.S. Olton. Dept. of Psychology, The Johns Hopkins Univ., Baltimore, MD 21218.

Memory in primates has been studied extensively with non-spatial visual discriminations. In order to conduct parallel research in rats, this experiment reports the effect of fimbria-fornix damage in rats upon performance of a delayed-match-to-sample task with objects. The usual procedures of a sample, delay, and choice were followed in a water maze. The objects were hung in two different locations; a hidden platform was placed below the water beneath the correct object. The choice accuracy of control rats was about 80%, demonstrating that this technique is useful to teach rats object discriminations. Postoperatively, the fimbria-fornix rats performed at chance, showing that the fimbria-fornix in rats is required for object-relevant / space-irrelevant memory.

96.12

HIPPOCAMPAL AND PARIETAL CORTEX LESIONS IMPAIR STONE-MAZE ACQUISITION IN RATS. R. F. Berman[1], R. P. Kesner[2], and H. J. Altman[3]. [1]Dept. Psych., Wayne State Univ., [2]Dept. Psych., Univ. of Utah, [3]Dept. Psychiatry, Lafayette Clinic, Detroit, MI.

The Stone-maze is a 14-arm sequential T-maze which rats learn to traverse for food reward. It has been used to assess learning deficits in aged rats, but little has been reported concerning the effects of specific brain lesions on acquisition of this maze task. Therefore, we have compared Stone-maze acquisition in young rats with either hippocampal, parietal, medial prefrontal or dorsolateral frontal cortex lesions with that of sham-lesioned and unoperated control animals. Hippocampal lesions were made electrolytically, all cortical lesions were made by aspiration, and all lesions were made bilaterally. Rats with hippocampal or parietal cortex lesions were significantly impaired in the acquisition of the Stone-maze compared to control animals. In contrast, the maze acquisition of medial prefrontal and dorsolateral frontal cortex lesioned animals was not impaired. These results demonstrate differential involvement of specific cortical areas in maze learning. The results also suggest a parallel involvement of the hippocampus and parietal cortex in maze learning, and thus are similar to previous findings obtained from studies using other complex maze tasks (e.g., water maze). (Supported by NIH Grant RR-08167).

96.13

PROPOSITION OF A NEW CONCEPT CONCERNING TO THE THREE-DIMENSIONAL NEURONAL CIRCUITRY IN THE RAT HIPPOCAMPUS. N. Tamamaki* and Y. Nojyo* (SPON: K. Yoshida). Dept. of Anatomy, Fukui Medical School, Fukui 910-11 Japan.

On the basis of physiological data, the excitatory pathways in the hippocampus has been said to be lamellarly organized. However, the neuroanatomical data, up to this time, did not support enough the existence of such organization. In this study, granular cells and pyramidal neurons in the serial hippocampal fields of rat brains were intracellularly labeled with HRP under anesthetia (pentobarbital 50 mg/kg i.p.), and the axonal projection patterns of these neurons were analyzed in three dimensions with computer graphics.

Caudally directed axon branches of CA1 pyramidal neurons were parallel to the alvear fibers. Most projection fibers of CA2 and CA3 pyramidal neurons to field CA1 were also arranged parallel to the alvear fibers, while these pyramidal neurons had wide spreading association fibers. Therefore, pyramidal neurons generally seemed to form lamellae parallel to the alvear fibers. On the other hand, the mossy fibers originating from the granular cells did not run parallelly to the alvear fibers along their full length. Therefore, the granular cells in the dentate gyrus seemed to form lamellae in the regio inferior intercrossing with the lamellae formed by pyramidal neurons. This study revealed that the serial excitatory pathways in the hippocampus can not be confined within a section of several hundred micrometers thickness.

96.14

INTERTRIAL INTERVAL AND SINGLE-ALTERNATION LEARNING IN INFANT RATS WITH LESIONS OF HIPPOCAMPUS OR OF HIPPOCAMPUS AND AMYGDALA. A. Amsel, P.L. Greene, N.J. Lobaugh, and T. Nick. Dept. Psychology, Univ. Texas, Austin, TX 78712.

These experiments were designed to investigate the role of the developing hippocampus and amygdala in long intertrial-interval (ITI) patterned (single) alternation (PA) in the infant rat. PA is a kind of go/no-go learning that produces differential responding on alternating reward and nonreward trials in the absence of differential external cues. In these experiments, rats were given two bilateral, electrolytic hippocampal lesions (HPPX) or sham surgeries (SHAM) at 10 or 11 days of age, and were trained 6 days later in a straight runway. In Experiment 1, rats received 120 trials in one day, with 8, 15, or 30-sec ITI. PA learning occurred in both HPPX and SHAM pups at the 8 and 15-sec ITIs, but only in SHAMs, to a reduced degree, at the 30-sec ITI. In Experiment 2, training was extended to 240 trials over two days, with 30 or 60-sec ITIs. Both SHAM and HPPX pups showed PA at the 30-sec ITI, while only the SHAMs showed PA learning at the 60-sec ITI. In Experiment 3, pups with combined hippocampal and amygdala lesions were trained at 8-sec ITI. The combined lesion significantly delayed the emergence of PA. The results of these experiments argue for a role of the hippocampus in single-alternation learning with long intertrial intervals and suggest that even at 16 days of age and an 8-sec ITI, the effects of combined hippocampal+amygdala lesion are stronger than hippocampal lesions alone.

96.15

SCOPOLAMINE AND FIMBRIA-FORNIX LESIONS IMPAIR CHOICE PERFORMANCE OF F-344 RATS IN A NEW COMPLEX SHOCK-MOTIVATED SIX DETOUR MAZE.

E. Bresnahan, P. Wiser*, N. Muth*, and D. Ingram*. Essex Community College, Baltimore, MD 21237 and Gerontol. Res. Center, NIA, Baltimore, MD 21224.

A new complex maze task is used for within-subject assessment of various cholinergic manipulations. Seven 6-mo male F-344 rats were trained to high active avoidance (AA) performance levels in the white straight runway portion of the maze. Next with the AA contingency still enforced, rats were required to learn different combinations of black alleyways representing detours from the straight path but leading to the goal. Additional brief shock was given for errors, entries into blocked alleyways. They were trained for 3 mo with one problem/day from a set of six 1-detour and twelve 2-detour problems, using a learning set approach (2 sample and 10 choice trials). Next using a 2/3 correct choice-trial criterion, performance was evaluated for 1 mo on 3 problems/day from the same 18-problem set. When all rats achieved criterion within 3 trials (weekly average), experimental manipulations were provided with this procedure. First, in a counterbalanced design, rats received 0.3 or 1.0 mg/kg scopolamine hydrocholoride (S) during 2 wks. Compared to 3 no-drug wks, S increased errors and trials to criterion in a dose-dependent manner ($p's < 0.01$). Using a more difficult problem set (2-detour only) in a second 5-wk period, 0.1 and 0.3 mg-Kg doses of physostigmine (P) did not affect errors or trials to criterion but did increase run-times ($p < 0.05$). Next, after 1-wk baseline performance using the original 18-problem set, rats received bilateral electrolytic fimbria-fornix (FF) lesions with the objective of interrupting septal-hippocampal cholinergic systems. Following 2 wks of recovery, testing continued for 6 wks. A deleterious effect of FF lesions ($p's < 0.001$) was observed on all performance measures. Recovery of function as observed in several measures was noted beginning with wk-4 of retest and clearly was evident by wk-6. Thus, this maze paradigm can be used to assess involvement of cholinergic systems in this flexible memory task utilizing a within-subjects design.

96.16

HIPPOCAMPUS, AMYGDALA, AND CROSS-MODAL ASSOCIATIONS. R. J. Sutherland, R. J. McDonald* and J. W. Rudy. Dept. of Psychol., Univ. of Lethbridge, Lethbridge, Canada, T1K 3M4 and Dept. of Psychol., Univ. of Colorado, Boulder, CO, 80309.

We propose that the hippocampal formation is necessary for acquiring and storing configural associations but not simple associations. Configural associations must be acquired to solve tasks in which prediction of a reinforcer requires an appreciation of the relationship among cues.

As a test of this idea we used a nonmatching-to-sample task for rats similar to one developed by Aggleton et al (1986). Three pairs of boxes contained distinct visual cues (white, black or striped walls) and olfactory cues (almond, patchouly or cheap aftershave, respectively). For each choice the rats were presented with two boxes, one identical to the box they had just exited and one that was different; they were rewarded for choosing the different one. Preoperatively, all rats solved the bimodal version and solved both unimodal versions. Half of the rats received hippocampal lesions; the other half received amygdala lesions. Postoperatively, all rats solved the bimodal and both unimodal problems. Hippocampal lesion rats failed to solve either cross-modal version (visual-olfactory or olfactory-visual). Amygdala lesion rats solved both cross-modal problems.

The results imply that the hippocampus is essential for solving tasks that require the animal to acquire configural associations among cues.

96.17

THE ROLE OF THE HIPPOCAMPUS IN THE ACQUISITION AND RETENTION OF CONFIGURAL ASSOCIATIONS. J.W. Rudy and R.J. Sutherland Psych Depts, University of Colorado, Boulder Colorado 80309, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

The negative patterning discrimination problem can be arranged by reinforcing the animal for bar pressing when either a light or tone is presented (L+/T+) but nonreinforcing the response when the compound stimulus consisting of the light and tone is presented (TL-). To solve this problem, the animal must be able to construct a unique configural representation of the compound (<TL>) that can be distinquished from the representations of the individual elements (T and L). We have proposed (Sutherland & Rudy, under review) that the hippocampal formation is essential for the acqusition and retention of associations involving configural representations. Consequently, our position predicts that animals with hippocampal damage will not solve the negative patterning problem and that damage to the hippocampal formation of animals who previously solved the problem will result in their failure to retain the solution. The results of two separate experiments support our position. Animals with hippocampal-formation damage neither acquired nor retained the negative patterning discrimination. These animals, however, were able to solve a simple discrimination in which they were rewarded for responding in the presence of a light but nonrewarded for responding in the presence of a tone.

96.18

HIPPOCAMPAL SIZE IN FOOD-STORING BIRDS. D.F. Sherry*, A.L. Vaccarino, K. Buckenham* and R. Herz*. (SPON: C. Thinus-Blanc). Department of Psychology, University of Toronto, Toronto, Ontario Canada M5S 1A1.

Black-capped chickadees (Parus atricapillus) recover their scattered caches of stored food by remembering the spatial locations of caches. Bilateral hippocampal aspiraton disrupts cache recovery in these birds without affecting the storing of food or the intensity of search for caches. Hippocampal birds search for caches they have made but search in the wrong places. The disruption is due to impairment of both memory for places and working memory (Sherry, D.F. & Vaccarino, A.L. Behav. Neurosci. in press).

Some species of birds cache food, but most do not. Volume of the hippocampus and telenchephalon were determined from serial brain sections for 29 species of birds from 9 taxonomic families. Sections were stained, enlarged and digitized to determine sectional areas and volumes of hippocampus and telencephalon. Three families that are predominantly food-storing, Paridae, Sittidae, and Corvidae, had a larger hippocampus relative to telencephalon volume and body weight than did non-storing families. Natural selection on memory capabilities, arising from food-storing behaviour, may have resulted in greater relative hippocampal size in food-storing birds.

96.19

INTRADENTATE COLCHICINE-INDUCED IMPAIRMENT OF REFERENCE MEMORY: SPATIAL VS. NONSPATIAL. Kevin P. Nanry*, William R. Mundy, and Hugh A. Tilson (SPON: Ronnie L. McLamb). National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, North Carolina 27709.

Male, Fischer-344 rats received bilateral injections of 2.5 µg of colchicine/site in the dorsal and ventral hippocampus. Intradentate colchicine preferentially destroyed dentate granule cells. Subsequent behavioral studies showed that three weeks after dosing, colchicine impaired the acquisition of a spatial, reference memory task in the Morris water maze. In a second group of rats which were trained in the water maze prior to dosing, intradentate colchicine impaired retention of this task when rats were tested three weeks later. The acquisition of nonspatial reference memory task, an autoshape response in an operant chamber, was facilitated by prior administration of colchicine. Facilitative effects of colchicine were seen if delays of 0, 4, or 6 sec were interposed between response and presentation of food reinforcement. If rats were trained on an autoshape response with either a 0 or 4 sec delay between response and reinforcement, intradentate colchicine had no effect on retention of the response 3 weeks later. These data are in accord with the conclusion that the dentate gyrus plays an important role in the acquisition of new information and retrieval of previously learned material and is an integral neural substrate for spatial, reference memory in the rat.

96.20

INTRADENTATE COLCHICINE IMPAIRS ACQUISITION OF A TWO-WAY ACTIVE AVOIDANCE RESPONSE IN A Y-MAZE. W. R. Mundy, R. L. McLamb, and H. A. Tilson. LMIN, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

The present experiment was designed to test the hypothesis that increased two-way avoidance responding after hippocampal damage may be related to a nonassociative process. Male Fischer-344 rats received bilateral injections of colchicine (2.0 ug/site) into the dorsal and ventral hippocampus to destroy dentate gyrus granule cells. Acquisition of a brightness discrimination, active avoidance task in a Y-maze was measured in 120-trial sessions given once weekly for three or four weeks. Colchicine lesions impaired the ability of rats to learn the task. If rats were trained prior to intradentate colchicine, significant effects on performance were not observed. In a third experiment, rats were tested in a modified procedure in which only the tone CS and light cue were presented; shock was not presented. Colchicine-treated rats showed a response bias under these conditions, entering the unlighted arm with greater frequency than the lighted arm when the tone and light were activated. These data are consistent with other reports indicating that rats with hippocampal lesions are impaired in the acquisition of a simultaneous discrimination if a response bias exists to one of the choices.

EXCITATORY AMINO ACIDS II

97.1

ROLE OF A G-PROTEIN IN THE REGULATION OF BINDING TO THE NMDA RECEPTOR COMPLEX. A.I. Sacaan, L.D. Snell* and K.M. Johnson. Dept. Pharmacol. & Toxicol., Univ. Texas Med. Br., Galveston, TX 77550.

We have recently reported that glutamate (GLU) and glycine (GLY) increase the affinity of the phencyclidine (PCP) receptor associated with the N-methyl-D-aspartate (NMDA) ionophore. To determine whether these receptor changes involve guanine nucleotides, we examined the effects of guanosine 5'-O-3-thiotriphosphate (GTPγS) on binding at this receptor complex. GTPγS potentiated GLU-induced ^{3}H-TCP binding while it inhibited GLY-induced ^{3}H-TCP binding. GTPγS decreased the binding of NMDA-specific ^{3}H-GLU and ^{3}H-GLY. The IC_{50} values of GLU and the selective NMDA receptor antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) for displacing ^{3}H-GLU were shifted to the right by the presence of 10µM GTPγS suggesting a decrease in the affinity of the NMDA receptor. The IC_{50} of GLY for displacing ^{3}H-GLY binding was not significantly altered by GTPγS, whereas that of CPP was shifted to the right. Since the CPP displacement of ^{3}H-GLY binding probably occurs via an indirect modulation of the glycine site by action at the NMDA receptor, these data suggest that GTPγS inhibition of GLY-induced ^{3}H-TCP binding and ^{3}H-GLY binding are also mediated through a decreased affinity at the NMDA receptor. Our data point to the possible involvement of a G-protein in the function of the NMDA/PCP/glycine receptor complex. DA-02073.

97.2

HIGH AND LOW AFFINITY SITES FOR ^{3}H-MK801: ALLOSTERIC EFFECTS OF GLUTAMATE AND GLYCINE ON RECEPTOR PROPERTIES. N. R. Mason, and L. G. Mendelsohn Lilly Research Labs., Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285

Electrophysiological studies have demonstrated modulation of excitatory amino acid receptors of the NMDA-type by phencyclidine (PCP) and MK801. Biochemical data provides further evidence of direct coupling between the NMDA receptor, PCP/MK801 receptor, and the non-strychnine sensitive glycine (GLY) receptor. These interactions suggest that drugs which act through these mechanisms may have utility in the treatment of diseases involving the excitatory amino acids, e.g. ischemia, epilepsy, psychosis, cognitive disorders and neural degeneration. We have established a receptor binding assay for the PCP/MK801 receptor using ^{3}H-MK801, in order to further study these phenomena. Rat forebrain membranes were characterized with respect to the effect of repeated washings to remove endogenous glutamate (GLU) and GLY, the effect of addition of GLU and GLY, and the interaction of a variety of drugs with the MK801 site. Repeated washing of forebrain membranes resulted in a decrease in the radioactivity of ^{3}H-MK801 bound to the receptor. The addition of GLU (100 µM) increased the amount of ^{3}H-MK801 associated with membrane receptors. Concentration response curves using GLU, GLY or the two together showed a greater than additive stimulation of binding. Competitive binding studies using unlabeled MK801 or PCP and well washed membranes produced biphasic displacement curves. Analysis with LUNDON software resolved two binding sites: a high affinity component (KD, 30 µM) and a low affinity site (Kd, 5 µM). GLU or GLY increased the affinity of MK801 for the high affinity site (Kd 3nM) with no affect on the low affinity site. Concentration response studies with CPP, D-AP5, and PCP showed that PCP could fully displace MK801 at both receptors. The NMDA blockers were ineffective at the low affinity site even at 100 µM although they are effective (non-competitive) blockers at the high affinity site. The results suggest that this second site is not coupled to the NMDA-type of glutamate receptor and that binding of MK801 or PCP is not affected by GLU or GLY agonists or antagonists at this site.

97.3

SCHILD PLOT ANALYSIS OF THE INTERACTIONS OF GLYCINE AND KYNURENIC ACID AT THE N-METHYL-D-ASPARTIC ACID RECEPTOR. Linda M. Pullan and Julie A. Cler*. Searle R & D/Monsanto, CNS Diseases Research, St. Louis, MO 63198.

Glycine and D-serine enhanced N-methyl-D-aspartic acid (NMDA) stimulated ^{22}Na flux from rat hippocampal slices. Glycine was slightly more effective than equimolar D-serine. The potentiation by glycine was concentration dependent and apparently not competitive to NMDA, with a maximal effect (30% enhancement) near 200μM. Glycine also reversed the kynurenic acid inhibition of NMDA stimulated ^{22}Na flux, increasing the flux several fold. The glycine potentiation was not competitive to kynurenic acid. Kynurenic acid was not competitive to NMDA with a Schild plot slope with an absolute value significantly different than 1. Addition of glycine resulted in a slope with an absolute value closer to 1. These results imply that kynurenic acid interacts at both the glycine recognition site and the NMDA recognition site of the NMDA receptor complex.

97.4

D-CYCLOSERINE EXHIBITS PARTIAL AGONIST CHARACTERISTICS FOR THE N-METHYL-D-ASPARTATE COUPLED GLYCINE RECEPTOR. W.F. Hood, R.P. Compton and J.B. Monahan. Searle CNS Diseases Research & Development, Chesterfield, MO 63198.

Using synaptic plasma membranes, we report that D-cycloserine displaces strychnine-insensitive [^{3}H]glycine binding (K_i=2.33μM) to a recognition site having properties consistent of a N-methyl-D-aspartate (NMDA) receptor modulatory site. Like glycine, D-cycloserine is a positive modulator of the NMDA receptor as shown by its dose-dependent enhancement of [^{3}H]1-(1-(2-thienyl)cyclohexyl) piperidine ([^{3}H]TCP) binding (EC_{50}=12.6μM). Further examination of this enhancement indicates that the maximal stimulation of [^{3}H]TCP binding by D-cycloserine is lower than that produced by other compounds acting at the glycine modulatory site (glycine and D-serine). Consequently, D-cycloserine can be shown to antagonize glycine stimulation. Hence, the stimulation of [^{3}H]TCP binding induced by D-cycloserine in the presence of various concentrations of glycine results in a family of dose response curves that asymptotically converge to 40-50% of the maximal glycine stimulation. These results are consistent with D-cycloserine acting as a partial agonist at the glycine modulatory site of the NMDA receptor complex.

97.5

KYNURENIC ACID UNMASKS GLYCINE MODULATION OF NMDA-EVOKED [^{3}H]NEUROTRANSMITTER RELEASE FROM RAT BRAIN SLICES. R.W. Ransom. Merck Sharp & Dohme, West Point, PA 19486.

Kynurenic acid (KYN) has been shown to be an antagonist at the glycine modulatory site associated with the NMDA receptor. The present experiments were designed to determine whether KYN can be used to detect glycine regulation of NMDA receptor activation in rat brain slices in vitro. NMDA-stimulated release of [^{3}H]norepinephrine (NE) was noncompetitively blocked by KYN (IC_{50} = 102 μM). Glycine, D-serine, L-serine, D-alanine, and L-alanine were able to reverse this inhibition with potencies that correlated with their abilities to displace strychnine-insensitive [^{3}H]glycine binding to rat cortex membranes. Glycine and D-serine had no effect on the inhibition of NMDA-stimulated [^{3}H]NE release by D-AP5, MK-801, or Mg^{2+}. Also, neither amino acid modified KYN inhibition of kainic acid-induced [^{3}H]NE release. Rat striatal slices were used to study NMDA-stimulated [^{3}H]acetylcholine and [^{3}H]dopamine release. KYN inhibition of the release of both neurotransmitters was prevented by low concentrations of either glycine or D-serine. These experiments confirm that, 1) KYN possesses glycine antagonist behavior at the NMDA receptor and, 2) the NMDA receptors mediating hippocampal [^{3}H]NE release and striatal [^{3}H]acetylcholine and [^{3}H]dopamine release are regulated by endogenous glycine.

97.6

GLYCINE REQUIREMENT FOR ACTIVATION OF N-METHYL-D-ASPARTATE RECEPTORS IN XENOPUS OOCYTES. **N.W. Kleckner* and R. Dingledine (SPON: B. Pallotta). Dept. of Pharmacology, University of North Carolina, Chapel Hill, NC 27599.**

Strychnine-insensitive glycine binding sites that co-localize with with N-methyl-D-aspartate (NMDA) binding sites in the brain are thought to be responsible for potentiation of NMDA receptor responses by glycine. We showed previously that rat brain mRNA injected into Xenopus oocytes encoded NMDA receptors that were regulated by glycine. Now we have found that glycine is required for NMDA receptor activation in oocytes. Xenopus oocytes were voltage clamped with one or two microelectrodes. At a holding potential of -60 mV in nominally Mg-free medium, application of NMDA by perfusion (300 μM) or brief pressure ejection (10 mM) elicited inward currents in the presence of glycine (66±13 nA and 5.1±0.7 nA, respectively), whereas in the same cells the NMDA-induced current in the absence of added glycine was negligible (0.6±0.3 nA and 0.04±0.02 nA, respectively). Dose-response curves for glycine at a fixed NMDA concentration (100 μM) showed an EC_{50} of 670 nM and a Hill slope of 1.35. The estimated current induced by NMDA in the absence of glycine was not significantly different from zero. D-serine and D-alanine mimicked the action of glycine while several other analogs did not. The obligatory nature of glycine at the NMDA receptor suggests that 1) its mechanism of action may more closely resemble that of essential cofactors of enzymes than of allosteric modulators of other neurotransmitter receptors, and 2) glycine uptake and perhaps glycinergic synapses may be important in NMDA receptor function.

97.7

POTENTIATION BY GLYCINE OF N-METHYL-D-ASPARTATE INDUCED EXCITOTOXICITY IN RAT CORTICAL CELL CULTURE. D. McNamara* and R. Dingledine. (SPON: R. Meeker). Dept. Pharmacol. Univ. North Carolina, Chapel Hill, NC 27599.

Glycine is known to potentiate current flow through NMDA channels. To determine whether glycine regulates the excitotoxic effect of NMDA, high density dissociated cultures prepared from E19 rat cortex were challenged on day 11 with 100 μM NMDA in the presence of 0-1000 μM added glycine or its analogues. The exposure period was limited to 5 min to minimize the effect of endogenous glycine released by the cells, after which cells were incubated overnight in fresh growth medium plus 100 μM D-APV to prevent toxic effects of residual NMDA. The degree of cell death was judged by the amount of lactate dehydrogenase (LDH) activity released into the medium, and was expressed as a fraction of the maximum amount of LDH released by exposure to NMDA plus 100 μM glycine for 1 hr. In the absence of added glycine, 100 μM NMDA released 12% of the releasable LDH pool; however, a few μM glycine was detected in the medium by HPLC after a 5 min incubation. Added glycine potentiated the toxic effect of NMDA, with an EC50 of 24 μM (95% CI 6-81). D-APV (100 μM) abolished the toxic effect of NMDA plus 100 μM glycine, but 5 mM Mg was without effect. Glycine by itself (1000 μM) was not toxic. Several glycine analogues, including D-serine and D-alanine, were ineffective. Thus the excitotoxic effect of NMDA is less sensitive to glycine and Mg than is the activation of NMDA receptors in oocytes or excised patches of membrane. It is possible that NMDA modulatory sites are themselves regulated in intact tissues.

97.8

GLYCINE POTENTIATES NMDA RECEPTOR MEDIATED QUINOLINIC ACID NEUROTOXICITY IN RAT STRIATUM

Kenton J. Swartz, Joseph B. Martin and M. Flint Beal

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Johnson and Ascher [Nature, 325, 5 (1987)] demonstrated that the putative amino acid neurotransmitter glycine allosterically potentiated the NMDA response in vitro. In the present experiments we examined whether glycine can potentiate excitotoxin lesions acting at the NMDA receptor in vivo. Glycine was co-injected at concentrations of 120 nmol, 240 nmol and 480 nmol with quinolinic acid (120 nmol) into the anterior striatum of the rat. All injections were made in a total volume of 1.0 ul. Controls consisted of quinolinic acid (120 nmol) alone, glycine (960 nmol) alone and 0.9 % saline alone. GABA and substance P-like immunoreactivity (SPLI) were measured as markers for medium-sized spiny striatal neurons and somatostatin-like immunoreactivity (SSLI) and neuropeptide Y-like immunoreactivity (NPYLI) were measured as markers for medium-sized aspiny striatal neurons. Quinolinic acid (120 nmol) lesions resulted in a 20.5 % decrease in GABA and a 23.0 % decrease in SPLI while co-injection of quinolinic acid (120 nmol) and glycine (480 nmol) resulted in a decrease of 37.8 % in GABA ($p < 0.01$) and a 52.2 % decrease in SPLI ($p < 0.01$). Increasing doses of co-injected glycine resulted in dose-dependent increases in the excitotoxin lesions as evaluated by decreases in GABA and SPLI. Saline and glycine (960 nmol) injected controls resulted in no significant change in the content of GABA and SPLI. SSLI and NPYLI were spared with all of the quinolinate lesions. Glycine therefore potentiates NMDA receptor mediated quinolinate neurotoxicity in vivo. This raises the possibility that a defect in glycine metabolism and/or neurotransmission could result in excitotoxic mediated neurodegeneration.

97.9

GLYCINE REGULATION OF THE NMDA RECEPTOR COUPLED CHANNEL IN HIPPOCAMPAL MEMBRANES: STRUCTURE/ACTIVITY RELATIONSHIPS. L.A. Skaryak*, D.W. Bonhaus and J.O. McNamara. Duke Univ. and V.A. Med. Ctr. Durham, NC 27705.

Glycine, an inhibitory neurotransmitter in the spinal cord, markedly potentiates NMDA evoked currents in cultured neurons and in frog oocytes injected with rat MRNA. We tested whether glycine also potentiates NMDA dependent channel activation in hippocampal membranes from adult rat. Using the apparent association rate constant of [3H]N-(1[thienyl]-cyclohexyl) piperidine (TCP) as an index of NMDA dependent channel opening, we found that: 1) glycine dose dependently increased NMDA dependent channel activation; 2) this response was strychnine insensitive; 3) D-serine, D-alanine, L-serine, L-alanine and B-alanine were all equally efficacious at activating the NMDA receptor coupled channel; 4) the rank order of potency of these glycine agonists in activating the channel matched their potency in displacing [3H]glycine from membranes derived from rat cortex (Kishimoto et al. 1981).

a.a.	gly	d-ser	d-ala	l-ser	l-ala	b-ala
EC50 (uM)	0.31	0.42	0.74	26.7	68.7	894.0

These results support the idea that a stereoselective glycine receptor, as part of the NMDA receptor/channel complex, regulates NMDA evoked currents in hippocampus.

97.10

RADIOHISTOCHEMICAL DEMONSTRATION OF NMDA/GLYCINE RECEPTOR COUPLED ION CHANNEL ACTIVATION. D.A. Hosford, D.W. Bonhaus & J.O. McNamara, Depts. Medicine(Neurology) & Pharmacol., Duke Univ. and V.A. Med. Ctrs., Durham, NC, 27705.

Kinetic analyses indicate that NMDA and glycine regulate ^{3}H-TCP binding to hippocampal membranes by controlling its access to a "guarded" binding site (Bonhaus, D.W. et al., this vol.), presumably within the NMDA channel. The intent here was to extend these findings from isolated membranes to slide-mounted sections of rat brain by using a radiohistochemical method.

Sections were preincubated in 50 mM Tris-HCl; incubated in 5 mM Tris-HCl with 2 nM ^{3}H-TCP and combinations of glycine, NMDA, PCP and other drugs; washed; dried; exposed to ^{3}H-sensitive film; analyzed with RAS/R1000 image analysis system (Loats). Specific ^{3}H-TCP binding was enhanced by NMDA (D-APV-blockable) and glycine (strychnine-insensitive) but not by AMPA or kainate. NMDA and glycine greatly (300 %) enhanced binding in dentate stratum moleculare and strata radiatum/oriens of CA1/CA3 of hippocampus but not in cerebellar granule cell layer. This parallels electrophysiologic studies that show NMDA channel activation in CA1 but not in adult cerebellar granule cells, and further validates the use of NMDA/glycine-regulated ^{3}H-TCP binding as a molecular marker of NMDA channel activation. This radiohistochemical method is a powerful tool that permits in situ functional analysis of the NMDA receptor/channel complex in discrete neuronal populations in physiologic and pathologic states. (funded by V.A. Merit Review & NIH grants)

97.11

AGONIST ACTIVATION OF THE NMDA RECEPTOR COUPLED CHANNEL IN HIPPOCAMPAL MEMBRANES: MEASUREMENT WITH [3H]N-(1[THIENYL]-CYCLOHEXYL) PIPERIDINE (TCP). D.W. Bonhaus and J.O. McNamara. Duke Univ. and V.A. Med. Ctr. Durham, NC 27705.

Phencyclidine and structural analogs (ketamine, MK-801) are uncompetitive NMDA antagonists (UCA). Numerous laboratories have shown that NMDA agonists increase [3H]UCA binding in membrane preparations. Electrophysiologic data suggest that UCA bind in the lumen of the receptor coupled channel and that access of UCA to the binding site requires opening of the channel. These findings raise the possibility that [3H]UCA can serve as biochemical markers of channel activation. We used a kinetic approach to test this idea. We found that NMDA receptor agonists increased both the apparent association and apparent dissociation rate constants for binding of the phencyclidine analog TCP. APV virtually eliminated both the association and the dissociation of the ligand. The effect of NMDA agonists and antagonists on both rate constants was of equivalent magnitude resulting in no change in the kinetically determined Kd. These results are consistent with the idea that NMDA regulates UCA binding by controlling access of the ligand to a binding site in the channel. The results are not consistent with NMDA increasing the affinity or number of TCP binding sites. Thus these results validate the use of [3H]UCA binding as a biochemical marker of channel activation.

97.12

GLYCINE MODULATION OF THE NMDA RECEPTOR: ANALYSES OF STOICHIOMETRY AND LIGAND SELECTIVITY. M. Benedict*, K. Thedinga* & G.E. Fagg, Research & Development Dept., Pharmaceuticals Division, CIBA-GEIGY Ltd., 4002 Basel, Switzerland. (SPON: P.L. Herrling)

Recent evidence indicates that glycine allosterically regulates the activity of the NMDA (N-methyl-D-aspartate) receptor complex in brain neurons. Here, we show that ^{3}H-glycine bound to a Triton X-100-treated synaptic membrane fraction from rat telencephalon with K_d 213 nM and B_{max} 17.0 pmol/mg protein. In the same membrane preparations, L-^{3}H-glutamate (NMDA-sensitive), ^{3}H-CPP and ^{3}H-MK-801 bound with K_d's of 102, 130 and 12 nM, and with B_{max}'s of 9.3, 6.8 and 8.1 pmol/mg protein, respectively. For all ligands, Hill coefficients were close to unity. These data suggest that the NMDA receptor complex comprises transmitter, glycine and channel units either in the ratio 1:2:1 or, if L-glutamate and CPP binding sites represent distinct but interconvertible states of the transmitter binding site (as recently proposed by Cotman's group), then in the ratio 2:2:1. However, dose-response curves for the stimulation of ^{3}H-MK-801 binding (a marker for channel opening) by glycine and L-glutamate were each characterized by Hill coefficients of unity.

Analyses of the ligand selectivity of the ^{3}H-glycine binding site indicated that it was strychnine-insensitive, but was inhibited by D-serine>D-alanine>L-alanine>L-serine (IC_{50}'s 0.4-40 μM). Cycloserine, HA-966 and kynurenic acid were also potent inhibitors (IC_{50}'s 2-10 μM), whereas a number of kynurenic acid analogues were inactive. Based on ^{3}H-MK-801 binding, D- and L-serine, D- and L-alanine and cycloserine were characterized as agonists at the glycine regulatory site (i.e., they increased MK-801 binding), whereas HA-966 and kynurenic acid antagonized the stimulatory effects of glycine.

97.13

GLYCINE INDUCES SPREADING DEPRESSION IN AREA CA3 OF RAT HIPPOCAMPAL SLICES THROUGH AN NMDA RECEPTOR-DEPENDENT MECHANISM. A.C. Bragdon, H. Kojima* and W.A. Wilson. Veterans Administration Medical Center, and Depts. of Medicine (Neurology) and Pharmacology, Duke University Medical Center, Durham, NC 27705.

Glycine was recently discovered to potentiate a variety of NMDA receptor-mediated actions in membranes and single neurons through a strychnine-insensitive mechanism. Reports of comparable effects in brain slices have been lacking. We now report that glycine induces spreading depression (SD) in area CA3 of rat hippocampal slices through an NMDA receptor-dependent mechanism. (We have also reported that glycine induces epileptiform bursting, Am Epilepsy Soc, 1988.)

Hippocampal slices from male Sprague-Dawley rats were studied submerged in a chamber perfused with ACSF (including K^+ 3.3 mM, Ca^{++} 1.2 mM, Mg^{++} 1 mM; 33°C). Extracellular potentials were recorded from s. pyramidale and stimuli were delivered to s. radiatum of area CA3. All drugs were bath-applied.

Glycine induced SD at concentrations ranging from 4-7 mM with an EC_{50} of 6 mM (N = 8 slices). Identical results were obtained in the presence of 10 uM strychnine (N=6) suggesting the effect was not mediated through the classical glycine receptor. The NMDA receptor antagonist D-APV at 50 uM prevented SD induced by 10 mM glycine when applied prior to glycine (N=6) and reversed SD when applied during glycine exposure (N=6). In medium lacking the channel blocker Mg^{++} SD occurred at 3 mM (N=4), a significant (P<0.005) reduction. D-serine, L-serine and beta-alanine also caused SD at similar concentrations; mannitol (5-30 mM) and glycerol (5-30 mM) had no effect. 10 mM KCl-induced SD was unaffected by D-APV.

These results suggest the NMDA receptor plays an important role in glycine-induced SD, but not in SD from all causes. The high amino acid concentrations required may reflect the presence of active uptake processes or an endogenous antagonist. Whether glycine acts through the NMDA receptor-associated glycine receptor or another mechanism awaits development of a selective antagonist.

Supported by the VA, Epilepsy Foundation of America, and NIH grant NS 17771.

98.1

LOW MAGNESIUM EPILEPTIFORM ACTIVITY IN THE DEVELOPING NEOCORTEX: LACK OF MODULATION BY ZINC AND GLYCINE.
J. J. Hablitz, E. Hegstad* and I. A. Langmoen*. Sect. of Neurophysiol., Baylor Col of Med., Houston, TX 77030 and Dept. of Neurosurgery, Rikshospitalet, Oslo, Norway.

Spontaneous epileptiform activity was induced in neocortical slices from rats 9-14 days old by perfusion with salines containing no added magnesium. It consisted, in extracellular recordings, of spike discharges superimposed on a slow negative field potential. Such events were 40-70 sec long, recuring every 4-5 min. Bath application of the NMDA receptor antagonist DL-AP7 (4-10 μM) completely suppressed spontaneous and evoked epileptiform activity (N=5). Addition of zinc (50-100 μM; N=10) produced no change in the frequency, amplitude or duration of discharges. Possible modulation by glycine was examined in three ways: (1) addition of glycine (100-500 μM), (2) blockade of glycine uptake by methylmalonic acid (.5-1.0 mM) and (3) blockade plus exogenous glycine. None affected epileptiform activity. The present results indicate that long duration paroxysmal bursts are observed in the developing neocortex following removal of extracellular magnesium. Involvement of NMDA receptors was indicated by the sensitivity to DL-AP7. Using this slice preparation, we were unable to demonstrate a modulatory effect of zinc or glycine. This may result from an immature state of the NMDA receptor during development or point to differences in synaptic vs nonsynaptic NMDA receptors. Supported by NS18145 and NS2373.

98.2

MULTIPLE EFFECTS OF CHANGES IN $[Mg^{2+}]_0$ ON RAT NEOCORTICAL NEURONS IN VITRO.
B. Sutor* and J. J. Hablitz. (SPON: A. C. Coats), Sect. of Neurophysiol., Dept. Neurol., Baylor Col. of Med., Houston, TX 77030.

Intracellular recordings were obtained from neurons in slices of rat frontal cortex using single-electrode current and voltage-clamp techniques. Following a decrease in $[Mg^{2+}]_0$ from 1.3 mM to a nominal 0 mM, the resting membrane potential (RMP) depolarized (3-5 mV), the input resistance (R_N) increased (15-25%) and the rheobase current decreased (50-60%). Simultaneously, graded EPSPs and IPSPs disappeared and were replaced by prolonged, spontaneous, all-or-none epileptiform bursting activity. The responses to iontophoretically applied NMDA were simultaneously enhanced. However, the NMDA-induced depolarization was still associated with an apparent increase in R_N. Upon return of 1.3 mM Mg^{2+} to the bathing solution, all effects were reversible. Following an increase in $[Mg^{2+}]_0$ to 3 mM, opposite effects, including hyperpolarization of the RMP, decreases in R_N, and direct excitability, depression of postsynaptic potentials and reduction in NMDA-induced responses were observed. The present results indicate that changes in $[Mg^{2+}]_0$ affect not only the neuron's response to NMDA, but in addition, intrinsic membrane properties and NMDA-independent synaptic transmission.

Supported by NS18145, NS22373, and by the Max Kade Foundation.

98.3

HIPPOCAMPAL SLICES EXPOSED TO QUISQUALATE SUBSEQUENTLY DEVELOP INTERICTAL ACTIVITY THAT IS INTENSIFIED BY AP4 AND AP6.
E.W. Harris, Dept. Pharmacology, Pharmaceutical Div., Pennwalt Corp., Rochester, NY 14603

It was recently reported (Robinson et al., Brain Res. **381**, 1986, 187; Harris et al., Brain Res. **418**, 1987, 361) that the glutamate analog quisqualate induces in hippocampal slices an atypical sensitivity to the otherwise innocuous glutamate analogs 2-amino-4-phosphono butyrate (AP4) and 2-amino-6-phosphonohexanoate (AP6). This report describes epileptiform activity that develops after bath application of quisqualate.

Bathing hippocampal slices briefly in 10uM quisqualate reduces extracellularly recorded synaptic responses by depolarizing postsynaptic neurons. Synaptic responses return 3-5 minutes after wash-out, by which time sensitivity to AP4 or AP6 is evident. Unexpectedly, spontaneous interictal discharges appear 20-30 minutes after quisqualate treatment. Upon re-application of quisqualate, AP4, or AP6, spontaneous discharge rate increases, culminating in a prolonged ictal discharge. The spontaneous interictal activity and elicitable ictal discharges persist for over 2 hours. Several ictal discharges can be elicited after each quisqualate treatment. The epileptiform activity has been observed in slices of septal and temporal hippocampus, but is always more pronounced in region CA3 than CA1.

98.4

DIFFERENTIAL ACTIONS OF ANTITUSSIVES: I. EFFECTS OF DEXTROMETHORPHAN AND CARAMIPHEN ON EPILEPTIFORM ACTIVITY AND NMDA RESPONSES IN PYRIFORM CORTEX SLICES.
J.P. Apland, S. Sparenborg and D.J. Braitman*, Neurotox Br, US Army Med Resrch Inst of Chem Defense, APG, MD 21010

The centrally acting non-narcotic antitussives dextromethorphan (DM) and caramiphen (CM) are potent anticonvulsants (Tortella and Musacchio, Fed.Proc, 46:708, 1987). DM blocks epileptiform activity by blocking NMDA receptors (Wong et al., Neurosci. Ltrs, 85:261, 1988). Here we report that, while DM blocks NMDA-induced epileptiform activity and responses in pyriform cortex brain slices, these responses were not blocked by the more potent antitussive anticonvulsant caramiphen.

Tangential slices of guinea pig olfactory (pyriform) cortex were placed in a total submersion chamber at 32° C in normal oxygenated Ringer's for extracellular recording. DM (100-300 uM) blocked responses elicited by NMDA as well as epileptiform activity evoked by Mg^{++} free medium, but had little effect on field potentials evoked by stimulation of the lateral olfactory tract (LOT). NMDA responses were blocked long after cessation of exposure to DM. In contrast, CM (100-300 uM) depressed LOT-evoked field potentials but did not block NMDA responses nor epileptiform activity in the short or long term. These results indicate that the antitussives dextromethorphan and caramiphen exert their anticonvulsant actions through different mechanisms.

98.5

DIFFERENTIAL ACTIONS OF ANTITUSSIVES: II. PROTECTION AGAINST SEIZURES INDUCED BY THE CHOLINESTERASE INHIBITOR SOMAN.
S. Sparenborg and D.J. Braitman*. Neurotox. Br., US Army Med. Res. Inst. Chemical Defense, APG, MD 21010

The antitussive dextromethorphan (DM) blocks epileptiform activity and NMDA responses (Wong et al., Neurosci. Let., 85:261, 1988). Since MK-801, a specific NMDA antagonist, blocks convulsions ,and seizures induced by soman (Braitman et al., SN Abstr. 1988), we tested DM and two other anticonvulsant antitussives, carbetapentane (CBP) and caramiphen (CM), for their efficacy against soman. Guinea pigs were surgically prepared under general anesthesia for chronic ECoG recording. One week later, they were pretreated with pyridostigmine and either CM edisylate (100 mg/kg), CBP citrate (100 mg/kg), DM HBr (30, 60 or 100 mg/kg), or saline, then were given 2 x LD_{50} soman, and treated with methylatropine and pralidoxime chloride. All saline control subjects exhibited severe convulsions and seizures within 15 min after receiving soman. DM attenuated seizures at 60 and 100 mg/kg, but death occurred within one hr after soman. CBP, and low dose DM, offered only slight protection against seizures and convulsions. In contrast, CM blocked ECoG seizures and provided substantial protection against convulsions and lethality, but CM does not appear to block NMDA responses (Apland et al.,SN Abstr., 1988). Although DM blocks NMDA responses, its anticonvulsant properties are inferior to those of the NMDA-blocker MK-801, and to CM, when used as a pretreatment for soman.

98.6

INVOLVEMENT OF THE NMDA RECEPTOR IN ABNORMAL DISCHARGES PRESENT IN HIPPOCAMPAL BRAIN SLICES FROM EPILEPTIC PATIENTS.
Leona M. Masukawa, Masato Higashima, Jung Kim and Dennis Spencer. Sections of Neuroanatomy, Neuropathology and Neurosurgery, Yale University Medical School, New Haven, Ct. 06510.

Hippocampal tissue was obtained from epileptic patients who underwent enbloc surgical resections of temporal lobe structures for the treatment of intractable epilepsy. These patients had complex partial seizures that were localized unilaterally to the hippocampus as determined by depth electrode placement along with neurological and neuropsychological testing. Structural abnormality was confirmed by neuropathological examination. The hippocampi were atrophied, sclerotic and displayed dramatic cell loss.

Conventional brain slice recording techniques were used on 450 um thick slices from these human hippocampi. Abnormal discharges were characterized by multiple population spikes (PS's) that were evoked in the dentate gyrus during stimulation of the perforant path (0.1Hz) in normal ACSF. Control rat brain slices generated only one PS at the same intensities. Excitability of the human dentate field response was potentiated by 1 Hz stimuli. The number of PS's was increased 4-5 times after 15 stimuli at this frequency. Multiple PS's were blocked by 5-100 uM APV and the effect was reversable for orthodromic stimuli at 0.1 Hz and 1 Hz. The potentiation at 1 Hz could be mimicked in rat brain slices by lowering Mg to 0.2 mM. These data suggest the involvement of NMDA receptor activation in the abnormal discharge of human epileptic brain slices. Suppported by NIH NS23077 to LMM and NIH NS06208-22 to DS and JK

98.7

THE EFFECTS OF EXTRACELLULAR CALCIUM ON THE EPILEPTIFORM ACTIVITY AND NMDA RESPONSES ARE DIFFERENT IN MATURE AND IMMATURE HIPPOCAMPAL SLICES. R.J. Brady and J.W. Swann, Wadsworth Ctr. for Labs and Res., NYS Dept. of Health, Albany, NY 12201.

The number of afterdischarges in penicillin-treated hippocampal slices from an immature rat 9-19 days old can be modulated by changing the concentration of extracellular calcium ([Ca]o) while holding the magnesium concentrations ([Mg]o) constant. Lowering [Ca]o increased the number of afterdischarges produced. Intracellular recordings in TTX-treated CA3 pyramidal cells investigated the effect of changing [Ca]o on the NMDA iontophoretic response. The result of changing [Ca]o paralleled that seen above, lowering [Ca]o increased the response.

The effects of changing [Ca]o on these parameters were also investigated in hippocampal slices taken from mature rats. In contrast to the previous results, changes in [Ca]o had little or no effect in mature slices. Lowering of the [Ca]o has been shown to decrease the voltage sensitivity of the NMDA response in immature but not mature CA3 cells. Since all investigations took place in constant [Mg]o it is likely that the immature NMDA receptor-channel complex is less sensitive to Mg and more sensitive to Ca than its mature counterpart. It is possible that this change in divalent cation sensitivity could underlie the observed developmental differences. **Supported by grants NS-23071 to RJB and NS18309 to JWS from NINCDS-NIH.**

98.8

CGS-19755: An NMDA antagonist in drug discrimination and catalepsy in pigeons. Baron, S.P.*, France, C.P.*, Hartman, J.* and Woods, J.H. Departs. Psychology and Pharmacology, Univ. of Michigan, Ann Arbor, Michigan 48109.

Separate groups of White Carneaux pigeons were trained to discriminate between i.m. injections of either 0.32 mg/kg phencyclidine (PCP) or 5.6 mg/kg N-methyl-D-aspartate (NMDA) and saline. The discriminative stimulus and cataleptic effects of the purported competitive NMDA antagonist 1-(cis-2-carboxypiperidine-4-yl)methyl-1-phosphonic acid (CGS-19755) were compared to the behavioral effects of other competitive and non-competitive NMDA antagonists. CGS-19755 produced catalepsy in all subjects and was three times less potent than PCP. CGS-19755 also failed to completely substitute for the PCP discriminative stimulus effects up to doses which suppressed responding, but did attenuate the discriminative stimulus effects of NMDA. Thus, antagonism of NMDA appears not to be sufficient for producing PCP-like discriminative effects in pigeons. In light of the reported neuroprotective effects of NMDA antagonists in animal models of hypoxic-ischemia, the present results suggest that CGS-19755, while sharing some behavioral effects with PCP, might function as an important therapeutic agent without producing the same subjective effects as PCP. This research was supported by NIDA grant DA/AG-05325-01.

98.9

NMDA RECEPTOR AFFINITY DETERMINES THE ANTICONVULSANT ACTIVITY OF NMDA ANTAGONISTS IN EPILEPTIC FOWL. S.C.J. Pedder, R. Wilcox, J.M. Tuchek and D.D. Johnson. Dept. of Pharmacology, Univ. of Saskatchewan, Canada S7N OWO.

The high seizure susceptibility in epileptic fowl is due to an autosomal recessive mutation. Epileptiform seizures can be evoked in homozygotes (epileptics) by intermittent photic stimulation (IPS) but not in heterozygotes (carriers). N-methyl-D-aspartate (NMDA) receptor antagonists have been shown to possess anticonvulsant properties in many animal models of epilepsy. However a correlation of *in vivo* activity to *in vitro* affinity has not been shown due to poor specific binding when using [^{3}H] glutamate as the radioligand. Recently a NMDA selective [^{3}H] glutamate radioligand binding assay has been described (Monahan and Michel, J. Neurochem., 1987). Using this procedure we have achieved NMDA specific binding levels of >90%. NMDA receptor antagonist (3-(±)-2-Carboxy-piperazin-4-yl)-propyl-1-phosphonic acid (CPP), 2-Amino-7-phosphonoheptanoic acid (2-AP7), 2-Amino-5-phosphonovaleric acid (2-AP5), ∝-Aminoadipic acid (∝AA), and ∝, ε-Diaminopimelic acid (DPA) were tested for their ability to protect against IPS-evoked seizures in epileptic chickens and to displace [^{3}H] glutamate from synaptic membranes. In both experiments the same order of potency was established, CPP=2-AP7>2-AP5>> ∝AA=DPA. These data provide evidence that NMDA receptor antagonism can be correlated with anticonvulsant effectiveness in a hereditary model of epilepsy. Supported by the MRC.

98.10

IN VIVO PHARMACOLOGICAL PROFILE OF NPC 12626 A PUTATIVE NMDA ANTAGONIST. **S.A. Borosky*, B.E. Jones, M.E. Guzewska*, S. Ellenberger*, W.J. Rzeszotarski*, and J.W. Ferkany. Div. Drug Discovery, Nova Pharmaceutical Corp., Baltimore, MD 21224.**

Evidence suggests that systemically active NMDA antagonists may be useful as anticonvulsants, anxiolytics and neuroprotective agents. The novel compound NPC 12626 [2-amino-4,5-(1,2-cyclo-hexyl)-7-phosphonoheptanoic acid] was synthesized and tested for NMDA antagonist effects. Administered ICV, NPC 12626 (ED_{50} = 0.72 nmoles) was 2- and 24-fold more potent in preventing NMDA-induced convulsions than CPP and AP7, respectively. NPC 12626 and CPP were equipotent (ED_{50} = 0.18 and 0.15 nmoles) in the PTZ test. Likewise, systemic administration of NPC 12626 prevented NMDA (ED_{50} = 43 mg/kg) and PTZ-induced (ED_{50} = 47 mg/kg) convulsions. Subchronic injections of NPC 12626 (100 mg/kg; 8 days; b.i.d) did not alter the profile or potency of the compound as an anticonvulsant. Like MK-801 and CPP, NPC 12626 was effective in attenuating damage to CA_1 neurons in the gerbil model of ischemia. Preliminary studies (L. Conti, this meeting) indicate an anxiolytic effect of NPC 12626 (6.25 mg/kg; i.p.). Injected at up to 3000 mg/kg (i.p.) no fatalities were observed in test animals. Similarly, subchronic administration of high doses (10days; 250 mg/kg; b.i.d.) were nonlethal to rats.

The data suggest NPC 12626 is a systemically active NMDA antagonist *in vivo*.

98.11

DEXTRORPHAN AND DEXTROMETHORPHAN ACT AS NMDA ANTAGONISTS IN VIVO: BLOCKADE OF NMDA CONVULSIONS IN MICE. J. Sepinwall, M. Smolen* ,S.S. Furman* and E. Mohacsi Research Division, Hoffmann-La Roche Inc., Nutley, NJ 07110

Several studies suggest that the neuronal degeneration seen in Huntington's Disease and after cerebral ischemia may be prevented by compounds acting as antagonists at the NMDA subtype of glutamate receptors. Morphinans, such as dextrorphan (DX), can bind to a high affinity site on the NMDA receptor channel complex, distinct from the NMDA recognition site (Wong et al., J. Neurochem. 50:274, 1988). DX and dextromethorphan (DM) can also prevent glutamate-induced neurotoxicity in neuronal cultures (Choi, Brain Res. 403:333, 1987). To determine whether DX and DM act as NMDA antagonists in vivo, we examined their ability to block convulsions induced by NMDA (175 mg/kg i.p.) in male CF1 mice. The known NMDA antagonist ketamine (K) (active between 30 and 100 mg/kg i.p.), DX (10-30 i.p.) and DM (20-30 i.p.) all blocked both the clonic and tonic convulsions induced by NMDA. MK-801 (0.03-1 i.p. or 0.3-1.6 p.o.) only blocked tonic convulsions; any possible anticlonic activity was obscured by MK-801 induced twitches, tremors and clonic seizures. In contrast to DX, DM and K, neither diazepam (1-30 p.o.) nor phenytoin (10-100 i.p.) blocked clonic convulsions although they did prevent the tonic seizures. Thus reversal of clonic seizures can be a selective endpoint for identifying NMDA antagonists. DX and DM should be pursued further as NMDA antagonists that may be useful as neuroprotective agents.

98.12

BEHAVIORAL AND EEG EFFECTS OF MK-801, AND NMDA RECEPTOR ANTAGONIST. R.G. Fariello and D. Garant*. Neurological Sciences, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612

MK-801 is a recently synthesized non-competitive NMDA receptor antagonist(Woodruff et.al. Neuropharmacology 26, 93.1987). We have studied the behavioral and EEG effects of MK-801 administered to cats. After 0.1 mg/kg MK-801 ip the animal assumes an abnormal posture with extensional ridigity of the neck and forepaws and squatting of the hindpaws. There is loss of postural reflexes and ataxia, with response to sudden auditory or tactile stimuli with massive myoclonic jerks. EEG from cortex and depth structures consisted of spike, polyspike and slow wave discharges with long periods of high voltage rhythmic slowing at 2-4 Hz. At 0.5 mg/kg the animal becomes totally unresponsive and presents erratic myclonic twitches. Bursts of polyspikes and electrographic seizures appear from cortical leads, whereas high voltage rhythmic waves and bursts of spikes and polyspike-and-wave are seen from cortical regions. After several hours the tracing turns into a burst surpression pattern, and then to severely depressed low voltage activity. Recovery is seen after 16-20 hours at 0.1 mg/kg and after 3-5 days after 0.5 mg/kg. There are some analogies between the effects of MK-801 and ketamine.

98.13

PAROXYSMAL DISCHARGES IN THE DENTATE GYRUS BLOCKED BY KETAMINE AND MK-801. J.L. Stringer and E.W. Lothman. Dept. of Neurology, Univ. Va. Med. Ctr., Charlottesville, VA 22908.

The NMDA sub-class of glutamate receptors is thought to be involved in events that depend on repetitive activation of neurons. Previous electrophysiological studies on the cellular and synaptic responses to activation of NMDA receptors have been restricted to *in vitro* systems. This study describes *in vivo* responses of the hippocampal formation to 30 sec trains of low intensity stimulation to the CA3 region. A distinct paroxysmal response, consisting of bursts of large amplitude population spikes (30-40 mV), was generated in the dentate gyrus but not in CA1. This dentate response was associated with a secondary rise in the extracellular potassium level and a negative shift of the DC potential. This paroxysmal dentate response was blocked by the NMDA antagonists ketamine and MK-801 in a dose dependent manner. However, the blockade by MK-801 developed only after activation of the paroxysms in the presence of the drug - a demonstration of use-dependence. These studies show that NMDA receptors are activated by repetitive, low intensity stimulation of hippocampal circuits to produce epileptiform discharges in the dentate gyrus. Supported by NIH grants, NS 21671, NS 07199, and NS 25605.

98.14

MK-801 PROTECTS AGAINST SEIZURES AND BRAIN DAMAGE INDUCED BY THE CHOLINESTERASE INHIBITOR SOMAN. D.J. Braitman*, N.K. Jaax* and S. Sparenborg (SPON: S. McMaster), Pathophysiology Div, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Grounds, MD 21010

Excitatory amino acids (EAA) have been shown to be involved in several models of epilepsy and brain damage (Meldrum, Clin. Sci., 68:113, 1985). We present evidence that the NMDA class of EAA receptors is involved in seizures and brain damage induced by the irreversible organophosphorus cholinesterase inhibitor soman. Guinea pigs were chronically instrumented for electrocorticogram recording under general anesthesia. One week later, each group of nine animals received i.p. injections of either MK-801 (LO, 1 mg/kg; HI, 5 mg/kg) or saline vehicle, and pyridostigmine. Thirty minutes later each subject received 2 X LD_{50} soman and was treated with atropine methylnitrate and pralidoxime chloride. All control subjects exhibited convulsions and seizure activity shortly after soman. LO MK-801 greatly attenuated and HI MK-801 completely blocked seizure activity. MK-801 treated animals did not convulse but were prostrate. Animals pretreated with MK-801 recovered faster and had a much greater probability of surviving for 48 hrs after soman exposure than did controls. Also, MK-801 reduced the severity of soman-induced brain damage. This is the first demonstration of the involvement of the EAA neurotransmitter system in seizures, convulsions, and brain damage induced by a cholinesterase inhibitor.

98.15

THE "MUSSEL TOXIN", DOMOIC ACID, PRODUCES ITS NEUROTOXIC EFFECT THROUGH KAINATE RECEPTOR: CLINICAL AND ELECTROPHYSIOLOGICAL STUDIES. G. Debonnel, J. Teitelbaum*, S. Carpenter*, L. Beauchesne*, J. Antel, A.N. Cashman and C. de Montigny. Institut P. Pinel and McGill University, Montréal, Québec, Canada.

Domoic acid (DOM), an excitatory amino acid structurally related to kainate (KA), was identified as being responsible for the toxic syndrome produced by mussels grown in Prince Edward Island (Canada). The main neurological symptoms were: memory deficits, confusion, altered state of consciousness and seizures. Six months later, patients still present severe neurological deficits. Two post-mortem examinations revealed extensive neuronal loss in the hippocampus, spotty loss in the thalamus (maximal in the dorso-medial nucleus), in the amygdala and the accumbens.

Unitary recordings were obtained in CA_1 and CA_3 regions of the rat dorsal hippocampus to compare the effect of DOM applied by microiontophoresis to those of KA, quisqualate (QUIS) and N-methyl-D-aspartate (NMDA). In both CA_1 and CA_3, the activation induced by DOM was three fold greater than that by KA. Both DOM and KA were 20 times more potent in CA_3 than in CA_1, whereas there was no such regional difference for QUIS and NMDA. These results provide strong evidence that DOM is an extremely potent agonist of KA receptors. Given the well-known neurotoxicity of KA, this finding is fully consistent with the clinical features of DOM intoxication in humans.

98.16

INHIBITION OF N-METHYL-D-ASPARTATE (NMDA)-INDUCED [^{3}H] NOREPINEPHRINE (NE) RELEASE FROM RAT HIPPOCAMPAL SLICES. **S. Leventer*, D. Giacomo*, S. McArthur*, and G. Metcalf. Wyeth-Ayerst Research, Princeton, NJ 08543**

Excitatory amino acids (EAA's) have been implicated in the pathology of several neurodegenerative diseases. EAA antagonists may be of benefit in such diseases. The purpose of this study was to test compounds for their ability to inhibit NMDA-induced [^{3}H]NE release from rat hippocampal slices as a test of NMDA antagonism.

Hippocampal slices (0.5mm) were preincubated for 30 min in Krebs-Ringer bicarbonate buffer (KRB) containing 10μM pargyline and 1mM ascorbate. Next, the slices were incubated in KRB containing 0.1μM [^{3}H]NE. Thirty min later, the slices were transferred to tubes with 0.5ml Mg^{+2}-free KRB containing 50 μM corticosterone and 10μM desipramine to minimize NE reuptake. At 5 min intervals, 250μl samples were analyzed for [^{3}H] and were replaced by fresh buffer. After 75 min, test drugs were added, and were present for the remainder of the experiment. Fifteen min later, NMDA was added for a 5 min period. After 125 min, the slices were homogenized and assayed for [^{3}H].

NMDA (30μM-3mM) caused a concentration-dependant increase in [^{3}H]NE release. $MgCl_2$ (1.25mM), as well as NMDA antagonists such as MK-801 (20nM), (±)-CPP (10μM) and (±)-AP-7 (100μM) inhibited the release induced by NMDA (300μM). These data support the use of this test for the determination of functional NMDA antagonism.

98.17

AMPA-INDUCED CONVULSIONS IN MICE: COMPARISON TO NMDA AND KAINATE INDUCED CONVULSIONS. **T. A. Emrey*, R. R. Notvest. (SPON: K. L. Keim) Department of Experimental Therapeutics, Wyeth-Ayerst Research, Princeton, NJ 08543**

The NMDA-induced convulsion model is an effective *in vivo* test for evaluating excitatory amino acid antagonists (EAAA) (Croucher, et al., 1982). Kainate (KA)-induced convulsion models have also been developed (Ben-Ari, 1985). This report characterizes a quisqualate receptor-mediated convulsion induced by administration of the quisqualate agonist, alpha-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoic acid HBr (AMPA), and compares it to NMDA- and KA-induced convulsions.

Male Swiss albino mice were habituated for 30 minutes to individual chambers (4 sq. in. plexiglass), treated with AMPA (50-175 mg/kg, i.p.), and observed for 30 minutes. Responses to 50-80 mg/kg doses included circling (ipsi- and contralaterally), tonic rigidity and tremors. Doses of 90-150 mg/kg also produced catatonia and catalepsy, generalized myoclonus and mortality. The AMPA-induced convulsion does not include hind limb scratching which is observed in both NMDA- and KA-induced convulsions.

Systemic administration of AMPA induces a distinctive and reproducible motor convulsive response which may be useful as a model for identifying EAAA acting at the quisqualate receptor.

98.18

EFFECT OF PROTOTYPE ANTICONVULSANT AGENTS ON QUISQUALIC ACID (QA)-INDUCED SEIZURES IN MICE. N.A. Singh*, E.A. Swinyard*, and H.S. White* (SPON: R.P. Tuckett). Dept. of Pharmacol. and Tox., Univ. of Utah, S.L.C., UT 84112.

Previous investigations have demonstrated that the QA antagonist glutamic acid diethyl ester (GDEE) and selected prototype anticonvulsants partially attenuate QA-induced tonic-extension seizures. The present investigation was designed to assess further the effect of phenytoin (PHT), carbamazepine (CBZ), phenobarbital (PB), valproate (VPA), ethosuximide (ETS), and clonazepam (CLZ) on forelimb tonic extension (FTE) induced by intracerebroventricular administration of QA. Groups of 5 to 14 CF #1 mice were injected i.p. with increasing doses of each anticonvulsant and at the predetermined time of peak effect challenged with the convulsive dose 97 of QA (42 μg/5 μl). Animals that did not display FTE within 30 min were considered protected. The effective dose 50 (ED50) was calculated by probit analysis. Except for ETS, all of the anticonvulsants tested were more effective in blocking QA-induced FTE than the QA antagonist GDEE. For example, CLZ was the most potent, followed by PHT, CBZ, PB, VPA, and GDEE (ED50's: 0.119, 6.05, 6.25, 6.41, 138.4, and 736.5 mg/kg, respectively). ETS was ineffective in minimally neurotoxic doses of 600 mg/kg. These results suggest that QA-induced FTE is blocked by preventing spread from a seizure focus. (Supported by NIH training grant GM07579 and NIH contract NO1-NS-4-2361).

98.19

QUISQUALATE ANTAGONIZES THE CONVULSANT ACTION OF KAINATE IN AREA TEMPESTAS. P. Zhong* and K. Gale (SPON: K. Woodbury). Department of Pharmacology, Georgetown University Medical Center, Washington, DC 20007.

Area tempestas (AT) is an epileptogenic zone within the deep prepiriform cortex from which clonic seizures are elicited by focal injections of GABA antagonists, NMDA receptor agonists, as well as kainic acid (KA). The present study examined the effect of quisqualate (QA), an agent that causes excitatory effects via receptors other than those activated by NMDA or KA.

QA was microinjected unilaterally into AT in freely moving, awake rats. Generalized clonic convulsions characterized by repeated facial and forelimb clonus, rearing and falling, were induced in 100% of the rats receiving 2500 pmol QA. These seizures were comparable to those obtained with 100 pmol KA in AT. QA, 500 pmol, produced convulsive responses in only 15% of the rats and these responses were very transient and mild, while QA, 250 pmol, was without convulsant effect.

Following 15 min pretreatment with QA (250 or 500 pmol) in AT, seizures induced by KA (100 pmol) in AT, were prevented. When the same rats were tested 24 hr later with KA alone in AT, a full convulsive response was obtained. Following 15 min pretreatment with QA (500 pmol) in AT, there was no modification of the convulsant effect of bicuculline (100 pmol) in AT, indicating that the anticonvulsant effect of QA is selective for seizures evoked by activation of KA receptors. These data support the in vitro observations of Wroblewski et al. (Fed. Proc. 46: 848, 1987) who found QA to be a non-competitive antagonist of KA receptor function in cultured cerebellar granule cells.

Supported by NIH Grant NS20576 and an RSDA (to K.G.) MH00497

98.20

N-METHYL-D-ASPARTIC ACID (NMDA)-INDUCED CONVULSIONS: AN IN VIVO MODEL TO IDENTIFY NMDA ANTAGONISTS. L. C. Johnson* and V. J. DeNoble (SPON: S. W. Tam). E. I. duPont de Nemours & Co., Inc., Wilmington, DE 19898.

NMDA receptors can mediate the neurotoxic actions of excitatory amino acids. Compounds that block NMDA receptor binding or prevent the resulting calcium ion influx prevent NMDA-induced cell death. Research has focused on in vitro studies to investigate the mechanism of cell death. This study was designed to develop an in vivo model of NMDA receptor activation useful in finding compounds that block NMDA-induced cell death. Such a model should be sensitive to NMDA antagonists, PCP receptor binders, and calcium channel blockers, while insensitive to anticonvulsants. Intravenous administration of NMDA to mice produces behavioral excitement leading to clonic convulsions. The ability of different pharmacological agents to block NMDA-induced convulsions in comparison to their ability to block electroshock-induced convulsions (ECS) was studied. Competitive NMDA antagonists (AP7, CPP) and PCP-like drugs (PCP, MK801) blocked NMDA and ECS convulsions at similar doses. Verapamil, a calcium channel blocker, blocked NMDA convulsions but was inactive against ECS. Anticonvulsants (phenobarbital, phenytoin) were either inactive or blocked NMDA convulsions only at doses higher than those active against ECS, while diazepam and valproate showed similar antagonist activity against NMDA and ECS convulsions. These data suggest that NMDA-induced convulsions can be used as an in vivo model of NMDA receptor activation.

TRANSMITTER UPTAKE, STORAGE, SECRETION AND METABOLISM I

99.1

TURNOVER OF MUSCARINIC CHOLINERGIC RECEPTORS IN GH_3 CELLS. C.P. Bolden and S.P. Baker. Department of Pharmacology, University of Florida, College of Medicine, Gainesville, FL 32610.

The recovery of muscarinic receptor (M-R) binding and function was determined after irreversible blockade with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Pretreatment of near confluent GH_3 rat pituitary tumor cells with 100 μM EEDQ plus 1 mM glycine ethyl ester decreased specific [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) binding 85-90% with no change in K_D of ([^{3}H]QNB) binding for the receptors left. This treatment had no effect on forskolin-stimulated adenylate cyclase activity, cell viability or cellular protein synthesis. Associated with the receptor loss was a 90% reduction in the maximal M-R mediated inhibition of forskolin-induced cAMP production. In addition, the carbachol dose-response curve for inhibition of cAMP production was shifted rightward about 5-fold. Recovery of receptor binding ([^{3}H]QNB) was a first-order process, the control levels being achieved within 24 hr and was blocked by the protein synthesis inhibitors cycloheximide and puromycin. In contrast, the maximum response (carbachol inhibition of forskolin-stimulated adenylate cyclase) fully recovered within 6 hr. The data suggests the half-life of the M-R in GH_3 cells is relatively short (approximately 7 hr) and a high coupling efficiency between the receptor and response is present.

99.2

IN VIVO ASSESSMENT OF THE COMPARTMENTALIZATION OF DOPAMINE IN STRIATAL NERVE TERMINALS G.W. Arbuthnott, I.S. Fairbrother, S. Butcher. (Spon: European Neurosciences Association) MRC Brain Metabolism Unit and Department of Pharmacology 1 George Square, Edinburgh EH8 9JZ, UK.

In rats anaesthetised with Halothane we have examined the release of dopamine (DA) into the perfusate passing through a dialysis fibre implanted into the neostriatum. The actions of various releasing agents including K^+, amphetamine, veratrine, ouabain and tyramine have been studied. The amount of DA release is altered to different extents by pretreatment of the animals with reserpine, or α-methyl-p-tyrosine (αmpt), according to the releaser administered. Similarly the drugs show markedly different sensitivities to EGTA or to TTX applied through the probe. Veratrine and K^+ cause release from a pool which is exquisitely sensitive to TTX, EGTA and αmpt. Tyramine results in release which is little affected by TTX, or by EGTA. Amphetamine and ouabain share an intermediate position.

The amount of DOPAC in the dialysate is also differently altered by the various releasing agents. At doses that cause the release of DA, K^+ causes a marked reduction of DOPAC, whilst tyramine does not.

Thus release in vivo seems to derive from different nerve terminal pools with differing sensitivities to pharmacological manipulations.

99.3

INFLUENCE OF SYMPATHETIC INNERVATION ON CATECHOLAMINE METABOLISM IN RABBIT CAROTID BODY. A. Gomez*, C. Gonzalez, B. Dinger and S. Fidone, Dept. of Physiol., Univ. of Utah, Salt Lake City, UT 84108

The present experiments examined the influence of sympathetic innervation on stimulus evoked ^{3}H-dopamine (^{3}H-DA) and ^{3}H-norepinephrine (^{3}H-NE; synthesized from ^{3}H-tyrosine) release from the carotid body superfused in vitro by media equilibrated with 10% O_2 or containing nicotine (10^{-4} M). In one group of animals, superior cervical ganglionectomy was performed 10 to 14 days prior to experimentation to eliminate the sympathetic innervation to the carotid body. The stimulus evoked release (stimulus minus basal release in 100% O_2-media) of each ^{3}H-catecholamine (^{3}H-CA) was expressed as a percent of the ^{3}H-DA or ^{3}H-NE remaining in the tissue. The ^{3}H-CA release evoked in low O_2-media, which is primarily ^{3}H-DA, increased 20.4x after sympathectomy, and likewise the nicotine evoked release of ^{3}H-CA (mostly ^{3}H-NE) averaged 4.9x that released from normal carotid bodies. These differences suggest that sympathetic fibers in normal carotid bodies decrease the sensitivity of type I glomus cells to natural and pharmacological stimuli. Alternatively, noradrenergic sympathetic fibers may participate in the uptake of catecholamines released from type I cells. (Supported by USPHS Grants NS12636 and NS07938).

99.4

MDMA AND MBDB POTENTIATE PHORBOL ESTER-STIMULATED CATECHOLAMINE RELEASE FROM PC-12 CELLS. J.A. Monti, J.M. Beaton, F. Benington*, R.D. Morin*, and S.T. Christian. Neuropsychiatry Research Program and Department of Psychiatry, University Station, Birmingham, Alabama 35294, U.S.A.

We have previously shown that MDMA (Ecstasy) and its congener MBDB, stimulated catecholamine release from PC-12 cells. The S(+) stereoisomer of both compounds is most potent in stimulating catecholamine release and MDMA appears to preferentially stimulate the release of NE as compared with DA. Phorbol dibenzoate stimulates NE and DA release from PC-12 cells with an EC_{50} ~200 nM. Phorbol didecanoate is inactive in stimulating catecholamine release. The release of NE and DA from PC-12 cells was measured (HPLC-EC) using increasing concentrations of Phorbol dibenzoate in the presence of 200 μM racemic MDMA or MBDB. Catecholamine release induced by the combination of Phorbol dibenzoate and racemic MDMA or MBDB was greater than that induced by the phorbol ester alone, and in the presence of MDMA or MBDB, the dose-response curves for Phorbol ester-stimulated catecholamine release were shifted to the left. Dose-response curves for Phorbol dibenzoate + MBDB-stimulated release of NE and DA were monophasic with EC_{50} ~50 nM, as was the dose-response curve for Phorbol dibenzoate + MDMA-stimulated release of DA (EC_{50} ~60 nM). However, the dose-response curve for Phorbol dibenzoate + MDMA-stimulated release of NE appeared to be biphasic with EC_{50}'s of 2 and 30 nM, respectively. Potentiation of Phorbol ester-stimulated catecholamine release of MDMA and MBDB was stereospecific with the S(+) isomer of each compound being more potent. Catecholamine release in the presence of S(+) MDMA or MBDB and Phorbol didecanoate was less than that induced by MDMA or MBDB alone. These data suggest the possibility that MDMA and MBDB-stimulated release of catecholamines from PC-12 cells may be mediated by protein kinase-C.

99.5

ATP-DEPENDENT UPTAKE OF GABA BY RAT BRAIN SYNAPTIC VESICLES. J.A. Near and W.J. Chang*. Medical Sciences Program and Department of Pharmacology, Indiana University School of Medicine, Bloomington, IN 47405.

A number of small neurotransmitters are sequestered in synaptic vesicles by transport systems coupled to a proton translocating ATPase. In order to determine if a similar uptake system for GABA exists in the mammalian CNS, association of [^{3}H]-GABA with a crude preparation of synaptic vesicles from rat brain was examined. ATP and GTP both supported the apparent transport activity, whereas other nucleotides and nucleosides were ineffective. Agents that disrupt proton electrochemical gradients, such as ammonium ion and CCCP, inhibited ATP-dependent activity. The activity was dependent on time, temperature, external pH, vesicle protein concentration and GABA concentration, with an apparent K_m of 13mM for GABA. Although plasma membrane transport systems for GABA are sodium dependent, the activity described here was not. Similarly, agents that are known to interact with GABA and glutamate receptors, synaptosomal and glial uptake systems for GABA or glutamate, and the vesicle catecholamine uptake system were all ineffective at relevant concentrations. These results suggest that a specific uptake system for GABA exists in synaptic vesicles from mammalian brain.

99.6

HIGH-AFFINITY UPTAKE OF L-KYNURENINE IN MOUSE BRAIN ASTROCYTES. K. Hares*[1], C. Speciale[1], R. Schwarcz[1] and N. Brookes[2] (SPON: F. Du). [1]Maryland Psych. Res. Ctr., Baltimore, MD 21228 and [2] Dept. Pharmacol. Exptl. Therap., Univ. Maryland Sch. Med., Baltimore, MD 21201.

L-kynurenine (KYN), the bioprecursor of the neuroactive brain metabolites quinolinic and kynurenic acid, can be taken up into rat brain slices by two processes, one Na^+-dependent and the other Na^+-independent (Soc. Neurosci. Abstr. 13:1675, 1987). Here, primary astrocyte cultures derived from the cerebra of newborn mice were equilibrated for 30 min in HEPES-Tris buffer (pH 7.4, 34.5°C) before addition of ^{3}H-L-KYN (8.9 Ci/mmol). Saturation curves for the initial velocity of KYN uptake conformed to a one-component saturable system with a K_m of a 32 μM and a V_{max} of 2.1 nmol/mg protein/ min. The KYN transporter did not recognize aspartate, GABA or taurine. However, the large neutral amino acids leucine, tryptophan and phenylalanine were accepted with somewhat higher affinity than KYN. In Na^+-free buffer, KYN uptake was equal to or greater than in the presence of Na^+. The spontaneous efflux of label from cultures loaded with ^{3}H-L-KYN was suppressed at 4°C, and was accelerated by the presence of unlabeled KYN or Na^+ in the buffer. KYN was notably concentrated by the astrocytes. After addition of 1 μM ^{3}H-L-KYN to the cultures, the steady-state (20-30 min) distribution ratio of labeled species was estimated to exceed 100-fold. (Supported by USPS grants NS 16102 and ES 03928).

99.7

ENHANCED KYNURENIC ACID SYNTHESIS IN CORTICAL AND HIPPOCAMPAL SLICES FROM AGED RATS. R. Schwarcz, W.A. Turski and J.B.P. Gramsbergen. Maryland Psych. Res. Ctr., Baltimore, MD 21228.

In aging, nerve cell loss may occur in particularly vulnerable brain areas, such as cortex and hippocampus. Kynurenic acid (KYNA), a regular brain constituent, has been shown to display neuroprotective properties. Therefore we have examined KYNA production and liberation of KYNA in brain slices of rats of different ages.

KYNA production and liberation from brain slices was examined upon exposure to 50 μM kynurenine (cf. Turski et al., this meeting). Experiments were performed with young adult (3 months), mid adult (1 year) and old (2 years) rats (N = 6 per group). After incubation, KYNA content (per mg slice protein) in medium of cortical and hippocampal slices from 1 and 2 year old rats was about twice as high as that from young animals ($p<0.05$). KYNA production in slices obtained from striatum, thalamus, cerebellum and liver was similar in all age groups. In urine, KYNA content decreased with increasing age (from 27±7 pmol/ μl at 3 months to 7±2 pmol/ μl at 2 years; $p<0.05$).

Our data show that large differences exist at all ages in the capacity of different organs and brain regions to synthesize and liberate KYNA. The specific increase in cortical and hippocampal tissue in the aged brain may be of relevance for an understanding of neuronal loss in advanced age.

Supported by USPHS grants NS 16102 and NS 20509.

99.8

DIFFERENTIAL REGULATION OF DOPAMINE AND NEUROTENSIN IN COLOCALIZED AND NON-COLOCALIZED NEURONAL POPULATIONS. A.J. Bean, T.E. Adrian*, I.M. Modlin*, and R.H. Roth. Depts. of Pharmacol. & Psychiat., Yale Univ. Sch. Med., New Haven, CT, and VA Med. Ctr., West Haven, CT.

The demonstration of coexistence of dopamine (DA) and neurotensin (NT) within a subset of ventral tegmental area neurons has prompted us to examine the regulation of DA and NT storage in colocalized (prefrontal cortex; PFC, and nucleus accumbens; NAS) and non-colicalized (striatum; CP, and periaqueductal grey; PAG) neuronal populations. We investigated the effects of reserpine (RES), alpha-methyl-p-tyrosine (AMT), and gamma-butyrolactone (GBL) on DA and NT content within the same sample of each brain region. RES (0.5-5.0 mg/kg i.p. -6, -18. -48 h) dose-dependently depleted DA in all brain regions at the 6 and 18 h time points and levels returned to control values by 48 h. NT levels dose-dependently declined in the PFC (18, 48 h), increased in the CP (6, 18 h), and NAS (18, 48 h) and were not altered at any time point in the PAG. AMT (200 mg/kg i.p. -4 h) reduced DA in all regions without altering NT. GBL (750 mg/kg i.p. -2 h) induced increases in PFC DA and NT which were reduced by RES. GBL-induced increases in CP and NAS DA, and decreased CP NT, effects which were reversed by RES. Thus, RES may increase NT synthesis and/or decrease NT release in NAS and CP. Furthermore, these data indicate that DA and NT may be co-stored in some PFC terminals in RES-sensitive vessicles. Supported by MH-14092, NIGMS-GM 07324, and the Veterans Administration.

99.9

STIMULATION-DEPENDENT RELEASE OF ATP FROM HIPPOCAMPAL SLICES. A. Wieraszko, G. Goldsmith, T. Seyfried, Biology and Physics Depts. Boston College, Boston, MA 02167

ATP is known to be released from K^+-stimulated rat brain synaptosomes (White, Nature 267: 67, 1977) and may act as a neuromodulator in the CNS. Here we demonstrate the release of ATP from hippocampal slices following the electrical stimulation of Schaffer collaterals. The slices were stimulated with bursts of pulses (300Hz for 50ms, 2 sec intervals) for 30 sec which caused a stable increase in the size of the population spike (LTP). The release of ATP was measured with a luciferase-luciferine system and the light emitted was recorded with a photomultiplier placed beneath the slice chamber. ATP release was observed shortly after the start of stimulation. No ATP release was detected in a Ca^{+2} free solution or after low frequency stimulation (1Hz). Glutamate (2 mM) applied without electrical stimulation did not evoke ATP release. Kynurenic acid (10 mM), which blocks population spike, did not block ATP release. We conclude that ATP is released from electrically stimulated hippocampal slices from presynaptic nerve terminals in a calcium-dependent fashion and may play a role in the modulation of synaptic efficiency.
Supported by NSF BNS-8604955 and NIH 23355

99.10

UNIQUE BIOCHEMICAL PROPERTIES OF DOG BRAIN MAO-A. P.W. Scates* and H.L. White. Dept. of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Monoamine oxidase occurs as two distinct enzyme forms, MAO-A and MAO-B. Both forms are found in brain mitrochondrial preparations, as characterized by substrate specificities and effects of selective inhibitors. Although MAO of Beagle dog brain can be classified as A and B using these criteria, certain properties of dog brain MAO-A distinguish it from the enzyme of other species. MAO-A in mitochondrial preparations from dog brain was inhibited by A-selective inhibitors. However, it was about 10-fold less sensitive to inhibition by clorgyline and pargyline than was MAO-A of human brain. After solubilization by nonionic detergent, dog brain MAO-A became much less sensitive to inhibition by clorgyline, harmine, and pargyline, while the Km with serotonin as substrate was increased approximately 15-fold. No such effects were observed with human or rat brain MAO-A in similar experiments. Solubilization of MAO-B of dog brain did not change its Km with phenethylamine as substrate. In addition, dog brain MAO-A was particularly sensitive to inactivation by trypsin under conditions which did not affect MAO of human or rat brain enzyme. These and other results reveal species differences in the structure and/or membrane microenvironment of MAO-A.

99.11

IMMUNOBLOT ANALYSIS OF HUMAN MAO A AND B FROM TISSUES CONTAINING WIDELY DIFFERENT RATIOS OF A TO B ACTIVITY.

L. A. Riley*, M. A. Waguespack*, R. M. Denney, Department of Human Biological Chemistry and Genetics, Graduate School of Biomedical Sciences, University of Texas Medical Branch, Galveston, TX, 77550.

Monoamine oxidases (MAOs; EC 1.4.3.4) oxidize important biogenic and xenobiotic amines. Previously, we reported an immunoblotting assay for MAO A using the monoclonal antibody MAO A-4D3 (Riley et al., Soc. Neurosci. Abs., 1987). Using the same system, the MAO B-specific antibody, MAO B-1C2 (Denney et al., Molec. Pharm. 22:500, 1982) immunoblots human MAO B. Binding of antibodies to MAO A or B on nitrocellulose is enhanced by the presence of Tween-20 in the primary antibody incubation. Both antibodies retain their specificities after antigen denaturation and apparent epitope regeneration. Neither antibody binds detectably to other proteins in placental mitochondria or platelets. Human placenta, widely reported to contain primarily MAO A, contains significant MAO B protein and activity [deprenyl-sensitive β-phenylethylamine (PEA) and benzylamine oxidizing activity]. In contrast, much more MAO B than MAO A protein was detected in liver, even though the rates of tyramine oxidation by A and B are approximately equal. These observations suggest that MAO A has a higher molecular activity than MAO B for many substrates, including shared substrates like tyramine and the reportedly MAO B-selective substrate, PEA. (Supported by grant NS19453.)

99.12

TIME- AND NH_4^+- DEPENDENT CHANGES OF AMINO ACID CONCENTRATIONS IN MEDIUM FOR ELECTROPHYSIOLOGICAL RECORDING FROM PRIMARY CULTURES OF CEREBELLUM.

J.E. Madl*, D.H. Smullin, S.R. Skilling*, A.A. Larson, and W.A. Raabe. Depts. Vet. Biol. and Neurology, Univ. of Minnesota and VA Med. Ctr., St. Paul, MN 55108.

Amino acids in the electrophysiological recording medium (ERM) used for tissue culture may affect membrane currents (e.g., effect of glycine on NMDA-activated channels). NH_4^+ depolarizes neurons and perturbs amino acid metabolism. We therefore studied the concentrations of glutamate (Glu), aspartate (Asp), glycine (Gly), taurine (Tau), glutamine (Gln) and asparagine (Asg) in ERM and ERM with 5mM NH_4Cl added. Primary monolayer cultures of the cerebellum were grown in 35 mm dishes. After discarding the growth medium, each dish was rinsed six times with ERM and sampled. By 60 min, the concentrations of Glu and Asp decreased to 30% and 15% of their original values, repectively. The concentrations of the other amino acids increased to 4-20 times their original concentrations within 30 min and remained stable thereafter. Addition of NH_4^+ to ERM resulted in an increase of all amino acids, including an 8-fold increase of Glu. These data indicate that after exposure of cultures to ERM, amino acid concentrations in ERM undergo significant changes within the first 30-60 min. In addition, agents affecting amino acid metabolism and neuronal function, such as NH_4^+, may produce significant changes in amino acids concentrations in ERM. Supported by NS 01105, DA 04090, DA 04190, DA 00124 and the Veterans Administration.

99.13

FUNCTIONAL INTRACELLULAR GLUTAMINASE ACTIVITY IN DIBUTYRYL C-AMP TREATED ASTROCYTES.

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The functional intracellular glutaminase activity measured by the release of ^3H_2O from L-(2-^{3}H)-glutamine is 5-10% of the activity measured in extracts of DiBcAMP treated and untreated astrocytes. The V_{max} was 181 nanomol/mg protein/h for untreated cells and 581 nanomol/mg protein/h for treated cells. The $K_{m(app)}$ for untreated and treated cells were similar: 169 and 132 nanomol/mg protein, respectively. Addition of glutamate to the medium inhibits intracellular glutaminase activity. However, a residual 30% of the enzyme activity was uninhibited. The $K_{i(app)}$ for glutamate was independent of DiBcAMP treatment, approximately 150 nanomol/mg protein. The glutamate inhibition curve as well as the calculated kinetic constants are consistent with published results for purified enzyme. After 4 h in the presence of increasing extracellular glutamine (0.2 to 6.4 mM), intracellular glutamine increased from 40 nanomol/mg protein and plateaued at about 140 nanomol/mg protein in untreated cells and increased from 20 nanomol/mg protein to 60 nanomol/mg protein in DiBcAMP treated cells. With increasing extracellular glutamate (40-200 M), intracellular glutamate concentration increased linearly to 600 nanomol/mg protein. These data indicate that glutaminase activity is regulated intracellularly by glutamine and glutamate as well as by additional effectors. (NICHD-16596)

99.14

IN VIVO METABOLISM OF 6-[^{18}F]-L-FLUORODOPA AND [^{3}H]-L-DOPA: DOUBLE LABEL EXPERIMENTS.

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To validate the assumption that 6-[^{18}F]-L-fluorodopa (FD) cerebral kinetics reflect the functional integrity of the endogenous dopamine (DA) system, we designed experiments with [^{3}H]-L-DOPA (HD), and FD.

Sprague-Dawley rats (275-325g) (jugular cannulae 1 day prior to the experiment) were pretreated with Carbidopa (5 mg/kg., s.c.) 60 min before i.v. injection of FD (400 μCi) and HD (50 μCi). Animals were sacrificed by decapitation at 15, 30, and 60 min. The striata and vermis were removed and were prepared for HPLC analysis (C-18 reverse phase column, 80% 0.1 M Na_2HPO_4, 2.6 mM OSA, 0.1 mM EDTA; 20% MeOH, pH 3.3); the metabolites were identified with authentic standards.

The striatum/cerebellum (s/c) total activity increased for ^{18}F and ^{3}H (2.0, 60 min); the metabolite profiles differed when expressed as a fraction of total radioactivity. [^{3}H]-Dopamine (HDA) and 6-[^{18}F]-fluorodopamine (FDA) were detected exclusively in striatum; none in plasma or in cerebellum. The FDA (striatum)/FD (plasma) ratio increased from 0.95 (15 min) to 3.2 (60 min); the HDA (striatum)/HD (plasma) ratio increased from 0.7 (15 min) to 1.0 (60 min). The relative plasma levels of HD remained higher than the FD levels, whereas there was an increase in the relative rate of O-methylation of FD when compared with HD. Both 3-O-MeFD and 3-O-MeHD rapidly appeared in the plasma, but the 3-O-MeDopa/Dopa ratio differed at 60 min (3-O-MeFD/FD is 6.5, 3-O-MeHD/HD is 1.0). The s/c ratio for 3-O-MeFD, and for 3-O-MeHD was approximately one at 15, 30 and 60 min. These data indicate that a quantitative correlation between 6-[^{18}F]-L-fluorodopa kinetics and cerebral DA metabolism can be established.

99.15

EFFECTS OF POLYMYXIN B AND SPHINGOSINE, PROTEIN KINASE C INHIBITORS, ON CATECHOLAMINE SECRETION FROM INTACT AND DIGITONIN-PERMEABILIZED ADRENAL MEDULLARY CELLS.

E. Tachikawa, S. Takahashi*, C. Shimizu*, N. Ohstubo*, and T. Kashimoto*. Department of Pharmacology, School of Medicine, Iwate Medical University, Morioka 020, Japan.

The effects of polymyxin B (PMB) and sphingosine, inhibitors of protein kinase C (PKC), on catecholamine (CA) secretion from intact and digitonin-permeabilized adrenal medullary cells were examined. Acetylcholine (ACh), 56 mM K^+, veratridine, Ca^{2+}-ionophore ionomycin or 12-O-tetradecanoylphorbol-13-acetate (TPA) induced the secretion of CA from the intact cells. PMB (0.1-100 μM) decreased the secretion of CA induced by these secretagogues in a concentration-dependent manner. Sphingosine (5-400 μM) also diminished the secretion of CA induced by ACh or veratridine but did not affect TPA- and ionomycin-induced CA secretion. Both PMB and sphingosine inhibited the rise in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) stimulated by ACh, 56 mM K^+ or veratridine. The degrees of both inhibition of ACh-, 56 mM K^+- or veratridine-induced rise in $[Ca^{2+}]_i$ and CA secretion by PMB or sphingosine were similar. PMB also reduced CA secretion from the digitonin-permeabilized cells evoked by an addition of free Ca^{2+} (1 μM) to the medium. Sphingosine had no effects on this Ca^{2+}-dependent secretion. These results suggest that an activation of PKC is involved in Ca^{2+}-dependent CA secretion from bovine adrenal medullary cells. Further, the inhibitory effects of PKC inhibitors on ACh-, 56 mM K^+- or veratridine-induced CA secretion can be considered as a result of blocking the influx of extracellular Ca^{2+} into the cells stimulated by these secretagogues.

99.16

EFFECTS OF MPP^+ ON THE RELEASE OF SEROTONIN AND 5-HYDROXYINDOLEACETIC ACID FROM RAT STRIATUM BY BRAIN DIALYSIS.

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In addition to the well documented parkinsonism inducing effects, high dose of MPTP causes acutely a serotonin syndrome. It is postulated that MPP^+, the major metabolite of MPTP in the brain, may increase 5-HT neurotransmission by blocking reuptake, inhibiting MAO and/or releasing 5-HT. Thus, an in vivo brain dialysis procedure was employed to investigate the effects of MPP^+ on the release of 5-HT and 5-HIAA in rats. Sprague-Dawley rats (280-300g) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and then Carnegie Medicin microdialysis probes were inserted into the striatum. The brain was perfused with Krebs-Ringer solution (1μl/min). MPP^+ (1mM) was applied through the dialysis tubing for 60 min. Perfusates were collected every 20 min and assayed simultaneously for monoamines and their metabolites by HPLC-EC. MPP^+ caused a 30-fold increase in the release of 5-HT which peaked within 30 min and lasted for more than 3 hours after the MPP^+ infusion. 5-HIAA decreased from 3.7 ± 0.4 to 2.0 ± 0.3 ng/20μl perfusate. In addition, MPP^+ increased dopamine level 55-fold and caused a concomitant increase in the release of 3-MT. While it decreased HVA and DOPAC. This MPP^+-induced increase in 5-HT release may be responsible for the acute 5-HT syndrome after MPTP.

99.17

FLUCTUATION OF STRIATAL DOPAMINE UPTAKE SITES DURING THE ESTROUS CYCLE: COMPARISON WITH BIOGENIC AMINE METABOLISM. M. Morissette*, A. Bélanger* and T. Di Paolo (SPON: M. Colonnier). Dept of Molecular Endocrinology, Laval University Medical Center, Québec, G1V 4G2 and School of Pharmacy, Laval University, Québec, G1K 7P4, CANADA.

Striatal dopamine (DA) uptake sites labelled with [^{3}H]GBR-12935 binding and biogenic amines concentrations measured by HPLC-EC were investigated during the rat estrous cycle and compared to ovariectomized (OVX) rats. Plasma prolactin concentrations peak in the afternoon of proestrus (PPM) while estradiol levels increase gradually to reach a peak on the day of proestrus. Progesterone concentrations are more phasic with one peak the day of diestrus I (DI) and one on PPM. In the striatum, DA levels increase in the morning of proestrus (PAM) compared to rats in estrus (E), DI, diestrus II (DII), PPM and OVX, the DA content being similar in the latters. Striatal DOPAC levels and the ratio DOPAC/DA increase in PAM ($p < 0.01$ vs E, DI, DII and OVX) with a minimum in E while HVA levels and the ratio HVA/DA remain unchanged. Striatal DOPAC levels during the estrous cycle were higher than in OVX rats ($p < 0.01$). Striatal 5-HT levels are decreased in E ($p < 0.01$ vs DI, DII, PAM, PPM) while no variation of 5-HIAA and 5-HTP, were observed. Density of striatal [^{3}H]GBR-12935 binding increases gradually to reach a maximum in PAM ($p < 0.05$ vs E, DI, PPM) while no variation of the dissociation constant were observed. In the striatum, both the maximum density of the DA uptake sites as well as DA and DOPAC levels peak in PAM, when estradiol levels are increased suggesting a causal-effect relationship although the primary site of action of estradiol and/or other hormones is yet to be determined. Thus, physiological hormone fluctuations, as occur during the estrus cycle, can influence DA uptake and biogenic release and metabolism in the striatum. Supported by the MRC.

99.18

VASOPRESSIN (VP) FUNCTION IN THE SEPTUM (S) AND HIPPOCAMPUS (H) OF ADULT MALE RATS.

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Different evidence indicate that vasopressin neurons (VPn) play an important modulatory role in various CNS functions. Herein, we describe the in vivo and in vitro functions of VPn innervating the S or H of adult male rats. Higher VP levels in the S (8.1 ± 1.4 pg/mg wet tissue, $\bar{X}$ ± SE, n=6) than in the H (0.32 ± 0.04, n=6) were determined with a sensitive and specific RIA for VP (0.1 pg/tube at 90% binding). However, in freely moving rats bearing a miniaturized push-pull cannulæ (PPC) in the lateral S detectable levels of VP were not measured (n=6). In contrast rats with PPC in the dorsal H released VP (1.45 ± 1.2 pg/10 min, n=6) in a pulsatile manner. Interestingly, pulsatile VP release was measured from S as well H fragments superfused in vitro. When VP was added either in vitro to S or H fragments or perfused directly into the lateral S or dorsal H of awake rats marked difference in the recovery of exogenous VP was found (31.8 ± 2.5%, n=6 vs 78.3 ± 5.6, n=5, respectively). In two experiments recovery of VP perfused through the PPC system was 89.5 and 86.8%. The data demonstrate that in spite of higher amounts of VP in the S than in the H it is only from the hippocampus that VP is released in a pulsatile manner in freely behaving rats.

99.19

NATURALLY OCCURING LIGAND INHIBITS BINDING OF [3H]-IMIPRAMINE TO HIGH AFFINITY RECEPTORS. T. Hauser*, K.G. Walton, J. Glaser* and R.K. Wallace. Departments of Physiology and Chemistry, Maharishi International University, Fairfield, IA 52556.

Numerous studies have suggested the role of serotonergic activity in various behavior such as depressive illness, anxiety, learning and memory. Our laboratory has been investigating possible endogenous and exogenous modulators of serotonin uptake in platelets. We have recently begun to look at specific herbal preparations known to influence mood which come from the traditional system of medicine of India known as Ayurveda.

An aqueous extract of one particular Ayurvedic preparation (Maharishi Amrit Kalash #5) yielded a factor that demonstrates striking inhibition of [3H]-imipramine binding to human blood platelet membrane receptors by Scatchard analysis. An ethanol extract was about an order of magnitude less effective at inhibiting [3H]-imipramine binding. The factor in the aqueous extract was concentrated using a reversed phase CYANO extraction column (Baker-10 SPE), eluting with methanol. Preliminary analysis using a Lineweaver-Burk plot indicates that inhibition is at least partially competitive. Characterization of the nature of the inhibition and purification of the factor are currently in progress.

Since an endogenous ligand for the imipramine receptor has never been clearly defined, and since the preparation under investigation has been demonstrated in previous human studies to influence mood and well-being and to contain ligands for other important neuro-receptors, it is possible that if a ligand can be isolated from this herbal formulation, it may prove to have imipramine-like effects on mood and behavior.

99.20

NICOTINIC AND MUSCARINIC RECEPTOR STIMULATION OF THE PHOSPHOINOSITIDE CYCLE IN CHROMAFFIN CELLS WITH ACCUMULATION OF INOSITOL TETRAKISPHOSPHATE. B.B. Sanborn*, O.E. Harish* and A.S. Schneider (SPON: R.A. Ferrari). Dept. Pharm. & Tox., Albany Med. Coll., Albany, N.Y. 12208.

Muscarinic or nicotinic stimulation of cholinergic receptors on adrenal chromaffin cells results in catecholamine secretion. Both receptor subtypes appear to interact with the phosphoinositide (PI) cycle, but by different mechanisms, and the resultant products (inositol phosphates and diacylglycerol) may be important in the mechanism of neurohormone secretion. Muscarine was found to cause a rapid breakdown of polyphosphoinositides (PIP_2, PIP, PI) with accumulation (in the presence of LiCl) of the corresponding inositol phosphates including inositol tetrakisphosphate (IP_4) (detectable within 10 sec). 2,3-DPG, a specific inhibitor of IP_4 and IP_3 monoesterase, should enhance accumulation of IP_4 and IP_3, and in cells pretreated with DPG prior to muscarinic stimulation, IP4 increased 42% and IP_3 224% above control. The nicotinic agonist, DMPP, stimulated IP, IP_2 and IP_3 production at 15 min, and the Ca^{2+} dependence of this effect was determined. The data suggest that elevated cytosolic calcium, secondary to nicotinic receptor stimulation, activates the PI cycle, whereas muscarinic stimulation of the PI cycle is receptor-linked. Calcium activation of the PI cycle may be relevant to the mechanism of calcium-mediated exocytosis.

99.21

UPTAKE OF PROLINE, PIPECOLIC ACID, LYSINE AND GLYCINE BY PC-12, C-6 AND CHO-K1 CELLS. M. Cordero*, O. Torres* and J.G. Ortiz. Dept. of Pharmacology, Univ. of Puerto Rico Medical School, San Juan, Puerto Rico 00936

The transport of proline (PRO), hydroxyproline, pipecolic acid (PA) and glycine (GLY) has been considered to be mediated by a common uptake system, (Wellner and Meister, 1980).

In PC-12 cells, preincubation of the cells in a salts/glucose medium resulted in a marked decrease in PRO uptake; moderate decreases in GLY and LYS uptake and essentially no change in PA uptake. PRO and LYS uptake were not altered by the preincubating C-6 cells in the salts/glucose medium, while the uptake of PA and GLY were significantly increased. On the other hand, in CHO-K1 cells, only the uptake of PRO was significantly reduced by the preincubation in the salts/glucose medium.

The results of these experiments are not consistent with the presence of a common uptake system for PRO, PA and GLY, nor with the existence of common regulatory mechanism(s) as evidenced by the differential effects of preincubation in a salts/glucose is medium. Detailed examination of the uptake process(es) necessary in order to determine the relative contribution of the different cell types to the endogenous levels of these compounds, as well as, their possible role in disorders of peroxisomal function(s). (supported by NIH/MBRS)

100.1

LOCALIZATION OF TYPE II CALCIUM/CALMODULIN-DEPENDENT KINASE IN CELLULAR LAYERS OF HIPPOCAMPUS. G.K. Feussner* and M.L. Vallano. (SPON: R.B. King). Dept. of Pharmacology, SUNY/Health Science Center, Syracuse, NY 13210.

Type II calcium/calmodulin-dependent protein kinase (CaM kinase II) is concentrated in brain and has been implicated in synaptic transmission, plasticity, and cytoskeletal function. The predominant form of the holoenzyme in cerebrum is 560-730 kDa containing two subunits of 50 kDa and 58-60 kDa in a molar ratio of 3-4:1, respectively. CaM kinase II appears to be present in all neuronal cell types, but its regional distribution and molar ratio of subunits comprising the holoenzyme are variable. CaM kinase II is particularly enriched in hippocampus where it represents ~2% of the total protein. The lamellar organization of the hippocampus maintained in the in vitro slice has provided a useful model for numerous structure/function studies. To ensure discrete sampling and to minimize post-mortem alterations, neuronal areas from freeze-dried tissue sections from rat brain frozen in situ were free hand dissected and assayed for CaM kinase II activity. The specific activity of CaM kinase II was 2-3 fold greater in area CA1, compared to areas CA3 and dentate gyrus. In addition, the dentate gyrus contained a distinct isozymic form of CaM kinase II compared to the pyramidal cell layers examined. These data provide a basis to examine differential regulation of CaM kinase II expression and activity within the hippocampus.
(Supported by NIH Grant NS 24705-01A1).

100.2

ALTERATION OF THE HIPPOCAMPAL CA1 RESPONSE TO CA3 INPUT BY SEPTAL STIMULATION. P.G. Newlon*, S.J. Goldberg, S. Miyazaki* and R.L. Hayes. Div. Neurosurg. & Dept. Anat., Med. Coll. of Va.-VCU, Richmond, Virginia, 23298.

Stimulation of the Schaeffer Collateral/Commissural path (SCC) from the CA3 region of hippocampus produces a characteristic field potential in CA1 which increases with stimulus intensity. We studied the effect of high frequency patterned stimulation of the septum on this CA1 response. Field potentials were recorded from the dorsal CA1 pyramidal layer with glass micropipettes (2-5 MOhm) in male rats anesthetized with urethane. Bipolar stimulating electrodes were placed in the ipsilateral CA3 region and the septum. CA1 field potentials were obtained from a range of stimulus intensities prior to, and 10, 30, and 60 min after a septal series of ten, 400 Hz trains (5 pulses/train; 200 msec intertrain interval). Septal stimulation reduced the CA1 population spike (PS) threshold by 30-40%. Lateral septal stimulation potentiated the PS amplitude while medial septum suppressed it. These effects persisted up to one hour. The influence of septal stimulation on the development of hippocampal long term potentiation is presently being investigated. This work was supported by NS-21458 and NSF BNS-8507610.

100.3

POSTSYNAPTIC TARGETS OF THE SEPTO-HIPPOCAMPAL GABAERGIC PROJECTION IN THE RAT. T.F.Freund* and M.Antal*(SPON:C.Beaulieu) MRC Anatomical Neuropharmacology Unit, Dept. of Pharmacology, Oxford OX1 3QT, United Kingdom.
The cholinergic component of the septo-hippocampal pathway has been pharmacologically, physiologically and anatomically well characterised, but much less is known about the role of the GABAergic component. Small injections of PHAL were delivered into the medial portion of the medial septum and diagonal band, where most of the noncholinergic septo-hippocampal cells are located. Two distinct populations of fibres were labelled in the hippocampus. One had small boutons, terminating mainly in the proximal dendrite-cell body region of both pyramidal cells and granule cells, whereas the other had large terminals, and formed numerous multiple basket-like synaptic connections with nonpyramidal cell somata and dendrites in all regions. The latter type of afferents as well as most of their postsynaptic targets were shown to be GABAergic by postembedding immunogold staining for GABA. Postsynaptic dendrites negative for GABA were also found. The GABAergic neurons surrounded by GABAergic septal baskets were further characterised by immunostaining for calcium binding proteins parvalbumin and calbindin D-28k (antisera kindly provided by Dr. K. G. Baimbridge), by which the dendritic tree of these cells were also visualised. Occasionally, more than 30 terminals of single septal axons were seen in contact with dendrites and perikarya of single target cells. GABAergic interneurons immunoreactive for calbindin in CA3 were far the most frequent targets. These target cells are likely to have extensive local axon arborisations controlling the activity of large groups of pyramidal and granule cells. The GABAergic component of the septo-hippocampal projection may thus have a crucial role in the septal induction of hippocampal rhythmic slow activity, by synchronising large populations of principal neurons via strong GABA-GABAergic disinhibition.

100.4

MODIFICATION OF EVOKED HIPPOCAMPAL DENTATE INHIBITION BY DIAZEPAM AND THREE ANTAGONISTS. **T.E. Albertson and R.M. Joy. U.C. Davis Medical Center, Sacramento, CA 95817**

Urethane anesthetized rats with perforant pathway stimulating electrodes and recording electrodes in the dentate gyrus of the hippocampus were exposed to increasing doses of either the agonist diazepam (DZP) or a benzodiazepine antagonist (PK-11195 (PK), CGS-8216 (CGS) and RO15-1788 (RO)). None of the four compounds significantly altered the excitatory postsynaptic potentials. The population spike (PS) threshold demonstrated minimal but significant increases after exposure to DZP and the central antagonists RO and CGS, but not to the peripheral antagonist PK. PS amplitude was reduced after exposure to RO, CGS and DZP, but not PK. DZP significantly increased early paired pulse $GABA_A$-mediated inhibition greater than it reduced later facilitation. The antagonist RO also significantly increased early $GABA_A$-mediated inhibition without altering later facilitation. Neither CGS nor PK altered early PS inhibition or later facilitation. The marked increases in $GABA_A$-mediated inhibition and smaller reductions in later facilitation seen with DZP was reversed by a second treatment with either RO, CGS, but not with PK, the vehicle control or DZP. When given first, both CGS and RO, but not PK prevented any further increase by DZP in $GABA_A$-mediated inhibition. These data point to the importance in vivo modulatory role of the benzodiazepine receptor on overall granule cell sensitivity and early presumed $GABA_A$-mediated PS inhibition.

100.5

VARIATIONS IN PHYSIOLOGY AND MORPHOLOGY OF HIPPOCAMPAL CA3 PYRAMIDAL CELLS. D.K. Bilkey* and P.A. Schwartzkroin (SPON: J.S. Lockard). Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

Cajal (1893) reported that hippocampal pyramidal cells vary in morphology depending on their location in the inferior or superior regions of stratum pyramidale (s.p.). We have attempted to determine whether these differences in morphology and location are correlated with particular electrophysiological characteristics. In vitro hippocampal slices were prepared from male guinea pigs. Intracellular recordings were obtained from pyramidal cells in area CA3a-c. Membrane potential, input resistance and firing characteristics were measured, and the cells were subsequently injected with the dye Lucifer Yellow. Preliminary data confirm that cells in the superior edge (i.e., s.p./str. oriens border) of s.p. have smaller soma, and also suggest that these cells have a longer time constant and are more likely to produce high frequency trains of action potentials followed by slow spikes. At the present time it is unclear whether this represents clear differences in electrophysiology between superior and inferior cells, or whether it is due to the greater probability of recording from the apical dendrites of cells in the superior region.

These studies were supported by NIH,NINCDS grant NS18895.

100.6

ULTRASTRUCTURE OF INTRACELLULAR LABELLED MOSSY CELLS IN THE RAT DENTATE GYRUS. D.D. Kunkel, H.E. Scharfman and P.A. Schwartzkroin. Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

Mossy cells of the rat dentate hilus are large, distinctive cells with extensive dendrites bearing complex spines (thorny excrescences), and an axon that projects ipsilaterally and contralaterally to the dendritic layer of granule cells. To investigate the circuitry of the region, we examined the morphology of mossy cells and other hilar neurons.

Mossy cells were recorded from transverse hippocampal slices maintained in vitro. Mossy cells were first identified physiologically and then injected intracellularly with Lucifer Yellow. These studies showed that mossy cell axons arborize profusely within the hilus. Subsequently, HRP was used to intracellularly label and then examine mossy cells ultrastructurally. Large terminals filled with small, round vesicles contacted mossy cell thorny excrescences. Elaborate cup-shaped spines were contacted by smaller terminals containing fewer, larger vesicles. HRP-positive synaptic contacts by mossy cell terminals were seen on a variety of hilar cell types. These terminals also appeared to be involved in axo-axonic contacts. Studies are ongoing to examine the relationship of HRP-filled mossy cells to hilar neurons immunoreactive with the antibodies to gamma-aminobutyric acid (GABA) and somatostatin (SRIF).

Supported by NINCDS, NIH grant NS 18895.

100.7

EFFECT OF CHOLINERGIC AGONISTS ON LACUNOSUM-MOLECULARE INTER NEURONS IN RAT HIPPOCAMPUS. L.J. Reece and P.A. Schwartzkroin. Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

Stratum lacunosum-moleculare (L-M) interneurons have been described in the guinea pig and rat hippocampus. Compared to other types of hippocampal interneurons, L-M interneurons have a low spontaneous firing rate and longer action potentials, in addition to differences in passive membrane properties. These L-M cells receive cholinergic innervation from the medial septum-diagonal band complex as evidenced by choline acetyltransferase and acetylcholinesterase histochemistry, and by degeneration studies after fimbria-fornix lesions. In order to evaluate the effect of this innervation, we applied acetylcholine and the cholinergic agonist carbachol to L-M cells while recording intracellularly from these cells in the *in vitro* hippocampal slice preparation. Drugs were applied iontophoretically near the cell soma or by the microdrop method to the surface of the slice. All cells were depolarized upon exposure to cholinergic agents. Unlike basket cells, which we have found to be extremely sensitive to these compounds, the responses of L-M cells were slow; despite large changes in membrane potential, cells did not begin discharging spontaneously or increase baseline firing rate.

These studies were supported by NIH NINCDS grants NS 15317 and NS 18895.

100.8

VIDEOMICROSCOPY AND SYNAPTIC PHYSIOLOGY OF CULTURED HIPPOCAMPAL SLICES. A.H. Ganong, D.E. Stevens* and T.H. Brown. Div. of Neurosciences, Beckman Research Institute, City of Hope, Duarte, CA 91010.

Culture of slices from postnatal vertebrate brains offer advantages including a high degree of organotypic cellular organization, the ability to visualize single neuronal somata and processes, and accessibility of neurons for recording electrodes and pharmacological manipulations. We have used a variation of the roller tube slice culture technique (B.H. Gahwiler, *J. Neurosci. Meth.*, 4:329, 1981) to culture 250-300 μm thick slices of rat hippocampus on collagen-coated coverslips.

During the first 1-2 weeks following plating many of the cells died leaving a thinner slice which still had recognizable pyramidal and granule cell layers. Slices that were damaged during the preparation often had large gaps or holes. The pyramidal cell layer was typically 3-8 cells thick in intact cultures after about 3 weeks in vitro. Individual neuron somata were difficult to discern in the thicker cultures using phase contrast optics. However, differential interference contrast optics (40X), especially when coupled with a video camera with adjustable contrast gain and offset, allowed for clear visualization of neuronal somata, nuclei, and nucleoli throughout individual cultures.

Intracellular recordings using potassium acetate electrodes were obtained from CA3 pyramidal neurons in cultures after 3 weeks *in vitro*. The neurons showed resting potentials in the range of -55 to -75 mV, overshooting action potentials, and input resistances in the range of 50-120 MΩ. When the cultures were superfused with Hank's salts (with 5.8 mM K^+, 0.9 mM Mg^{2+}, and 1.26 mM Ca^{2+}) spontaneous EPSPs and IPSPs could be recorded. Both the spontaneous IPSPs and evoked IPSPs had a reversal potential near -70 mV. The presence of 10 μM picrotoxin in the recording medium eliminated the ipsps, but also resulted in paroxysmal depolarizing shifts. This epileptiform bursting could be reduced in medium with 2 mM K^+, 4 mM Mg^{2+}, and 3 mM Ca^{2+}. The use of this medium coupled with low intensity stimulation allowed the study of excitatory synaptic events in isolation from the large inhibitory components present in these cultures. *(Supported by the AFOSR).*

100.9

HILAR NEURONS: GATEKEEPERS OF THE DENTATE GYRUS B.Y. Wong and D.A. Prince. Dept. of Neurology Stanford University, Stanford, CA 94305-5300.

Dentate hilar cells (HCs) are postulated to inhibit dentate granule cells (DGCs) by excitation of intermediary GABAergic interneurons. We therefore performed experiments to determine whether HC discharges are associated with DGC inhibition.

Intracellular HC recordings were obtained from longitudinal guinea pig hippocampal slices. Nearly continuous spontaneous EPSPs were observed in all neurons. Stimulus trains delivered to the hilus evoked large (>20 mV, 1.0 sec) bursts of summated EPSPs, followed by >25 mV post-burst hyperpolarizations which lasted up to 20 sec. In about 25% of slices, spontaneous bursts occurred after stimulation. Responses were associated with hilar field potentials. Using Anti-Lucifer Yellow immunocytochemistry we identified neurons with these electrophysiological properties as large cells with spiny dendrites whose morphology is consistent with the mossy cell as seen in Golgi studies of the hilus.

In other experiments, we found that spontaneous hilar field potential bursts were associated with large inhibitory synaptic events in DGCs. Since HCs have long associational and commissural hippocampal projections, they may have a critical role in gating information flow through the dentate by amplifying excitatory inputs and collectively activating potent local inhibition of DGCs. Supported by NIH grants NS01179 and NS06477.

100.10

IN VIVO ELECTROPHYSIOLOGICAL EVIDENCE FOR MONOSYNAPTIC INPUT FROM THE ENTORHINAL CORTEX TO PYRAMIDAL CELL REGIONS OF THE HIPPOCAMPUS M.F. Yeckel, G. Barrionuevo and T.W. Berger, Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, PA 15260

Efferent fibers of the entorhinal cortex (EC) project to the molecular layer of the fascia dentata (FD), where they can initiate a sequential excitation of the dentate granule cells, CA3 pyramidal cells, and CA1 pyramidal cells. Serial propagation through this tri-synaptic pathway traditionally has been regarded as a fundamental characteristic of intrinsic hippocampal physiology. However, anatomical evidence also indicates a monosynaptic projection from the EC to the molecular layers of CA3 and CA1, suggesting simultaneous rather than sequential activation of these cell regions.

We investigated this possibility using electrical stimulation to the angular bundle of halothane anesthetized rabbits while recording from FD, CA3, and CA1. Antidromic responses due to reciprocal pyramidal cell projections to EC were identified and dissociated through high frequency stimulation and knife transections of the alveus. Latencies to orthodromic activation of single units and population spikes were 4-6ms and 6-7ms in FD (medial and lateral perforant path, respectively), 4-6ms in CA3, and 5-7ms in CA1.

These data demonstrate that input from EC can activate all hippocampal subfields nearly simultaneously, and suggest that FD and CA3 provide feedforward modulation of EC-driven CA1 activity. Supported by NSF, NIMH, ONR.

100.11

GABAERGIC CONTRIBUTION TO NONLINEAR REPSONSE PROPERTIES OF THE IN VIVO DENTATE GYRUS. R.L. Port, T.A. Blanpied*, R.J. Sclabassi, and T.W. Berger. Departments of Behavioral Neuroscience, Psychiatry, and Neurosurgery, University of Pittsburgh, Pittsburgh, PA 15260.

GABAergic interneurons provide feedforward and feedback inhibition in all regions of the hippocampal formation. The role of GABA in determining nonlinear response properties of dentate granule cells was examined in the rabbit. Systemic manipulations were used to alter GABA throughout all hippocampal regions: 3-mercaptopropionic acid (3-MP; 16 mg/kg, i.p.), a GABA synthesis inhibitor and aminooxyacetic acid (AOAA; 30 mg/kg, i.p.), a GABA aminotransferase inhibitor. Local pressure injection of bicuculline (BMI; 1 mM) was used to antagonize GABAergic effects primarily in the dentate gyrus. Perforant path (PP) input to granule cells was activated using random impulse train stimulation. The relationship between interstimulus intervals (ISIs) of the train and evoked population spike amplitude was determined through computation of first and second order kernels.

First order kernels represent the average population spike amplitude to all impulses, and were slightly reduced by AOAA (1.5 mV vs. 1.6 mV in control preparations) and were increased by 3-MP (2.1 mV). Second order kernels reflect the modulatory influence of a preceding stimulus as a function of ISI. Control preparations exhibited suppression of spike amplitude to ISIs of 10-50 ms, and facilitation to ISIs of 60-400 ms. Spike suppression was reduced by 3-MP, and eliminated totally by local injection of BMI. Facilitation was reduced by AOAA, and occurred to ISIs <40 ms after BMI. Supported by ONR and NIMH.

100.12

GABAERGIC CONTRIBUTION TO NONLINEAR RESPONSE PROPERTIES OF THE IN VITRO HIPPOCAMPAL DENTATE GYRUS. T.P. Harty, T.W. Berger, R.J. Sclabassi, and G. Barrionuevo. Depts. of Behavioral Neuroscience, Psychiatry and Neurological Surgery, Univ. of Pittsburgh, Pgh., PA 15260

Using random impulse train stimulation and nonlinear systems analytic procedures, we previously have compared the nonlinear response properties of the in vivo and in vitro dentate gyrus. Second order kernel analyses showed that in vivo granule cell population spikes evoked by perforant path stimulation display a marked decrement in amplitude when inter-stimulus intervals (ISIs) in the train are short (10-50 ms). Longer ISIs (50-400 ms) lead to a facilitation of spike amplitude. In contrast, in vitro population spikes are facilitated rather than suppressed when ISIs are short. This facilitation occurs over a shorter range of intervals (10-150 ms) than in vivo facilitation.

The lack of suppression of in vitro preparations to short ISIs may reflect the transection of GABA-containing dentate interneurons during slicing. Consistent with this hypothesis, the GABA antagonist bicuculline (5-20 uM) had minimal effects on dentate nonlinearities. The GABA agonists pentobarbital (10-100 uM) and alphaxalone (1-5 uM) produced a suppression of population spike responses to short ISI's, thus restoring inhibition to in vivo levels. However, facilitation similar to the in vivo preparation (i.e. to ISI's of 50-400 ms) was not observed, suggesting that this component of dentate network properties is mediated by connectivity with other hippocampal subfields. Supported by ONR, NIMH, and NIH.

100.13

AN INPUT/OUTPUT MODEL OF THE HIPPOCAMPAL FORMATION. R.J. Sclabassi, D. Krieger, J. Solomon*, S. Levitan*, G. Barrionuevo, and T. Berger. Departments of Electrical Engineering, Behavioral Neuroscience, Neurosurgery, and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15213.

We are utilizing an integrated theoretical and experimental approach to characterize the functional properties of the hippocampal formation through the use of an input/output neural network model. We conceive of this network as a set of interconnected elements or nodes, each of which may be characterized by its own input/output function. We have established that nonlinear input/output characteristics of each network element can be measured experimentally by recording electrophysiological responses to random impulse train stimulation and expressing the relationship between input signal and node output as the kernels of an orthogonalized functional power series expansion. The kernels are estimated experimentally by cross-correlation techniques for Poisson-distributed point process inputs. The resulting functions may be interpreted physiologically as generalized recovery functions and theoretically as n^{th} order impulse responses. N^{th} order convolution integrals are computed on transputer elements which represent populations of neurons interconnected according to known anatomical relationships. This paper will report the result of three different simulation studies: the closed-loop system, the partially closed system, and the open-loop system.

100.14

OPTICAL RECORDING OF NEURONAL ACTIVITY IN ORGANOTYPIC SLICE CULTURES

T. Knöpfel*, H.J. Kasper*, B. Kohler*, Z. Zglinski*, L. Zeller* and B.H. Gähwiler; Brain Research Institute, University of Zürich, CH-8029 Zürich (Switzerland)

Previously, multi-site recordings of neuronal activity using voltage-sensitive fluorescent dyes have been obtained from a variety of preparations. We report here the application of this technique to organotypic cultures of rat hippocampus and cerebellum. These cultures offer unique opportunities since optical signals with a high signal-to-noise ratio could be recorded from single neurons which were integrated in complex synaptic networks.

For optical mapping with high temporal resolution (0.2 ms), a 10 x 10 photo-diode array was used. With this detector, we recorded synaptically evoked activity from single cerebellar Purkinje cells and population responses from the more densely packed hippocampal pyramidal neurons using dye RH 414.

Optical imaging with high spatial resolution (256 x 244 points) was achieved with a charge coupled device (CCD). At present, the temporal resolution of our CCD detector is too low (20 ms) to resolve single action potentials. The CCD allows, however, mapping of slow synaptic potentials, while their time course could be recorded with the diode-array.

This work shows that using a combination of diodes and a CCD allows recording with high temporal, as well as spatial, resolution neuronal activity in organotypic slice cultures.

100.15

Compartmental modelling of hippocampal neurones. J.E.Chad, H.M.Cole *, E.W.Stockley *, H.V.Wheal * and T.R.Harris *. Dept. of Neurophysiology, University of Southampton, SO9 3TU and IBM Scientific Centre, Winchester, UK.

We have created a database structure that enables us to accurately represent the three-dimensional morphology of individual neurones. The description of membrane surfaces within the database is used to generate a compartmental model. This model is analysed by a circuit simulator, allowing us to predict the electro-physiological properties of these neurones. Our model is being refined by recursive testing against the experimentally measured properties of the digitised cells. We have analysed pyramidal neurones both in, or dissociated from adult rat hippocampal slices. The simplified morphologies of dissociated cells enable a more accurate determination of their electrophysiological properties due to the improved space clamp. These neurones have a greatly increased input resistance (300-400 Mohms) compared to intact cells (30-40 Mohms), presumably due to the removal of many dendritic processes. Thus far the model can predict the relative contributions of the soma and the dendritic system to the passive properties of the cells. We are using this system to study the relative contributions of distal and proximal synaptic inputs to cell function.

100.16

SHAPE AND AREA OF HIPPOCAMPUS/PARAHIPPOCAMPUS IN SCHIZOPHRENIC, SUICIDE AND CONTROL BRAINS. L.L. Altshuler*. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892. M. Casanova*, N. Sachder*, T.E. Goldberg*, J. Kleinman (SPON: J. Stevens). NIMH Neurosciences Center at St. Elizabeths Hospital, Washington, D.C. 20032.

Anatomic abnormalities of limbic system structures have been demonstrated in schizophrenia by neuroimaging and postmortem studies. Postmortem reports reveal smaller volumes of the hippocampus proper and parahippocampal cortex as compared to controls. The present study was undertaken in an attempt to replicate these findings using a method that minimizes shrinkage due to fixation and paraffin embedding. Coronal sections were made at the level of the mammillary bodies (pes hippocampi) in 18 schizophrenic, 17 non-schizophrenic suicide and 17 non-psychiatric control brains, matched for sex and age at death. The tissue blocks were then photographed and digitized with a LOATS computer image analysis system. The illumination and resolution were maximized and the area measurements calibrated. Sections were evaluated for total area (mm^2) and for shape (harmonic) with LOATS. Analysis for shape in the dominant harmonic revealed no statistical differences between the groups in either the hippocampus (H) or parahippocampal cortex (PH). However, analysis for area revealed significant differences between groups in the PH (ANOVA $F=5.23$, $P<.02$). By post hoc t-test the schizophrenic PH area was significantly smaller than suicide ($p<.05$) and control ($p<.05$) PH. No significant differences in hippocampal area were found.

Our positive findings are consistent with previous studies and further support the existence of structural pathology in schizophrenia.

100.17

MEASUREMENT OF THE SOMATIC SHUNT IN HIPPOCAMPAL GRANULE CELLS. D. Durand and G.Yuen. Applied Neural Control Laboratory, Dept of Biomedical Engineering, School of Medicine, Case Western Reserve University, Cleveland, OH. 44106.

Intracellular recordings in neurons have revealed the presence in some neurons of a shunt at the soma. This shunt could be caused by injury from electrode penetration or by a lower membrane resistance at the soma compared to the membrane resistance in the dendrites. Theoretical derivations of the equation for the electronic parameters of cell with a somatic shunt have allowed not only the measurement of this shunt but have also shown that electrode penetration cannot solely be responsible for the shunt.

Intracellular recordings were obtained from hippocampal granule cells. A short hyperpolarizing current pulse (.5ms, 1 to 7nA) was injected in the cells and the resulting voltage filtered, averaged and digitized. Peeling analysis and least-square non-linear fit methods were used to extract the coefficients and the time constants of the multiexponential decay. A computer program was then used to solve numerically the shunt model equations and to determine the best fit the data.

The results from 13 cells show that the ratio of the somatic time constant to the dendritic time constant was equal to .32 $\pm$ .22. This ratio normally assumed to be equal to 1, suggests that, on average, the granule cells have a dendritic membrane resistance equal to three times the somatic membrane resistance (assuming that the membrane capacitance is constant). The presence of this shunt could be important for the interpretation of the measurements of electrotonic parameters in granules cells.

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100.18

MODELLING THE EFFECT OF EXTERNAL APPLIED ELECTRIC FIELDS ON THE EXCITABILITY OF HIPPOCAMPAL CELLS. E. Warman and D. Durand, Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH. 44106

Recent studies have shown that electrical fields applied to the cerebellum and hippocampus can modulate the excitability of the neurons provided that the electrical field is properly oriented with the dendritic structure. We have studied the effect of applied extracellular fields on the excitability of hippocampal granule and pyramidal cells using computer simulations.

Both cells were modeled as compartmental cylinders with active channels at the soma compartment. The extracellular intercompartment voltages were set by calculating the product of the constant electric field applied and the intercompartmental distances. In the presence of the applied field, the new steady-state transmembrane potentials were calculated and the cell response to stimulation was tested.

The results indicate that in the presence of a constant field it is possible to modify the somatic steady state potential. This induced membrane potential can lead to a change in the excitability of the cells. A 10mV/mm field changed the rheobase current by 11% in granule cells and 14% in pyramidal cells. Preliminary studies indicate that the effect of the electric field is dependent on the morphology of the cell models. The contribution of dendritic length, branching and variations in dendritic diameter are currently under investigation.

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100.19

EIGENFUNCTIONS OF THE CHARGING CURVE IN MULTIPOLAR NEURONS OF NONUNIFORM DENDRITIC LENGTH.

L.L. Glenn, B.G. Samojla, and P.J. Rebeta*. *Department of Physiology, Ohio College of Podiatric Medicine, Cleveland, Ohio 44106-3082.*

A general solution to the cable equation (Rall, Biophys. J. 9:1483, 1969) describes the voltage response of a passive membrane cylinder to current stimulus as

$$V = C_0 \exp(-t/\tau_0) + C_1 \exp(-t/\tau_1) + \ldots + C_i \exp(-t/\tau_i) + \ldots$$

We present below the equations for the C_i and τ_i of a multipolar cylinder model with cylinders of nonuniform electrotonic length, when the stimulus is a step of current applied or withdrawn at t=0.

$$C_0 = \frac{\Sigma G^{\infty}_j \tanh L_j}{\Sigma G^{\infty}_j L_j} \qquad C_i = \frac{2\ \Sigma G^{\infty}_j \tanh L_j}{(1 + \alpha_i^2)\ \ \Sigma G^{\infty}_j L_j \sec^2 \alpha_i L_j}$$

where L_j is the electrotonic dendritic length for the j^{th} dendrite, G^{∞}_j is the input conductance that the j^{th} dendrite would have if it were semi-infinite, and the α_i and τ_i are obtained from

$$\Sigma G^{\infty}_j \tan \alpha_i L_j = 0 \qquad \tau_i = \tau_0 / (1 + \alpha_i^2)$$

The significance of these equations is that the charging curve for a current step can be calculated for all t in multipolar neurons with dendrites of arbitrary number and length.

LEARNING AND MEMORY: PHARMACOLOGY II

101.1

MK-801 IMPAIRS OLFACTORY DISCRIMINATION LEARNING IN 16-DAY-OLD RATS. M.E. Stanton and K.F. Jensen. Neurotoxicology Division, U.S. EPA, Research Triangle Park, NC 27711.

NMDA receptors are thought to play a special role in neural processes associated with development, learning, and neurotoxicity (TINS,10[7],1987). These receptors are widely distributed in a variety of forebrain regions, including the olfactory system and hippocampus. Here we report that administration of MK-801, a potent non-competitive antagonist of the NMDA receptor, impairs olfactory discrimination performance in 16-day-old rat pups.

Pups were taken from their nest, injected i.p. with MK-801 (.1 mg/kg) or saline vehicle, and 40-70 min later, were tested on an odor-aversion task (see Kucharski and Spear, Dev. Psychobio, 1984, 17, 465-479 for details). Rat pups were trained with two odors (lemon and methyl), one (CS+) paired with footshock and the other (CS-) not, and then given a simple preference test involving the two cues. MK-801 treated animals showed less of an aversion to CS+ than did those treated with saline. Control experiments indicated that this impairment did not reflect general anosmia and/or motor effects of MK-801.

These findings suggest a role for the NMDA receptor in early learning.

101.2

NMDA ANTAGONISM AND WORKING MEMORY PERFORMANCE. M.J. Pontecorvo and D.B. Clissold* NOVA Pharmaceutical Corporation, Baltimore, MD 21224

Although recent data suggest that N-methyl-D-aspartate (NMDA) sensitive glutamate receptors may be in important for learning and memory, their particular contribution to memory processes remains to be delineated. Therefore, we trained rats to perform a two choice continuous non-matching to sample working memory task.

A variable number of trials with one stimulus (tone) alternated with trials with the second stimulus (light). The rats were reinforced for responding on one lever if the present stimulus was the same as the previous(match), and the second lever when the present stimulus differed from the previous (non-match). Thus, the rats had to remember across the intertrial interval (retention intervals: 2.5, 10 and 20 sec) which stimulus was presented most recently.

After achieving asymptotic levels of accuracy the rats received either saline, 3(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), a direct acting NMDA antagonist, or MK801, a non-competitive antagonist that acts through the PCP site. Although high doses of CPP disrupted response accuracy, these effects were accompanied by a marked reduction in response probability. In contrast, MK801 reduced accuracy at doses that did not produce a reduction in response probability. Interestingly, the reduction in accuracy following MR801 was evident across all retention intervals and resulted primarily from an increase in false alarms (non-match choices on match trials), rather than from a change in both hits (correction-match responses) and false alarm, or a selective reduction in hits, as has previously been observed in this test with other amnestic treatments. We believe this pattern of results indicates that disruption of NMDA transmission produces a general disorientation rather than a specific failure of working memory.

101.3

EFFECTS OF MK 801, AN ANTAGONIST OF THE N-METHYL-D-ASPARTATE TYPE OF EXCITATORY AMINO ACID RECEPTOR, IN TWO-TRIAL MEMORY TESTS. N.Venable*and P.H.Kelly. Preclinical Research, Sandoz AG, CH-4002 Basel, Switzerland

The effects of the N-methyl-D-aspartate (NMDA) type receptor antagonist, MK 801, in passive avoidance learning and in a new test, described below, were examined. Systemic administration (0.03-0.3 mg/kg) to mice (i.p.) or rats (s.c.) shortly before a single step-through passive avoidance training trial did not significantly affect step-through latencies on the training day, but resulted in a dose-dependent reduction of latencies in the retention test performed 24 hr later. In parallel experiments in mice the substance slightly reduced the threshold for shock-induced vocalization, suggesting that the impairment of passive avoidance was not due to drug-induced analgesia. In the second test rats were placed in a swimming pool for a short (3-5 min) swim in order to assess the pattern of their exploratory activity on two consecutive days. Animals received drug (0.1 mg/kg s.c.) or vehicle shortly before the first swim only. On the first day animals initially swam predominantly around the perimeter, and then spent an increasing proportion of time in the central region. This increase was less in the drug-treated group. On the second day, during the first minute of the test, animals which had received vehicle spent a much higher proportion of time in the central region than they had spent in the first minute of their first swim. In contrast, animals which had received drug before their first swim spent only the same low proportion of time in the central region during the first minute of their second swim as they had during the first minute of their first swim. These results suggest that in both tests memory formation was impaired under the influence of MK 801, and thus are consistent with either a direct or indirect role of NMDA receptors in memory formation.

101.4

A BEHAVIORAL EFFECT OF MK801 MIMICS A DEFICIT ASSOCIATED WITH HIPPOCAMPAL DAMAGE. G. Robinson*, G. Crooks*, P. Shinkman, and M. Gallagher. Dept. Psychology, Univ. North Carolina, Chapel Hill, NC 27599.

N-methyl-D-aspartate (NMDA) receptors appear to play an important role in the induction of long-term potentiation in the hippocampal formation. The anticonvulsant compound MK-801 {(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclo- hepten-5,10-imine maleate} belongs to a class of antagonists that bind to phencyclidine (PCP) sites and block activation of NMDA receptors. This experiment examined the effects of systemically administered MK-801 on a spatial learning task that is sensitive to hippocampal dysfunction.

All animals received an habituation session (90 sec) during which they were allowed to swim in a pool of tepid, opaque water. Rats were then trained for 3 daily sessions (6 trials each) to locate an escape platform submerged 1 cm below the water surface in the pool. During this "place" training, the platform remained in the same position across trials. A final session consisted of a free swim (60 sec) to analyze the search pattern with the platform removed from the pool. This trial was immediately followed by 6 "cue" training trials during which animals were allowed to escape to a visible platform that was positioned in different locations from trial to trial. Animals received subcutaneous injections (0.00, 0.01, 0.05, or 0.08 mg/kg MK-801) prior to each session.

At the 0.08 mg/kg dose of MK801, a general performance decrement was evident on both the place and cue tasks. At the 0.05 mg/kg dose, rats were impaired in place, but not in cue learning. The latter results parallel the effects of damage to the hippocampal formation. Other behavioral tests are currently underway to assess the range of learning/memory tasks that are sensitive to low-dose MK-801 administration.

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101.5

GLYCINERGIC COMPOUNDS FACILITATE MEMORY FORMATION AND RETRIEVAL IN RATS. G.E. Handelmann, L.L. Mueller, and A.A. Cordi*. CNS Diseases Research, G. D. Searle and Co., St. Louis, MO 63198.

Glycine and other neutral amino acids (e.g. serine) have been shown to potentiate neuronal responses to N-methyl-D-aspartate (NMDA; Johnson and Ascher, Nature, 325: 529, 1987), and to bind to a recognition site in rat brain which has the pharmacological characteristics (Monahan et al., FASEB J., 2:A1401, 1988) and anatomical distribution (Bristow et al., Eur. J. Pharmacol., 126:303, 1986) consistent with an NMDA-receptor coupled modulatory site. As the NMDA receptive neural pathways in the forebrain are believed to play a role in processes of learning and memory (Collingridge, Trends Pharmacol. Sci., 6:407, 1985) glycinergic neurotransmission may be expected to enhance these functions. We studied the effects of two glycinergic compounds on performance of a shock-motivated passive avoidance task in rats. Milacemide, a prodrug which increases brain glycine concentrations (Christophe et al., Life Sci., 33:533, 1983), enhanced memory consolidation and retrieval at 10 mg/kg i.p. D-cycloserine, which binds with high affinity to the glycine receptor (Monahan et al., submitted), was effective at 3 mg/kg. These studies suggest the importance of the glycine neurotransmission in cognitive processes.

101.6

THE DIHYDROPYRIDINE CALCIUM CHANNEL AGONIST BAY K 8644 ENHANCES WORKING MEMORY IN THE RAT AND MOUSE. D.G. Spencer, Jr. and J. Traber*. Neurobiology Dept., Troponwerke (Bayer AG), Neurather Ring 1, 5000 Köln 80, FRG.

BAY k 8644 (1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) has high affinity for the dihydropyridine site on calcium channels, whereupon it exerts agonistic effects, enhancing calcium ion flux. This mechanism is associated in the periphery with vasoconstriction and positive inotropic effects, while facilitation of neurotransmitter release and of long-term potentiation in the hippocampus have been observed in the central nervous system. In the present study, the effects of BAY k 8644 on rat and mouse memory performance were studied. Rats were trained to select arms without repetition in a conventional 8-arm radial maze, with an interval of 1 or 4 hours between the first 6 and last 2 arm choice opportunities. BAY k 8644 (1.25 mg/kg i.p.) injected during the interval consistently and significantly improved post-interval arm choice accuracy. BAY k 8644 at 0.64 and 1.25 mg/kg i.p. also significantly antagonized scopolamine-induced deficits in passive avoidance retention performance in mice. These data support on a behavioral level the hypothesis of calcium channel activity involvement in the neurobiology of memory.

101.7

Physostigmine, Tetrahydroacridine, and 3,4-Diaminopyridine protect against hypoxia-induced passive avoidance retention deficit: Comparison of safety margins. V. J. DeNoble, K. Strek*, L. Johnson*, and L. Cook. E. I. DuPont de Nemours & Co., Med. Prod. Department, Wilm., DE 19898.

The applicability of acetylcholinesterase (AChE) inhibitors for intervention of a cholinergic deficit is limited by the imbalance between acetylcholine (ACh) and the absence of AChE at the synapse. DAP is a compound that releases ACh in vitro without effects on AChE. In this experiment post training administration of two AChE inhibitors and DAP on hypoxia-induced amnesia was evaluated. During PA acquisition, rats were placed in a lighted compartment and given access to a dark compartment. Ten sec after entering the dark side rats were given two, three sec foot shocks (1.5 mA). Retention (300 sec max) was tested four hr later. At 21% O_2 retention latencies were at max, however, exposing rats to 6.5% O_2 for 30 min prior to PA training reduced median retention latencies to less than 30 sec. All three compounds produced a dose related increase in retention latencies. The compounds were evaluated in an overt side effects test battery. A comparison of the peak effective dose in hypoxia and minimum dose which produced tremors revealed the following ratios. DAP had a safety margin of 5, Ph 1, and THA 2. The results suggest that DAP, a compound that induces non-stimulated release of ACh offers no safety advantage over AChE inhibitors.

101.8

Comparative Effects of Putative Cognitive Enhancing Compounds. G. P. Alberici and G. F. Steinfels. E. I. Dupont de Nemours & Co., Med. Prod. Dept., Wilmington, DE 19898 USA.

Electroencephalographic (EEG) activity in man and animals has been shown to change with age and pathological states, such as Alzheimer's Disease. Generally this change consists of a shift in EEG spectral power with a relative decrease in the high frequencies (alpha and beta) and a relative increase in the low frequencies (delta and theta). This decrease in the alpha and beta bands is believed to be involved with a deterioration of vigilance and is correlated with deficits in cognitive performance. It is thought that a compound that would reverse this shift would also improve cognitive performance. Tetrahydroacridine (THA), amphetamine, physostigmine and aniracetam have been shown to improve some elements in the cognitive process. In this study the cortical EEG profile of these compounds in the Long Evans rat was examined. The profiles place the compounds into two general groups; those that increase alpha (aniracetam and amphetamine) and those that increase beta (THA and physostigmine) relative power. Amphetamine and physostigmine produced an inverted U-shaped dose response curve for relative change in spectral power in the alpha or beta bands. The highest doses of amphetamine (1.0 mg/kg) and physostigmine (1.0 mg/kg) decreased relative power in all bands except theta, which increased. These profiles suggest that differences among the compounds may reflect the relative degree of pharmacological efficacy.

101.9

SELECTIVE EFFECTS OF N-methyl-D-aspartate (NMDA) ON ACQUISITION OF A PASSIVE AVOIDANCE RESPONSE IN RATS. K.W. Jones*, C.L. Schaeffer*, G. Steinfels, G. Alberici, and V.J. DeNoble (SPON: W.K. Schmidt). E.I. duPont Medical Products Dept., Wilmington, DE 19898.

The NMDA receptor is suspected to play an important role in learning and memory. In the following studies the function of NMDA in learning a passive avoidance (PA) task was assessed. During acquisition male CD rats were placed in the illuminated side of a two compartment apparatus. When they went into the dark side, they received two three sec .75 mA footshocks. Retention testing occurred 24 hr later. The rat was placed in the illuminated side and the latency was recorded (≤300 sec). Administration of NMDA 30 min prior to acquisition resulted in significant amnesia at doses of 3, 10, 30, and 50 mg/kg s.c. When administered immediately after acquisition NMDA had no effect on memory and the same doses given 30 min prior to retention testing had no effect on recall. An increase in low frequency EEG was found following 15-48 mg/kg s.c. NMDA, indicating a direct effect on brain function. The 30 mg/kg NMDA-induced amnesia was not blocked by the NMDA antagonist 3-((+)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) at doses of .001 to .05 mg/kg. The results suggest that NMDA can interfere with the acquisition of a PA task, but not consolidation of memory or recall for the task.

101.10

INFLUENCE OF AP5 ON MEMORY FORMATION IN THE CHICK. T.A. Patterson, K. Scharre, E.L. Bennett, & M.R. Rosenzweig. Brain Research Group, Open University, Milton Keynes, MK7 6AA England and Department of Psychology, University of California, Berkeley, CA 94720.

Several experiments examined the influence of AP5, a NMDA receptor antagonist, on memoryin chicks using a one-trial taste-avoidance task.

To determine the dose response function for AP5, chicks were given bilateral injections into the medial hyperstriatum ventrale (MHV) of either saline or different doses of APV, 5 min before training. Chicks were tested 24 hr after training. Results show that 250 picogram to 25 microgram (dose per brain) AP5 produced significant amnesia.

Experiment 2 examined the pre- and post-training susceptiblity gradients of AP5. Groups of chicks were given bilateral injections into the MHV of either saline or AP5 (250 nanogram per brain) at various times before and after training, and the chicks were tested 24 hr after training. The results indicate that AP5 produced amnesia when given between 60 and 5 min before training, but was not amnestic when given after training.

The time course of amnesia development following pretraining injection of AP5 (250 nanogram per brain) was determined. Chicks were given 5 min pretraining bilateral injections into the MHV of either saline or AP5, and groups of chicks were tested at various times after training. The results show that AP5 produced permanent amnesia that developed between 10 and 30 seconds after training.

These results indicate that NMDA receptor activation plays a role in memory formation in the chick. The results suggest that activation of NMDA receptors may be important for either acquisition, or formation of a very early stage of memory. Experiments are in progress to decide between these possibilities.

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101.11

A COMPARATIVE STUDY OF THE LPO AND MHV IN MEMORY FORMATION IN THE CHICK BRAIN. P.A. Serrano, S.J. Ramus*, E.L. Bennett and M.R. Rosenzweig. Department of Psychology, University of California, Berkeley, CA 94720.

The medial hyperstriatum ventrale (MHV) of the chick brain is involved with memory formation (Gibbs and Ng, 1977). When injected into the MHV, glutamate (GLU), ouabain (OUAB), and emetine (EME) are effective in disrupting the formation of short-term, intermediate-term (ITM), and long-term memory, respectively (Patterson et al., 1986). These agents were injected into another area of the chick brain, the lobus parolfactorius (LPO), which showed increased [^{14}C]2-deoxyglucose uptake in chicks after training on a one-trial taste avoidance task (Rose, et al. 1986). Injections of 50mM GLU, .027 mM OUAB, 2.25mM EME or saline (10µl per hemisphere) were given bilaterally into the LPO or MHV. Five min after injection, the chicks were trained on a one-trial taste avoidance task and then tested 24 hr later. Chicks injected into the MHV showed significant amnesia for all three drugs ($p<.05$). Only chicks injected with OUAB in the LPO showed significant amnesia ($p<.05$). The results show that a depolarizing agent (GLU) or a protein synthesis inhibitor (EME) are not effective at producing amnesia, whereas the Na+/K+ ATPase inhibitor OUAB does produce amnesia. The LPO may be an area primarily involved with ITM formation, suggesting that not all areas are involved with the three stages of memory formation. To test this further, we are determining comparative time courses for the appearance of amnesia when OUAB is injected into the MHV or LPO. Effects of other ITM inhibitors are presently being studied in the LPO, and other brain regions are being investigated.

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101.12

MEMORY FOR A ONE-TRIAL PASSIVE AVOIDANCE TASK IN CHICKS GIVEN ANISOMYCIN IS NOT STATE DEPENDENT. D.W. Lee, A. M. Perlmutter*, E.L. Bennett and M.R. Rosenzweig. Dept. of Psychology, Univ. of California, Berkeley, CA, 94720.

Research has shown that drugs such as glutamate, ouabain, and anisomycin inhibit the formation of short-term, intermediate-term, and long-term memory, respectively (Gibbs & Ng, 1977; Patterson, et al., 1986). The experiment described here investigates the possibility of state dependency of amnesia caused by the protein synthesis inhibitor anisomycin (ANI). Further experiments will examine both glutamate and ouabain.

Two-day-old XCell white leghorn cockerels were trained in a one-trial passive avoidance task. Bilateral injections (10µl per hemisphere) of saline (SAL) or ANI (3.0 mg/ml) were made into the medial hyperstriatum ventrale 5 min pretrain and 5 min pretest. Training consisted of one 10 sec exposure to a 3mm metal bead dipped in either methylanthranilate (M), a bitter tasting liquid, or water (W). Chicks peck readily at the bead lure during this 10 sec period. Twenty-four hours later, testing consisted of one 10 sec exposure to the same dry bead. Retention for this task was measured as the avoidance of the M bead during test; animals given the water bead during training were expected to peck during test. Six groups were formed: SAL-W-SAL, SAL-M-SAL, ANI-W-ANI, ANI-M-ANI, ANI-W-SAL, ANI-M-SAL (pretrain drug - training bead - pretest drug). As expected, ANI-M-SAL chicks showed significant amnesia; ANI-M-ANI chicks did not. However, the ANI-W-ANI group also showed significantly higher rates of avoidance of the water bead when compared to that of controls. These findings suggest that ANI administration results in generalized avoidance behavior but not necessarily state dependency. The design and interpretation of state dependency tests of ANI will be discussed.

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101.13

POSTTRAINING INJECTION OF GABAERGIC ANTAGONISTS ENHANCE RETENTION OF AVERSIVELY MOTIVATED TASKS: ROLE OF THE AMYGDALOID GABAERGIC SYSTEM.

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The effect of sub-convulsive doses of GABAergic antagonists on the retention of two aversively motivated tasks, inhibitory avoidance and Y-Maze discrimination, was investigated in adult CFW mice. Posttraining IP injections of picrotoxin (0.1-3.0 mg/kg) and bicuculline (0.1-3.0 mg/kg), but not bicuculline methiodide (BMI) (1-30 mg/ kg), induced a dose-dependent increase in retention of both tasks. Neither picrotoxin nor bicuculline affected testing latencies when the animals were trained in the absence of footshock punishment in the inhibitory avoidance task.

The participation of the GABAergic system in the amygdala was also examined. Adult Sprague-Dawley rats with bilateral implanted amygdala cannulae were trained in the inhibitory avoidance task and tested 48 hr later. Rats that received posttraining injections of BMI (0.1-1.0 nmoles per cannula) showed a significant increase in retention as compared to control animals.

Our results indicate that GABAergic antagonists enhance the retention of aversively motivated tasks through central mechanisms, and suggest the involvement of the intrinsic GABAergic system of the amygdala in memory consolidation.

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101.14

DIAZEPAM AND LONG-TERM HABITUATION OF THE ACOUSTIC STARTLE RESPONSE IN RATS. B. J. Young*, F. J. Helmstetter, and R. N. Leaton. Department of Psychology, Dartmouth College, Hanover, NH 03755.

It is well known that fear increases the amplitude of the acoustic startle response. This effect is usually shown in a potentiated startle paradigm following explicit fear conditioning (e.g., Davis, 1978). We have shown significant correlations between freezing (a behavioral index of fear) and startle amplitudes in a potentiated startle paradigm (Leaton & Borszcz, 1985), and without explicit fear conditioning we have found increased freezing over the early trials in acoustic startle habituation paradigms (Borszcz,Cranney,& Leaton, 1988). Fear occurring over the early trials of habituation training may mask the appearance of long-term habituation (LTH), and we have shown that manipulations that reduce fear, lesions of the amygdala (Leaton & Supple, 1987) or of the central gray (Borszcz, et al.), appear to facilitate the rate of LTH. We now report that diazepam, a widely used benzodiazepine with marked anxiolytic properties, appears to facilitate LTH. Rats tested daily for 4 days (10 trials/day, 60-sec ISI, 125-dB, 100-ms white noise stimulus) following 2.5 mg/kg of diazepam showed significantly greater rates of habituation from Day 1 to Day 2 than vehicle injected controls. Diazepam reduced overall response levels, but the significant rate of decrement was accompanied by a larger absolute response decrement. Diazepam injections represent still another procedure for reducing fear and producing what appear to be increased rates of LTH.

101.15

ENHANCING EFFECT OF A β-CARBOLINE ON MULTIPLE-TRIAL LEARNING IN MICE. P. Venault*, M.J. Raffalli-Sebille*, R.H. Dodd** and G. Chapouthier*. Départ. de Psychophysiologie, Lab. de Physiologie Nerveuse* and Inst. de Chimie des Substances Naturelles**, CNRS, Gif-sur-Yvette, France.

In contrast to diazepam (DZ), which impairs memory processing, methyl β-carboline-3-carboxylate (β-CCM), administered before the training session, has an enhancing effect on performance in the retention test (Venault et al., Nature, 1986, 321, 864). However, this action of β-CCM has been demonstrated only in single trial or single session learning protocols. The present report confirms these results in a multiple trial learning procedure in mice (brightness discrimination in a T maze, with negative reinforcement). The animals were trained for 10 trials per day for 6 consecutive days. The sessions during the first 3 days took place after a subcutaneous injection of β-CCM (0.3 mg/kg), DZ (2.5 mg/kg) or saline. Performance was improved by β-CCM and impaired by DZ in the first 3 sessions as well as in the 3 final sessions (no further treatment). These results provide evidence that the opposite effects of β-CCM and DZ observed in single trial learning protocols, during the retention test, are already observable during the drug treatment. They confirm that both drugs have an action on the acquisition (learning) side of memory processing rather than on retention (memory) itself. These effects being suppressed by the coadministration of Ro 15-1788 (15 mg/kg, s.c.), our results suggest a specific role for benzodiazepine receptors in learning.

(SPON: M. Amalric)

101.16

ENHANCEMENT OF LEARNING AND MEMORY IN MICE BY CGS 8216, A BENZODIAZEPINE RECEPTOR ANTAGONIST.

H. Lal, B.A. Kumar* and M.J. Forster. Dept. of Pharmacology, Texas Coll. of Osteopathic Medicine, Fort Worth TX 76107-2690.

Benzodiazepine receptor antagonists are known to reverse diazepam-induced amnesia, but may also improve learning and memory when administered alone (Lal, H. et al. *FASEB J.* 2, 1988, in press). Based on these findings it was proposed that diazepam-like endocoids may exert a tonic inhibitory influence over learning/memory processes. In order to further test this hypothesis, the present study examined the mnemonic effects of CGS 8216, a benzodiazepine antagonist with a chemical profile different from flumazenil. Mice pretreated with CGS 8216 (2.5, 10, or 40 mg/kg, i.p.) were tested for acquisition and retention of a discriminated escape response in a T-maze (Forster, M.J. et al. *Drug Dev. Res.* 11:97, 1987). All CGS 8216-treated groups required fewer trials to reach the acquisition criterion than controls. When the mice were tested for retention one week later (without further treatment), the previously CGS 8216-treated mice had greater difficulty reversing the maze habit than controls and showed improved first-trial recall. The effects of CGS 8216 on retention were dose-dependent and similar to the memory-enhancing effects reported previously for flumazenil. These findings suggest that the enhancement in learning and memory seen after CGS 8216 and flumazenil are mediated specifically via an antagonism of benzodiazepine receptors. [supported by a Grant from CIBA-GEIGY Corp. (H.L.) and NIH grant AG06182 (M.J.F.)].

101.17

Ro 15-4513 ENHANCES DISCRIMINATION LEARNING AND MEMORY IN MICE. B.A. Kumar*, H. Lal and M.J. Forster. (SPON: I.M. Korr).Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

The benzodiazepine receptor antagonist, flumazenil, was previously shown to facilitate acquisition and retention in mice (Lal H. et al., *FASEB J.*, 2, 1988, in press). The memory-enhancing effect of this drug was proposed to be mediated via antagonism of diazepam-like endocoids. In the present study, we investigated the mnemonic effects of Ro 15-4513, an anxiogenic drug (Harris, C.M. & Lal, H. *Neuropharmacol.*, 26:1545-1547, 1987) with inverse agonist properties at benzodiazepine receptors (Harris, C.M. & Lal H. *Drug Dev. Res.*, 13, 1988, in press). Mice received Ro 15-4513 (2.5 and 10 mg/kg, i.p.) 15 min prior to training under a T-maze, discriminated escape paradigm described previously (Forster, M.J. et al. *Drug Dev. Res.*, 11:97, 1987). All mice were tested for retention one week later without additional treatment. Ro 15-4513 resulted in a dose-dependent decrease in the number of trials required for initial acquisition of the maze habit. In retention tests, the Ro 15-4513-treated mice had better first-trial recall and required more trials to reverse the previously acquired habit than controls. These findings are consistent with the hypothesis that memory processes could be modulated by endogenous ligands acting on benzodiazepine receptors.

101.18

REVERSAL OF CHLORDIAZEPOXIDE-INDUCED IMPAIRMENT IN DISCRIMINATION PERFORMANCE BY RO 15-1788. S.O. Cole*(SPON: J.L. Falk). Dept. of Psychology, Rutgers University, Camden, NJ 08102.

Chlordiazepoxide (CDP) 10 mg/kg, administered on 6 weekly sessions, impaired the performance of a previously-learned go-no go successive discrimination in male, Sprague-Dawley rats. The impairment was accompanied by an increase in responding during no go periods of the task, indicating that CDP-drugged animals have difficulty withholding incorrect responses. The benzodiazepine (BDZ) receptor antagonist Ro 15-1788 (5 and 10 mg/kg) reversed the impairment in discrimination performance on sessions 2-6 (but not session 1) when co-administered with CDP. While the reversal of impairment by Ro 15-1788 was not dose-dependent, it was accompanied by a significant reduction in responding during no go periods of the task. These findings suggest that the impairment in discrimination performance produced by CDP is mediated by central BDZ receptor sites. When administered alone, Ro 15-1788 10 mg/kg (but not 5 mg/kg) produced a mild BDZ-like impairment in discrimination performance and increase in no go period responding. These findings suggest that Ro 15-1788 may have some intrinsic action of its own which needs to be assessed independently of its use as a mediational research tool.

101.19

NEUROCOGNITIVE FEATURES OF MIDAZOLAM INDUCED AMNESIA. J. French, E.F. Domino, R. Pohorecki*, C.F. Galus*, and S.K. Pandit*. Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109-0626.

Subhypnotic doses of midazolam were evaluated in healthy adult volunteers using a neurocognitive assessment battery. Volunteers were assigned randomly to medicated and control groups (10 subjects each). Midazolam HCl in a dose of 0.02 mg/kg was injected i.v. three times at 15 min intervals after obtaining baseline electrophysiological recordings and memory test results.

The incidence of EEG beta activity from the frontal lead F_3 was increased while alpha activity from the occipital O_1 lead was decreased by midazolam in a dose dependent manner. The amplitude of the P300 component of the auditory evoked potential progressively decreased with increasing doses of midazolam as did the accuracy of counting the number of rare tones. The N100 data was progressively augmented. Twenty four hr after midazolam, the treated subjects were impaired in their ability to recall memory cards presented, particularly following the second and third dose. The ability to recall misplaced objects was impaired relative to baseline. The results support the conclusion that midazolam induced amnesia is better correlated with an attention deficit than gross sedation.

101.20

ROLE OF CHOLINERGIC AND GABAERGIC NEURONAL SYSTEMS IN CYCLOHEXIMIDE-INDUCED AMNESIA IN MICE. T. Nabeshima, Y. Noda*, K. Itoh* and T. Kameyama*. Dept. of Chemical Pharmacology, Fac. of Pharmaceutical Sciences, Meijo Univ., Nagoya, 468 Japan.

The role of cholinergic and GABAergic neuronal systems on the cycloheximide (CXM)-induced amnesia was investigated using the step-down type passive avoidance task in mice. CXM (7.5 - 120 mg/kg, s.c.) given just after the training caused amnesia (indicated by short latency to step down from the platform on the gride floor) in the retention test conducted 24 hr later in a dose dependent fashion. In the CXM (60 mg/kg)-treated mice, a choline esterase inhibitor, physostigmine (PHY; 0.125 and 0.25 mg/kg, i.p.), or GABA agonists, muscimol (1 and 2 mg/kg, i.p.) and baclofen (6 and 12 mg/kg, i.p.) given just after training markedly prolonged step down latency (SDL), indicating reversal of amnesia. The antiamnesic action of PHY (0.125 mg/kg) was almost completely antagonized by a central acetylcholine antagonist, scopolamine (3 mg/kg, s.c.), but not by a peripheral acetylcholine antagonist, butylscopolamine (3 mg/kg, s.c.). Furthermore, the antiamnesic action of muscimol (2 mg/kg) was reversed by GABA antagonists, picrotoxin (0.5 mg/kg, s.c.) and bicuculline (0.5 mg/kg, s.c.), while the effect of baclofen (12 mg/kg) was reversed by picrotoxin (0.5 mg/kg), but not by bicuculline (0.5 mg/kg). These results suggest that the dysfunction of cholinergic and GABAergic neuronal systems play an important role in the CXM-induced memory impairment on the passive avoidance task.

EPILEPSY II

102.1

MUSCARINIC ACETYLCHOLINE RECEPTORS IN THE GENETICALLY EPILEPSY-PRONE RATS (GEPRs) by P.C. Jobe, R. Duffield* and A.K. Ho*. Department of Basic Sciences, Univ. of Illinois College of Medicine at Peoria, Peoria, IL 61605

The possible role of the central muscarinic receptors (AChRs) in seizure predisposition was studied in the GEPRs under phosphorylating conditions. Brain homogenates and synaptic membranes were prepared from naive and acoustically screened GEPRs of the moderate (GEPR-3) and severe (GEPR-9) seizure types. Results obtained showed that the specific [^{3}H]-QNB binding sites were significantly decreased in the naive GEPR-3 and GEPR-9 brain when compared with the controls, with GEPR-9s ($p<0.01$) showing significantly greater reduction than the GEPR-3s ($p<0.05$). Total binding sites remained significantly lower in GEPR-9 brain during seizures and 1 hour after seizures. Under phosphorylating conditions, both the controls and GEPR-3s showed a reduction in binding in the range of 20% approximately whereas in the GEPR-9 brain [^{3}H]-QNB binding showed no significant reduction. The number of [^{3}H]-pirenzepine binding sites were significantly higher in the GEPR-9 than the GEPR-3 and the controls. Binding under phosphorylating conditions showed little or no effect on the [^{3}H]-pirenzepine binding in the GEPRs. These findings suggest an abnormal response of the GEPRs to phosphorylation and a differential effect on the M_1 and M_2 receptor subtypes. [Supported in part by BRSG, University of Illinois, AFAR and a grant from the NIH (NS 16829).

102.2

APAMIN-SENSITIVE POTASSIUM CHANNEL AND CHOLINERGIC INFLUENCES ON INFERIOR COLLICULAR SEIZURE ACTIVITY. **Thomas J. McCown and George R. Breese Univ. of North Carolina Sch. Med. Chapel Hill, N.C. 27599-7250.**

The role of apamin-sensitive potassium channels, as well as nicotinic and muscarinic cholinergic function, on neural excitability was investigated within the inferior collicular cortex. Inferior collicular microinjection of apamin(21 pmol) significantly reduced the stimulation current necessary to elicit seizure activity from the inferior colliculus, yet higher doses did not cause spontaneous seizure activity. Likewise intermediate doses of d-tubocurarine (0.22 umol), gallamine(1.7 umol) or α-bungarotoxin(0.33 nmol) reduced the threshold current, while higher doses caused spontaneous seizure activity. Conversely, atropine caused a dose-related increase in the seizure threshold. However, atropine pretreatment totally blocked seizure threshold reduction effects of α-bungarotoxin, partially attenuated d-tubocurarine or gallamine effects, yet did not alter the actions of apamin. Thus, both apamin-sensitive potassium channels and cholinergic function significantly influence seizure activity. (Supported by USPHS HD-031110).

102.3

MATHEMATICAL MODELLING OF THE HUMAN EPILEPTIC SPIKE COMPLEX IN MAGNETOENCEPHALOGRAPHY. C.Baumgartner* (1,3), S.Di* (1,2), W.W.Sutherling (1) and D.S.Barth (1,2) (SPON: C.H.Markham). Dept. of Neurology (1) and Psychology (2), Univ. of California, Los Angeles, CA 90024, Neurological Univ. Clinic, Vienna, Austria (3).

The interictal spike complex in epileptic patients is often difficult to interpret because of its origin in different brain regions and a substantial spatial and temporal overlap of the generated potentials. This poses a major problem for the localization of the abnormally discharging neuronal populations by non-invasive measurements as scalp-EEG and magnetoencephalography (MEG).

We therefore developed new methods of computer-based analysis of the epileptiform MEG using both physical models and multivariate statistical methods to investigate spatially and temporally overlapping extracranial neuromagnetic fields over the entire course of the interictal spike complex. These methods were applied to MEG measurements from five patients with partial seizures. We could estimate the localization of several different brain regions which generated the epileptic spike discharges and were active simultaneously.

It is concluded that with the help of these new models the investigation and localization of epileptic discharges from several different brain regions overlapping both in space and time can be performed by non-invasive measurements. These methods of analysis should also readily be applicable to data from scalp-EEG.

102.4

NEUROBIOLOGICAL BASES OF EPILEPSY IN MAGNETOENCEPHALOGRAM (MEG). S.Di*, C. Baumgartner*(1) and D.S.Barth. (SPON: W.W. Sutherling). Dept. of Neurology and Psychology, Univ. of California, Los Angeles, CA 90024. (1).Neurological Univ. Clinic, Vienna, Austria.

The magnetoencephalogram (MEG) is a recently developed method of localizing and studying the synchronized cellular currents produced by epileptic paroxysms in animal and man. Clinically, MEG is a potential tool to noninvasively localize human epileptic foci for neurosurgical treatment of seizures. However, based on the theoretical prediction that neuromagnetic fields are induced predominantly by intracellular currents, MEG animal studies may also provide valuable insights into the electrophysiological mechanisms of seizure disorders.

The present study uses a penicillin model of epilepsy in rat cortex to explore the neurogenesis of epileptiform magnetic fields in man. Laminar electrical recordings of rapid and slow cellular currents were obtained from 5 rats using glass microelectrodes and analyzed by current source density (CSD) method. MEG measurements were done on the same animals. combining MEG with CSD maps from detailed electrical recording, a qualitative relationship has been established between recorded neuromagnetic field and the spatial-temporal characteristics of the electrical activity of the underlying neural structures.

102.5

RAT STRAIN DIFFERENCES IN KAINIC ACID NEUROTOXICITY. G.T. Golden, T.N. Ferraro, G.G. Smith*, J.H. Kulp* and P.F. Reyes*. VA Medical Center, Coatesville, PA 19320 and Thomas Jefferson Medical College, Phila., PA 19107.

Genetic factors may influence the neurotoxic effect of Kainic acid (KA). Sanberg (1979) reported that the neostriatum of Wistar rats is more sensitive to intrastriatally injected KA than that of Sprague-Dawley rats. Systemic administration of KA elicits multifocal seizures and causes biochemical changes and neuronal loss. Here we report behavioral, EEG and biochemical data indicating strain differences in sensitivity to KA and, in addition describe a strain of rats which reliably and consistently demonstrate electrographic and behavioral status epilepticus to a 10 mg/kg, sc dose of KA. Male Sprague-Dawley (SD, n=11), Wistar-Furth (WF, n=10) and Long-Evans (LE, n=10) rats had electrodes implanted in the hippocampus and cortex for chronic EEG recordings. Ten days after recovery rats received a sc injection of 10 mg/kg KA. Behavior and EEG were monitored for 240 min. The # of subclinical (only EEG) and electroclinical seizures, # of wet dog shake episodes (WDS), the total time in ictal activity and latency to onset of status epilepticus were compared in the three strains. Results showed that WF strain demonstrated the most consistent and least variable response to systemically administered KA. WF rats had the shortest latency to onset of status epilepticus, spent the greatest amount of time in seizure activity and had more behavioral and electrographic seizures. (Supported by VA funds.)

102.6

EFFECTS OF REPEATED AUDIOGENIC SEIZURES (AGS) ON SEIZURE SEVERITY AND EEG IN TWO SUBSTRAINS OF THE GENETICALLY EPILEPSY-PRONE RATS (GEPRs) D.K. Naritoku*, L.B. Mecozzi*, and C.L. Faingold. (SPON: J.R. Couch) So., IL Univ. Sch. Med., Depts. Neurol. and Pharmacol., Springfield, IL.

GEPRs show increased susceptibility to seizures induced by numerous stimuli. GEPR-9s exhibit wild running, clonus, and tonic seizures with hindlimb extension (HLE) in response to loud sounds. AGS in GEPR-3s do not include HLE. Effects of daily AGS induced by electrical bell sound (110 dB SPL) were studied in both substrains. Behavior and EEG were simultaneously recorded in unrestricted animals. All GEPR-9s uniformly developed prolonged seizures and cortical spike-wave EEG abnormalities by day 3. GEPR-3s showed an erratic response to daily AGS. Most GEPR-3s developed prolonged seizures and spike-wave abnormalities; however, spike-wave onset varied from day 3 to 10 and seizure behaviors were inconsistent. GEPR-3s with prolonged AGS exhibited clonic activity which differed from the usual running clonic seizures, and profound postictal states. Tonic seizures with HLE were never seen in the GEPR-3. These data demonstrate increased seizure severity following repeated AGS in GEPR substrains which have distinctly different behavioral seizure patterns. This suggests that mechanisms of AGS propagation from brainstem initiation sites may be dissimilar in the two substrains despite increased severity. Seizure propagation to cortex appears to be induced by repeated AGS in the GEPR.

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102.7

ACCELERATED KINDLING IN THE MUTANT EPILEPTIC MOUSE El R.C. Green, B.J. Elizondo, T.N. Seyfried Department of Neurology and Division of Neuroscience, The Children's Hospital and Harvard Medical School and Department of Biology, Boston College, Boston, MA 02115

The El mouse carries an autosomal dominant mutation on a ddY inbred backround. Homozygous mutants (El/El) develop generalized motor seizures after 2 months of age when tossed in the air. El/El mutants (n=7) and non-epileptic ddY +/+ controls (n=6) were implanted with bipolar electrodes in the left olfactory bulb and kindled with daily stimulations through six consecutive stage 5 seizures. All mice were kindled at 4-6 weeks of age, prior to the development of any inherent seizure activity in El.

Kindling to first stage 5 seizure required 14.1 ± 1.223 stimulations in the epileptic El mice versus 19.5 ± 0.764 stimulations in the ddY mice (mean ± SEM, $p < .005$). The kindling rate to the sixth consecutive stage 5 seizure was similarly accelerated in El. The mean afterdischarge (AD) duration at the initial stage 5 seizure in the El mice (23.3 ± 1.085 seconds) was significantly shorter than that of the non-epileptic controls (38.7 ± 2.14 seconds) (mean ± SEM, $p < .0001$). The shorter AD duration was entirely accounted for by a shorter mean latency to onset of motor seizure in the El mice ($p < .005$).

These results demonstrate that there is accelerated electrical kindling in the El mouse in comparison to ddY controls, at an age prior to the development of motor seizures in El.

Supported by the Penfield Fellowship of the Epilepsy Foundation of America (RCG) and by NIH grants NS-23355 and NS-24826 (TNS).

102.8

SOUND-INDUCED SEIZURE RESPONSIVENESS OF THE MODERATE SEIZURE COLONY OF THE GENETICALLY EPILEPSY-PRONE RAT. **C.E. Reigel, P.K. Mishra, J.W. Dailey and P.C. Jobe. Dept. of Basic Sci., Univ. of Ill. Col. of Med. at Peoria, Peoria, IL 61656.**

The moderate seizure genetically epilepsy-prone rat (GEPR-3) has been bred to exhibit generalized clonic convulsions following a single running episode in response to sound. Such a convulsion receives an audiogenic response score (ARS) of 3 according to the method of Jobe and coworkers (J. Pharmacol. Exp. Ther. 184: 1-10, 1973). The GEPR-3 colony currently reflects 25-28 generations of brother-sister inbreeding for this characteristic clonic seizure. This has resulted in a highly consistent degree of seizure responsiveness in the progeny of the GEPR-3 colony. The purpose of the present report was to document the seizure characteristics of the recent GEPR-3 colony. Routine seizure assessments of eleven generations of GEPR-3s spanning a 3 year period were examined. The majority of GEPR-3 progeny are sound stimulated at 3 weekly intervals to confirm their seizure responsiveness and for the selection of new breeders. Latencies to the onset of wild running and convulsion are recorded in addition to the ARS score. Collectively, the GEPR-3s tested exhibited a 99.5 percent incidence of sound-induced seizures on the first test. Over 86 percent of the GEPR-3s exhibited seizures rated at an ARS of 3 on the first test. Latencies to running and convulsion decreased and incidence of ARS of 3 increased in the second and third tests, suggestive of an experiential effect on seizure severity.

102.9

CLASSICAL GENETIC ANALYSIS OF KINDLED SEIZURE SUSCEPTIBILITY IN DBA/2J AND C57BL/6J MICE. C. Applegate, S. DiFazio*, K. Spanknebel*, J. Burchfiel and P. Neumann*. Dept. Neuroscience, The Children's Hospital, Boston, MA 02115.

We examined the susceptibility of DBA/2J (D2) and C57Bl/6J (B6) inbred strains of mice to kindling stimulation of the olfactory bulb. The kindled seizure phenotypes of F_1 (N=14) and F_2 (N=19) hybrids and offspring of backcrosses (D2xF1; N=16; B6xF1; N=14) also were defined.

In comparison with B6 (N=18) mice, D2 (N=10) animals required significantly fewer stimulations to elicit the first generalized seizure, exhibited significantly longer afterdischarge durations at all kindled seizure stages and exhibited significantly longer motor seizure durations. F1 hybrids represented an intermediate seizure phenotype on these measures.

Phenotypic distributions in F2 and backcross generations were inconsistent with a single gene hypothesis suggesting that 2 or more genes are responsible for strain differences in kindled seizure susceptibility. We are currently defining seizure phenotypes in BXD recombinant inbred strains to determine the mode of inheritance of susceptibility to kindling and to identify and map the genes involved.

102.10

CHRONOBIOLOGICAL VARIATIONS IN THE CONVULSIVE EFFECT OF MONOSODIUM GLUTAMATE (MSG) WHEN ADMINISTERED TO ADULT RATS. R. Gutiérrez-Padilla*, F. Alfaro*, G. Tapia-Arizmendi* and A. Feria-Velasco. Unidad de Investigación Biomédica de Occidente, I.M.S.S. Guadalajara, Jalisco. MEXICO.

The convulsive effect of MSG administered intraperitoneally (i.p.) to various animals is well known and the convulsive pattern varies according to animal age. This work was designed to know whether the convulsive effect of MSG in rats would vary when the drug is given at different day times. Three groups of rats were given i.p. 4mg/g MSG at 7 am, 3 pm and 11 pm, whereas 15 rats were injected with NaCl (eqNaCl) equimolar to MSG solution at 7 am, 3 pm and 11 pm in groups of 5 as controls. Animal behavior was recorded in all experiments. Results from both groups were compared to those from similar groups of rats injected i.p. with isotonic saline solution (ISS). No convulsions appeared in animals injected with ISS or eqNaCl at the 3 times studied. With MSG, no variations were seen in the latency period when data from the 3 times studied were compared among them. Duration of convulsive period when rats were injected at 7 am was shorter (88.5$^+$27 min) than those obtained at 3 (199.4$^+$4) and 11 pm (203.5$^+$5). No significant variations were seen in total number of convulsive episodes in the 3 groups, but the number of seizures per hour and their intensity were significantly greater when animals were injected at 7 am than those seen when rats were studied at 3 and 11 pm. Nearly 70% of animals of the group injected at 7 am died in status epilepticus, whereas no deaths were recorded in animals injected at 3 or 11 pm. Results could be explained in terms of variations of physiological processes at both, the brain and extracerebral tissues involved in MSG metabolism related to circadian rhythms. Whether the behavioral variations correlate with variations of brain biochemical parameters related to the production of convulsions in the MSG model is unknown.

102.11

INDUCTION OF STATUS EPILEPTICUS IN NAIVE RATS BY ELECTRICAL STIMULATION OF VENTRO-LATERAL FOREBRAIN. J. Rick, J. Heitzner, G.O. Ivy, and N.W. Milgram. Div. of Life Sciences, Univ. of Toronto, Toronto, Ontario, M1C 1A4.

In an attempt to induce status epilepticus using electrical stimulation in experimentally naive rats, we implanted electrodes in a variety of limbic and cortical structures and stimulated continuously for up to 2 hours, using bursts of pulses delivered at 3Hz at progressively higher intensities. Thus far, status epilepticus was produced in 80% (4/5) of a group of animals with electrodes placed in the ventrolateral forebrain, adjacent to or in the endopiriform nucleus. In contrast, recurrent seizures persisting after the offset of stimulation developed in 13% (1/6) of the rats stimulated in the perforant path, and in none of the animals with neocortical electrodes. Histological analysis using both cell stains and immunocytochemical markers of astroctye reactivity revealed relatively widespread patterns of damage which was largely unilateral in the animals with endopiriform nucleus electrode placements. In contrast, the damage produced by stimulation of perforant path was bilateral and symmetrical. These findings support the claim of a unique seizure sensitive system in the pyriform cortex. Supported by NSERC.

102.12

3D ANALYSIS OF SEIZURES PATHWAYS IN CORTICALLY-KINDLED RATS

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In control and electrically kindled subjects, the metabolic responses to bicuculline-induced focal cortical seizures were studied. These experiments were undertaken to determine whether kindling of forelimb cortex alters its functional anatomy.

Rats were kindled within the forelimb sensorimotor cortex. Over a two month period experimental subjects received 39 to 50 stimulations; controls underwent afterdischarge testing at the start and end of experiment but were not stimulated during the kindling period.

Four hours prior to 14C-2-deoxyglucose administration, electrode headsets were replaced with epidural wells. Metabolic traces were obtained on experimental subjects and non-kindled controls while focal cortical seizures were induced by the topical application of (-) bicuculline methiodide (1 mg/ml).

The autoradiographic data were reconstructed to 3 dimensions. Each of 600 coronal sections was assigned a value corresponding to its rostral-caudal position. Geometric reassembly was performed in accurate 3-space. Anatomic surfaces were rendered to orient the viewer and statistics computed on metabolic data. Isodensity contours were established at several metabolic rates and volumetric measurements taken.

No striking differences between the kindled and control patterns of metabolic activation were observed. A more detailed analysis of the topology and volume of these metabolic effects is being performed and will be presented.

102.13

ELECTROGRAPHIC KINDLING IN AN ISOLATED PYRIFORM BLOCK IN A CHRONIC PREPARATION. M. Mosher and R. J. Racine. Dept. of Psychology, McMaster Univ., Hamilton, Ontario, CANADA, L8S 4K1.

Previous work has shown that pyriform lobe structures are heavily involved in the kindling process, regardless of which sites are kindled (Kairiss, E.W., et al. Brain Res. 1984, 322, 101-110). It is still not clear, however, whether the pyriform lobe structures require participation of other structures for these changes to develop. To test the ability of the pyriform cortex and amygdala to develop a chronic epileptogenic response pattern, we isolated a block of tissue containing a portion of the pyriform cortex and amygdala. These blocks retained a blood supply and remained viable for the duration of the experiment. An electrode was implanted into the center of the block. Kindling stimuli were then applied via this electrode while the electrographic response was monitored. We have found that these blocks support normal looking epileptiform afterdischarges (AD's) and that these discharges develop in strength in much the same way as in the intact preparation. The AD's increased in duration, the AD spike frequency increased, the AD spike amplitudes increased and the AD spike morphology became more complex. Spontaneous interictal spike activity also appeared within the isolated blocks. These changes were long-lasting. Also of some interest was the finding that convulsions would develop (though more slowly) in animals in which the isolation was not complete, indicating that propagation to outside structures required only a small bridge of tissue.

102.14

INFERIOR COLLICULUS (IC) UNIT ACTIVITY AND AUDIOGENIC SEIZURES (AGS) IN BEHAVING GENETICALLY EPILEPSY-PRONE RATS (GEPRs). C.L. Faingold and C.A. Boersma Anderson*, Dept. Pharmacol., Southern IL Univ., Springfield, IL 62794-9230

The GEPR displays AGS in response to intense acoustic stimuli. Recording neuronal activity during seizures is difficult, but microwire electrodes have been used recently during kindling. In this study microwires were implanted into the IC of GEPRs under anesthesia. At least one week later, multiunit and single unit activity were recorded in unrestrained GEPRs during acoustic stimulation. Responses were examined to acoustic stimuli (12 kHz tone bursts, 60-100 msec at 2/sec) that initiate AGS in the GEPR with high intensities. The number of action potentials in the response initially increased as acoustic intensity was increased, and an afterdischarge-like response was observed at the offset of the tone as AGS threshold was approached. The afterdischarge resulted from increased firing of the unit and recruitment of additional units. The overall responses of many units decreased with higher sound intensity [(non-monotonicity (NM)] as observed in normal animals. GABA is implicated in NM in normal IC neurons. However, NM in the GEPR was often overcome with further intensity increases. This reduced effectiveness of NM may be another manifestation of the reduced efficacy of GABA observed in GEPR IC neurons. Neuronal responses after AGS were greatly reduced reversibly during the post-ictal depression of behavior. (Support: NIH NS 13849; NS 21281).

102.15

NONCONVULSIVE STATUS EPILEPTICUS IN KINDLED RATS: 2-DG UPTAKE & NEUROPATHOLOGY. G.G. Buterbaugh, B.E. Jones, J.S. Kiefer* and G.M. Hudson*. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Pilocarpine (20 mg/kg)-facilitated status epilepticus (pfSE) was evoked by amygdala stimulation of amygdala kindled rats. All rats (N=14) remained upright and immobile to the onset (55.4 ± 2.4 min) of slow leftward circling continuing through 2 hrs with generalized EEG seizures when pfSE was terminated. Three weeks later, histology showed enlarged ventricles and bilateral cell loss, especially in the substantia nigra pars reticulata (SNpr) and the paleolimbic cortex; hippocampal regions were relatively spared. Other rats received i.v. ^{14}C-2-DG after 2 hrs pfSE, 45 min before decapitation; brains were frozen sectioned for autoradiography. 2-DG uptake showed a uniform hypometabolic pattern (relative to rats after 10 min of pfSE), most notable in SNpr and paleolimbic regions. These results support a relationship between diminished 2-DG uptake and regional brain damage in a model of nonconvulsive SE. Moreover, dorsal hippocampal kindled rats (2 hrs pfSE) showed similar behavior and brain damage as amygdala rats and supported generalized seizures and pfSE three weeks post-pfSE. Since pfSE continues for 4-6 hrs if not terminated, the results also question the role of SNpr and paleolimbic regions in the support of long duration pfSE.

102.16

FLASH EVOKED POTENTIALS (FEP) FOLLOWING PROLONGED STATUS EPILEPTICUS IN KINDLED RATS. L.M. Mayo*, R. Gussio*, G.A. Young, C.U. Eccles and G.G. Buterbaugh. Department of Pharmacology, University of Maryland, School of Pharmacy, Baltimore, MD 21201.

Amygdala-kindled male Sprague-Dawley rats were subjected to pilocarpine-facilitated status epilepticus (pfSE) for one or two hrs duration. FEP's were then obtained at three day intervals for four weeks. Three days post-pfSE, FEP waveform patterns were grossly altered with markedly diminished amplitudes. These changes were related to the duration of pfSE. Consequently, the six conventional positive-negative waveform components could not be identified. For several weeks following pfSE, temporal changes in waveform pattern and amplitude varied between rats. A predominant positive peak transiently emerged in all animals. Preliminary signal analysis suggests frequency modulation of the general FEP waveform. In the 1 hr pfSE rats, FEP's resembling pre-pfSE waveforms appeared after 2-3 weeks. Histological examination (at 4 weeks) revealed widespread bilateral gliosis and cell loss which was more severe in 2 hr pfSE rats. These results suggest alterations in the FEP after pfSE are, like cell damage, related to seizure duration. They are compatible with the reports of transient blindness following SE and support the use of FEP's as a tool to monitor seizure induced brain damage and the effect of potential cytoprotective agents.

102.17

DECREASE IN CEREBELLAR IODOCYANOPINDOLOL BINDING SITES IN 31-DAY-OLD GENETICALLY EPILEPSY-PRONE RAT. S. Razani-Boroujerdi, D.Y. Tso-Olivas and D.D. Savage. Dept. Pharmacol., U. New Mexico Sch. of Medicine, Albuquerque, NM, 87131.

Studies from other laboratories have revealed deficits in neurochemical and electrophysiological indicators of noradrenergic neurotransmission in the cerebellum of the Genetically Epilepsy-Prone (GEPR-9) rat. For example, the ability of norepinephrine to potentiate GABA-mediated inhibition of Purkinje cell firing is decreased in GEPR-9 rats compared to non-epileptic controls. One possible explanation for this observation is a decrease in the number of GEPR-9 rat Purkinje cell β-adrenergic receptors. To test this hypothesis, ^{125}I-iodocyanopindolol (CYP) binding to monoaminergic receptor binding sites was measured in horizontal sections of cerebellum in non-epileptic control and GEPR-9 rats using *in vitro* autoradiography.

CYP binding was measured in the absence and presence of 1 μM propranolol to determine the total amount of specific CYP binding. In addition, sections were incubated with CYP in the presence of 22 different concentrations of the β_2-adrenergic antagonist ICI118,551 to discriminate CYP binding to β_2-adrenergic receptors and serotonin 5HT$_{1B}$ receptors. The number and affinity of β_2-adrenergic and 5HT$_{1B}$ subtypes of CYP binding sites in the cerebellum was determined by analysis of the displacement of binding data using the non-linear curve fitting program LIGAND. The results indicated a significant decrease in total CYP binding in the stratum moleculare of cerebellar cortex in GEPR-9 rats compared to control. LIGAND analysis of the displacement of CYP binding data indicated a 25% decrease in the number of β_2-adrenergic binding sites with no change in the number of 5HT$_{1B}$ binding sites or the affinity of CYP for either subtype of CYP binding site. (Supported by NIH-RR08139).

TROPHIC INTERACTIONS I

103.1

TARGET-SPECIFIC REQUIREMENTS OF IMMATURE AXOTOMIZED CNS NEURONS FOR SURVIVAL AND AXONAL ELONGATION AFTER INJURY
B.S. Bregman, M.B. Clark, and H.W. Muller. Dept. Anatomy, Univ. Maryland Sch. Med., Baltimore, MD 21201

This study examined the degree to which the requirements of immature axotomized neurons for survival and axonal elongation are target specific. Following spinal cord hemisection at T6 in rat pups <48 hours of age, transplants of embryonic target (spinal cord) or non-target (cortex, hippocampus, cerebellum, astrocytes and Schwann cells) tissue were placed into the lesion site. We used neuronal cell counts in the red nucleus (RN) to assay for cell survival and the serotonergic (5-HT) raphe-spinal projection for the analysis of axonal elongation at acute (7 days post-operative, dpo) and chronic (30 dpo) survival times. Target and non-target transplants survived within the cord at both 7 and 30 dpo. At 7 dpo, both target and non-target transplants rescued immature axotomized RN neurons. In contrast, at 30 dpo, only target specific transplants (spinal cord) supported the survival of axotomized RN neurons. At 7 dpo, immature 5-HT axons grew into both target and non-target tissue, but only the spinal cord transplants maintained the projection. These results suggest that components contained within a variety of immature tissues can substitute for the loss of the normal target immediately after injury, but permanent survival and maintenance of axonal projections are dependent upon target specific factors. Supported by NIH NS19259, NS01249, March of Dimes and NATO.

103.2

TWO MECHANISMS MAY CONTRIBUTE TO THE NEURONAL SURVIVAL-PROMOTING ACTION OF VIP: NEUROTROPHIC FACTOR RELEASING ACTIVITY AND GLIAL MITOGENESIS.
D.E. Brenneman, Lab. of Develop. Neurobiology, NICHD, Natl Institutes of Health, Bethesda, MD 20892

Vasoactive intestinal peptide (VIP) has been shown to increase neuronal survival by an action involving non-neuronal support cells in CNS cultures (Brenneman, D.E., J. Cell Biol. 104:1063, 1987). We now present evidence that low concentrations of VIP can produce an increase in the number of astroglia and a rapid increase in the availability of neurotrophic material in conditioned medium from nonneuronal spinal cord cultures. A combination of GFAP (glial fibrillary acidic protein) immunocytochemistry and ^{3}H-thymidine autoradiography was used to assess the astroglial population. Compared to controls, a 20-30% increase in the number of astroglia was observed during a five day test period. In addition, nonneuronal cultures from fetal mouse spinal cord tissue were incubated with 0.1 nM VIP for 30 min. High molecular weight (>30 kD) substance(s) were found to be released into the incubation medium that significantly increased the survival of spinal cord neurons during a critical period of development in cultures. Conditioned medium from nonneuronal cultures that were not stimulated with VIP had no detectable neurotrophic activity. These data suggest both a long term (glial mitogenesis) and a short term (trophic factor releasing activity) regulatory role for VIP during development.

103.3

INTERACTION OF SCHWANN CELLS AND TRIGEMINAL NEURONS IN LONG-TERM COCULTURE. K.Y. Chan and J.H. Chang*. Dept of Ophthalmology, University of Washington, Seattle, WA 98195.

Previous studies in this laboratory have shown that trigeminal neurons (TN) of rabbit are induced to extend neurites and remain viable in culture conditions by a neurono-trophic factor (ENF) secreted from corneal epithelial cells (innervated target). In this study, the trophic relation between TN and Schwann cells (SC) was investigated using LM, EM and immunocytochemistry (IC). Neonatal TN were mechanically dissociated and cultured in serum-free medium containing ENF for 5 days. During this period TN extended a profuse network of neurites. Subsequently the cultures were maintained in culture medium containing 2% serum for several months. Within 1 wk, SC enveloping the TN migrated to the substratum and proliferated slowly. These stellate cells associated spatially with the soma and neurites of TN. During the following weeks some SC aligned themselves along the neurites which gradually increased in diameter and ran a linear course instead of wavy pattern. After 4 wks of coculture, myelin layers were detected around the neurites by EM. IC studies showed that NGF receptor was expressed in both TN and SC. Neurofilament was identified in the neurites only. Vimentin was expressed by both SC & fibroblasts (when present). Thus, differentiated TN enhanced the survival and proliferation of SC, which depended on neuronal contact to initiate myelination. This in vitro model would be useful for studying cellular interaction during corneal nerve regeneration. (NIH EY04538)

103.4

Cyclic AMP Analogs and Cortical Astrocyte Conditioned Medium Increase [^{125} I] Tetanus Toxin Binding in Mouse Hippocampal Cultures. Ralph F. Alderson. (SPON: J.D. Clements). Lab. Developmental Neurobiol., NICHD, NIH, Bethesda. MD 20892.

Basic fibroblast growth factor (bFGF) and analogs of cyclic AMP; 8-bromo-cAMP (8Br-cAMP), and 8-(4-chlorphenylthio)-cAMP (CPT-cAMP), promote neuron survival in the hippocampus (Walicke et.al., PNAS, 83:3012, 1986), or sympathetic and sensory neurons in culture (Rydell&Green, PNAS, 85:1257, 1988) respectively. Tetanus toxin (TT), a discrete early marker for neurons in culture was iodinated and used in a celisa assay to evaluate the potential survival or neurite promoting effects of the cAMP analogs and conditioned medium on hippocampal cells in culture. These effects were compared to those produced by bFGF.

Dissociated cell cultures were prepared from the hippocampi of E18 mouse embryos. The cells were grown in defined medium and treated with the various ligands for 48 hours. B-FGF (Collaborative Research) at a dose of 100 ng/ml produced a 60% increase in TT binding (3535.5 dpm/well) as compared to the vehicle treated controls (2215.2 dpm/well). 8Br-cAMP at a dose of 10 or 1 uM increased TT binding by 36 and 53%, respectively. A similar dose response curve was obtained with CPT-cAMP. When the hippocampal cells were treated with 16.4 ng of defined medium conditioned by purified cortical astrocytes, TT binding was enhanced by 80% (2888.10 dpm/well) as compared to the vehicle treated controls (1607.9 dpm/well). The active factor(s) in conditioned medium are now under investigation.

103.5

HIPPOCAMPAL NON-NEURONAL CELLS INCREASE CHOLINE ACETYLTRANSFERASE ACTIVITY IN BASAL FOREBRAIN CULTURES. M. Yokoyama, C.F. Dreyfus, and I.B. Black. Div. Devel. Neurol., Cornell Univ. Med. Coll., N.Y., N.Y. 10021.

To define the role of central targets in development of the basal forebrain (bf)-hippocampal system, we grew bf from embryonic day 17 (E-17) rat as dissociated cultures in a fully defined system. Maturation of bf cells grown alone was compared to development in co-cultures with the appropriate hippocampal or the inappropriate cerebellar target. Cholinergic development was monitored using the activity of choline acetyltransferase (CAT), the acetylcholine biosynthetic enzyme.

Control bf cultures grown alone exhibited a significant increase in CAT activity over 10 days in culture, the longest period studied. Addition of the hippocampus elicited a 5-fold increase in CAT activity over the control cultures grown alone, and a 2-fold increase above the bf-cerebellar cultures. The increase in CAT activity was reproduced in co-cultures of bf neurons with hippocampal non-neuronal cells. CAT activity in these cultures was significantly higher than that observed in co-cultures with cerebellar or basal forebrain non-neuronal cells.

Our study suggests that target, hippocampal non-neuronal cells may stimulate development of bf cholinergic neurons. Future studies will be directed to further characterization of target influences. (supported by NS 20788, HD 23315, and the Alzheimer's Disease and Related Disorders Assoc., Inc.)

103.6

EVIDENCE THAT THE LOSS OF A RETROGRADELY TRANSPORTED MUSCLE-DERIVED FACTOR INITIATES THE AXON REACTION. B.G. Gold and C. Dark* Neurotoxicology Labs., Rutgers College of Pharmacy, Piscataway, NJ 08854

The mechanism whereby the response of the neuronal perikaryon to axonal injury (axon reaction) is initiated is unknown. Somatofugal axonal atrophy represents one readily quantifiable component of the axon reaction arising from a selective reduction in neurofilament synthesis and delivery to the axon. In the present study, colchicine (5 mM) was unilaterally applied (30-40 min) to the sciatic nerve using soaked cotton balls to determine whether this is a sufficient alteration to produce somatofugal axonal atrophy. This protocol was shown to impair the retrograde axonal transport of wheat germ agglutinin-horseradish peroxidase to dorsal root ganglia (DRG) cells without producing extensive (<1%) axonal degeneration. Rats were perfused with 5% glutaraldehyde at two weeks for quantitative morphometry. A significant ($p<0.05$) reduction in axonal area of dorsal root fibers in the L5 DRG was demonstrated compared with contralateral controls. Neurofilament number was also reduced for axonal perimeter in these atrophic fibers. Preliminary studies indicate that the atrophy is not as marked following application of homogenized muscle to the proximal stump of a transected nerve. These studies suggest that a muscle-derived factor is required for normal neurofilament synthesis.

103.7

NEURON-SUPPORT CELL INTERACTIONS INCREASE DOPAMINERGIC (DA) CELL NUMBER IN THE SUBSTANTIA NIGRA (SN). E.K. O'Malley, C.F. Dreyfus, and I.B. Black. Div. Devel. Neurol., Cornell Univ. Med. Coll., New York, N.Y. 10021.

To examine local interactions regulating SN development we have been growing dissociated SN under a variety of conditions. Tyrosine hydroxylase (TH), a dopamine biosynthetic enzyme, was used to monitor SN development.

Initially cells were plated at low (1x), medium (2x), and high (4x) densities in serum-containing media. After 7 days increased density increased TH activity per culture suggesting that high density fosters DA cell development. The number of Neuron Specific Enolase (NSE)-positive cells, a measure of total neuron number, increased linearly with increased cell density. Conversely, the number of TH immunopositive cells rose disproportionately, suggesting a selective effect on the DA subpopulation.

To define specific cellular interactions virtually pure neuron cultures were compared to neuron-support cell cultures in serum-free medium. In pure neuron cultures TH^+ cell number increased proportionally to plating density. In marked contrast, neuron-support cell cultures reproduced the disproportionate rise in TH^+ cell number noted previously. Our observations imply that neuron-neuron interactions specifically increase TH activity per cell, while neuron-support cell interactions selectively increase DA cell number. (supported by NIH grant NS 20788.)

103.8

INJURY-DEPENDENT UPREGULATION OF CHOLINE ACETYLTRANSFERASE IN RAT BASAL FOREBRAIN BY EXOGENOUS NERVE GROWTH FACTOR. L.R. Williams, K.S. Jodelis*, and M.R. Donald. CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

After transection of the rat fimbria/fornix (F/F), treatment with Nerve Growth Factor (NGF) is reported to increase choline acetyltransferase (ChAT) activity in dissections of the "septal area"; dissections that included both right and left forebrain (Hefti et al., 1984). In the present report, accurate microdissections of the right and left forebrain were analyzed separately for ChAT activity 2 weeks following unilateral aspiration of the right F/F and continuous infusion with 2.5s NGF (1.2 μg/day). Although there was a small, 40% stimulation of activity by NGF on the uninjured left side (compared to untreated animals), there was a larger, previously unidentified, 2-fold stimulation of ChAT on the axotomized right side.

ChAT Specific Activity according to Fonnum (1975)
(n) (pmole ACh/μg protein/min, Avg. ± S.D.)

	Left	Right
Untreated (8)	1.9±0.2	1.5±0.2
NGF-treated (8)	2.7±0.2*	3.9±0.3**

These results indicate that neuronal injury triggers a sensitization to exogenous NGF that results in, and is requisite for, the larger stimulation of ChAT activity. Such stimulation may be mediated by an upregulation of NGF receptor.

103.9

PLASTICITY OF NGF RECEPTORS IN THE RAT BASAL FOREBRAIN FOLLOWING AXOTOMY AND EXOGENOUS NGF TREATMENT. H. Yip and L.R. Williams. Department of Anatomy, Univ. Utah, School of Medicine, Salt Lake City, Utah 84132, and CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Exogenous NGF infusion into the rat brain is known to prevent the death of basal forebrain cholinergic neurons after axotomy and to stimulate choline acetyltransferase activity (ChAT), particularly in injured neurons. We tested the hypothesis that such upregulation of ChAT activity was mediated via an upregulation of NGF receptor in basal forebrain neurons. Rat brains were frozen 2 wk after unilateral right fimbria/fornix transection and continuous NGF infusion (2.5s, 1.2 µg/day, Williams et al. 1986). Coronal sections were cut through the basal forebrain and prepared for quantitation of NGF receptor by receptor binding autoradiography using both ^{125}I-NGF and ^{125}I-anti NGF receptor antibody as markers (Yip et al. 1987). A significiant decrease of receptor density (33%) was observed in the forebrain on the side of axotomy in untreated animals. However, in NGF-treated animals, the receptor density was at least equal to that of uninjured neurons, indicating survival of the axotomized neurons by the NGF treatment. In some animals there is a 50% increase in receptor density, indicating an upregulation of NGF receptors. These results suggest that the observed stimulation of basal forebrain ChAT by NGF is mediated by an upregulation of NGF receptor.

103.10

NGF-MEDIATED NGF RECEPTOR RE-EXPRESSION AND CHOLINERGIC NEURONAL HYPERTROPHY IN THE DAMAGED ADULT NEOSTRIATUM. F.H. Gage, P. Batchelor,* K.S. Chen,* D. Chin,* S. Deputy,* T. Shaw,* M. Rosenberg, W. Fischer,*[2] and A. Bjorklund[2]. Dept. of Neurosciences, UCSD, La Jolla, CA 92093, USA, and [2]Department of Histology, University of Lund, Lund, Sweden.

Adult cholinergic interneurons of the neostriatum are not immunoreactive for monoclonal antibodies to NGF receptor (courtesy of E. Johnson), whereas the developing neostriatum is immunoreactive to this same antibody. Chronic NGF infusion to the adult neostriatum results in hypertrophy to some of the cholinergic interneurons. NGF infusion and chronic damage to the adult striatum also results in re-expression of the NGF receptor such that many cholinergic interneurons become immunoreactive for NGF receptor. Infusion of anti-NGF, will partially inhibit the re-expression of these NGF receptors. Infusion of the NGF will dramatically increase the size and ChAT-immunoreactivity of these same cholinergic neurons.

These findings distinguish two separate trophic mechanisms in the damaged adult neostriatum. 1) Developmentally expressed NGF receptors are once again re-expressed in response to damage as a result of the release of trophic factors including NGF. 2) Cholinergic interneurons which are upregulated to express NGF receptor immunoreativity, as well as those that do not show NGF receptor immunoreactivity, will show a robust hypertrophy in response to NGF. Additional evidence supports the contention that damage to the striatum results in availability of trophic factors in addition to NGF.

103.11

MIXED SUSPENSIONS OF CRYOPRESERVED FETAL MIDBRAIN TISSUE AND C6 GLIOMA: ENHANCED DOPAMINE NEURON VIABILITY IN CULTURE AND NEURAL GRAFTS. T.J. Collier, J.E. Springer, M.F.D. Notter, C.D. Sladek, M.J. Gallagher, B.F. Daley and J.R. Sladek Jr. Departments of Neurobiology and Anatomy and Neurology, University of Rochester School of Medicine, Rochester, N.Y. 14642.

Cryopreservation provides a convenient method for storage, transportation and viability-testing of fetal brain tissue to be used in cultures and neural grafts. However, our experience with cryopreservation of the developing dopamine (DA) neurons of the ventral midbrain indicates that this procedure yields fewer cells than fresh tissue (Brain Res. 436:363, 1987; Soc. Neurosci., 13:784, 1987). In particular, immunocytochemical analysis of cultures for the glial marker GFAP, indicates that a large glial population is lost during cryopreservation. In response to the accumulating evidence for important trophic support provided by glia, we assessed whether the viability of frozen-stored DA neurons could be improved by addition of C6 glioma cells (derived from a rat astrocytoma) to DA neuron cultures and grafts. Cultures stained for the neuronal marker, neuron-specific enolase, and the DA neuron marker tyrosine hydroxylase (TH) indicated that the addition of C6 glioma cells enhanced process outgrowth from TH-positive neurons, and appeared to promote neurite fasciculation. Our initial observations in mixed suspension grafts of cryopreserved fetal rat DA neurons and C6 glioma placed into the denervated striatum of adult rat hosts also suggest improved yield of grafted neurons and enhanced TH fiber staining in the adjacent striatum. However, the findings in vivo are as yet equivocal: it is unclear whether the enhanced fiber staining is related to graft-derived or host-derived neurites. Taken together, these findings support the view that supplementation of glial-derived factors enhance the viability of cultured and grafted cryopreserved DA neurons. ADRDA FSA 85-015 (TJC), Pew Foundation (JRS).

103.12

TARGET DEPENDENCE OF HYPOGLOSSAL MOTOR NEURONS DURING DEVELOPMENT AND IN MATURITY. S. Thanedar* and W. Snider (spon: C. Hunt). Dept. of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The extent to which somatic motor neurons in maturity depend on connections with skeletal muscle for survival is controversial. We have reinvestigated this issue using the hypoglossal nucleus of rats as a model system. The peripheral projections of these neurons via the hypoglossal nerve were interrupted, deflected, and prevented from reinnervating their normal target for periods up to one year. We then counted large neurons in the hypoglossal nucleus and myelinated profiles in the cranial nerve XII rootlets.

After transection of the nerve and prevention of reinnervation in early postnatal life, approximately 60% of hypoglossal motor neurons die and surviving neurons are markedly atrophic compared to contralateral controls. In maturity there is also substantial neuronal atrophy and death (approximately 30%) after this procedure, but the majority of neurons survive for periods of up to one year. The adult response is present by three weeks of age. We also investigated the time course of neuronal atrophy and death after permanent target deprivation in adult animals. One month after the hypoglossal nerve was deflected, there was marked axonal atrophy and some degree of cell death, although somatic atrophy was minimal. By three months after the procedure, substantial neuronal atrophy and cell death were apparent. There was little change between three and six months.

We conclude that hypoglossal motor neurons are influenced by connections with their targets in postnatal life. Even in maturity, neurons require target connections for maintenance of somatic and axonal morphology. However, the majority of motor neurons in adult animals can survive target deprivation for prolonged periods.

103.13

NGF INDUCED INCREASES IN THE EXPRESSION OF CELL SURFACE GLYCOPROTEINS IN PC12 CELLS ARE DIFFERENTIALLY MODULATED BY INCREASED INTRACELLULAR cAMP. P. Doherty, D.A. Mann* P. Seaton* and F.S. Walsh. Dept Neurochem, Inst Neurology, Queen Square, London WC1N 3BG, England.

Specific antibody reagents have been used to quantitate the relative expression of the NGF-receptor (NGF-R), the neural cell adhesion molecule (N-CAM) and the Thy-1 and L1 antigens for PC12 cells grown in SATO media in the presence of NGF and/or cholera toxin (see Doherty et al, 1987, J Neurochem 49:1676-1687). Whereas NGF treatment induced transcription dependent increases in the cell surface expression of all four glycoproteins, treatment with cholera toxin specifically increased the expression of L1. Over the first few days of culture cholera toxin acted synergistically with NGF in both promoting neuritic outgrowth and an increased synthesis and cell surface accumulation of the 140 and 180Kd subunits of N-CAM. In contrast, over the same time period, cholera toxin fully inhibited the NGF induction of its own receptor, and partially inhibited the induction of both L1 and Thy-1. Over longer periods of culture (3-5d) cholera toxin inhibited the NGF induction of N-CAM and neurite outgrowth. This longer term inhibition may be directly related to the shorter term inhibition of NGF-R expression. A similar pattern of synergistic and inhibitory responses were observed when differentiation was induced by FGF, rather than NGF.

103.14

EFFECT OF TESTOSTERONE IMPLANT ON SURVIVAL OF MOTONEURONS FOLLOWING AXOTOMY IN MALE CASTRATED RATS. W.H.A. Yu. Dept. of Anat. Sci., CUNY Med. Sch., New York, NY. 10031.

It was shown previously that axotomy-induced neuronal loss in motor nuclei of cranial nerves was more severe in females and male castrates than in male rats (Yu, W.H.A., Anat. Rec., 218:152A, 1987). In order to test the hypothesis that androgens promote survival of motoneurons, this study was made whereby neuronal loss in castrated males was compared to castrated males given androgen replacement therapy. Twelve male Sprague-Dawley rats weighing 160-180 g were orchidectomized bilaterally under light ether anesthesia. Eleven days later, these rats were anesthetized with chloral hydrate, subjected to unilateral transection of the hypoglossal and facial nerves, and received either subcutaneous implant of 2 or 4 testosterone-filled Silastic capsules (each measuring 10 mm in length and 2.0 mm in diameter), or no replacement therapy as control (n=4 for each group). Neuronal population in the respective motor nuclei were quantified 12 weeeks after axotomy on serial paraffin sections cut through the extent of the motor nuclei and stained with cresyl violet. Total number of neurons in the motor nucleus of the axotomized side was expressed as a percentage of the contralateral, intact side. Results indicated that the percent neuronal loss in the two replacement therapy groups did not differ from each other nor from that of male castrated control. In view of the previous finding that injection of testosterone to gonad-intact females was effective in attenuating neuronal loss (Yu, W.H.A., Anat. Rec. 219:152A, 1987), it is postulated that gonadal substance(s) facilitated effects of androgens on motoneurons. This possibility is being tested on ovariectomized females receiving testosterone implants.

Supported by PSC-CUNY Research Award.

103.15

INSULIN-LIKE GROWTH FACTOR 1 AND INSULIN DECREASE CHOLINE ACETYLTRANSFERASE ACTIVITY AND INCREASE GLUTAMIC ACID DECARBOXYLASE ACTIVITY IN CULTURES OF RAT STRIATAL NEURONS. B.J. Brass* and J.N. Barrett. Dept. of Physiology & Biophysics, Univ. of Miami School of Medicine, Miami, FL 33101.

Neuron-rich cultures were prepared from the striata of embryonic rats (day 15-16). Exposure to insulin-like growth factor 1 (IGF 1) or insulin for 1-3 weeks produced up to a 40% decrease in the activity of choline acetyltransferase (ChAT), the rate-limiting enzyme for acetylcholine synthesis. In the same cultures the hormones produced up to a 40% increase in the activity of glutamic acid decarboxylase (GAD), the rate-limiting enzyme for GABA synthesis. The hormonal effects were dose-dependent; half-maximal reductions of ChAT activity were observed with 1 nM IGF 1 and 22 nM insulin. The magnitude of the hormonally induced reduction of ChAT and its speed of onset increased with cell density. This suggests that the effects of IGF 1 and insulin on cholinergic neurons may be indirect, possibly mediated by secreted molecules or cell-to-cell contact that would be more prevalent in denser cultures. In contrast, the hormonally induced increase in GAD activity was not cell density dependent. IGF 1 and insulin are both present in the brain, and these results suggest that they may play a role in regulating the balance between cholinergic and GABAergic transmitter systems. Supported by NIH grant NS 12207.

103.16

INVOLVEMENT OF NERVE GROWTH FACTOR (NGF) IN GLIOMA-INDUCED ANGIOGENESIS S.E. Kennedy (*), M. S. Fiandaca, J.H. Kordower, M.F.D. Notter, and J.E. Springer (SPON: R. Herndon) Dept. of Neurobiology and Anatomy, University of Rochester Med. Ctr., Rochester, New York 14642.

Neovascularization is a hallmark of many central nervous system (CNS) gliomas. This angiogenic response is critical for the development and growth of the glioma, and has been attributed to the presence of a tumor-derived soluble factor(s). We now report the possible involvement of NGF in glioma-induced angiogenesis, and suggest a mechanism by which this soluble factor may direct the growth of newly formed vessels via specific NGF receptors. C6 gliomas (derived from rat CNS and obtained from the American Type Culture Collection) were injected as a 1 µl suspension (20,000 cells/µl) into the hippocampal formation of the rat. At 2-4 weeks following surgery, animals were perfused, and 30 µm brain sections prepared for NGF receptor (NGFR) immunocytochemistry. C6 gliomas grown as monolayers in culture were also analyzed for the presence of NGFRs. Both cultured and transplanted C6 cells exhibited a wide range of NGFR immunoreactivity (NGFRI) across cells, and may reflect the differential expression of NGFRs at different developmental phases of cell mitosis. Following transplantation, NGFR-positive cells (presumably gliomas) appeared to form tube-like structures, some of which extended into the tumor mass from blood vessels in the surrounding brain parenchyma. Electron microscopic analysis revealed NGFRI in cell membranes closely associated with endothelial cells. A similar pattern of NGFRI was observed in centrally-derived human gliomas. There is evidence to indicate that one of the factors secreted by rat C6 gliomas, and possibly human gliomas, has a biological activity identical to NGF. From these results, it is proposed that glioma-derived NGF may bind to elements associated with migrating endothelial cells as well as NGFRs located on NGF-secreting tumor cells, thus providing a mechanism for directing and maintaining the proliferation of glioma-induced neovascularization. JES is an NRSA postdoctoral fellow. Supported by a grant from the American Federation for Aging Research (JES).

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CIRCUITRY AND PATTERN GENERATION II

104.1

NONSPIKING INTERNEURONS IN THE VENTILATORY CENTRAL PATTERN GENERATOR OF THE SHORE CRAB. R. A. DiCaprio. Dept. of Zoological & Biomedical Sciences, Ohio Univ., Athens, OH 45701.

The central pattern generator controlling ventilation in Crustacea was originally thought to consist of a single nonspiking neuron driving scaphognathite levator and depressor motor neurons (Mendelson, M., Science 171:1170, 1977).

This study describes eight nonspiking interneurons which are elements of the crab ventilatory CPG. The membrane potential of all CPG interneurons oscillated in-phase with the extracellularly recorded ventilatory motor pattern and this oscillation ceased during ventilatory pauses. In all cases, a rhythmic phase-locked modulation of the membrane potential was also present during periods of reversed ventilation. Injection of intracellular current pulses into these interneurons caused a resetting of the ongoing rhythm, indicating that they are a part of the ventilatory central pattern generator. Structurally, all of the interneurons were restricted to a single hemiganglion and perturbation of these cells with intracellular current only effected the motor output of the hemiganglion containing the interneuron.

104.2

PATTERN GENERATING NEURONS SWITCH BETWEEN DIFFERENT NEURAL CIRCUITS. P. Meyrand*, J.M. Weimann*, and E. Marder. Biol. Dept., Brandeis Univ., Waltham, MA 02254.

The crustacean stomatogastric ganglion (STG) produces two different rhythmic outputs, the pyloric rhythm and the gastric rhythm. It has been thought that these two rhythms were generated by two independent groups of neurons, and that weak interactions exist between these two different neural circuits. However, in the crab, Cancer borealis, components of the pyloric and gastric networks can switch among a variety of output patterns. Specifically, three classes of neurons considered part of the gastric network, the Lateral Gastric (LG), Medial Gastric (MG), and Lateral Posterior Gastric (LPG) neurons are sometimes part of the pyloric circuit when the gastric system is inactive. At other times they participate in the gastric rhythm. The Ventricular Dilator (VD) and Inferior Cardiac (IC) neurons of the pyloric network, take on gastric rhythm properties under some conditions. We are currently investigating the mechanisms controlling the switch of these neurons between different motor outputs. These data argue that many neurons of the STG can be used to form part of several, operationally distinct neural circuits, and that the composition of the circuits is defined by modulatory neural inputs to the ganglion. Supported by NS-17813 and AFSOR F49620-86C0131 and a Gillette Fellowship (JMW).

104.3

PATTERNS OF SPONTANEOUS ACTIVITY IN CULTURED NETWORKS OF HIPPOCAMPUS AND SEPTAL REGION NEURONS. M.Siebler*, H.Koeller*, C.Schmalenbach*, H.W.Mueller, H.J.Freund, Dept. of Neurology, University of Duesseldorf, FRG

Dissociated cultured neurons from the hippocampus and septal region of embryonic rat (E18) developed neuritic networks under the trophic influence of spatially separated astrocytes. In hippocampal neurons at 3 - 6 days in vitro (DIV) network driven postsynaptic potentials (psp) as well as action potentials (ap) appeared. The interspike intervals varied randomly from ms up to minutes. The impulse rate increased during development and after 8 - 10 DIV some neurons displayed combined firing patterns with two preferred impulse intervals: 20-100 ms and 200 - 400ms, frequently interrupted by long silent periods. Septal neurons or mixed cultures of dissociated hippocampal and septal neurons showed no spontaneous activity prior to 7 DIV and nor did impulses exhibit a preferred frequency between 200 - 400 ms within 20 DIV. Fast impulse rates were associated with regenerative firing during psps while low frequencies could be a phenomenon of inhibition specific for hippocampal neurons. During reversible blockade of spontaneous activity by tetrodotoxin miniature-psps could be registered, suggesting that mpsps are driving force behind spontaneous activity in neuronal networks.
Supported by the DFG. M.S. is recipient of a fellowship from the DFG.

104.4

ENHANCEMENT OF RECURRENT EXCITATION OF THE EARTHWORM MEDIAL GIANT FIBER BY SLOWLY CONDUCTING PROJECTIONS WITHIN THE VENTRAL NERVE CORD. J.L. Johnson. Dept. of Physiol. and Pharm., USD School of Medicine, Vermillion, SD 57069.

In the earthworm, head end escape is centrally programmed by the medial giant fiber (MGF). While action potentials are initiated in the MGF by segmental mechanoreceptive inputs, the recurrent excitatory effects exerted upon this giant interneuron remains to be adequately appreciated. The data obtained in this study suggests that the interneuronal tract fibers within the cord that are activated by segmental sensory inputs markedly enhance recurrent excitatory affects exerted upon the MGF. When the slowly conducting intersegmental projections were activated, recurrent excitation of the MGF was enhanced for a time period of 79 ± 3 msec (M ± SEM) after a conduction latency of 16 ± 1 msec (M ± SEM; conduction distance = 10 mm). Thus the ability of sensory inputs to induce MGF discharge may depend, in part, upon the ability of the sensory input to facilitate excitatory recurrent feedback effects exerted upon this command cell. This indicates that, besides initiating MGF discharge, afferent inputs may enhance self-excitation of this giant interneuron through recurrent feedback circuits.

This work was supported by a General Research Funds Grant from the University of South Dakota.

104.5

DIFFERENTIAL EFFECTS OF NEUROMODULATORS ON PATTERNED MOTOR ACTIVITY IN THE BUCCAL GANGLIA OF HELISOMA. A.Don. Murphy, J.E. Richmond and K. Lukowiak. Dept. of Biol. Sci., University of Illinois at Chicago, Chicago, Illinois. 60680

We have recently established that buccal motor output in Helisoma is driven by three pattern generating sub-units (S1, S2 and S3) within the buccal ganglia. Previously we have shown that several neurotransmitters can initiate patterned activity in buccal motor neurons based on observations of only one of these subunits, the S2 or cyberchron group. We can now monitor all three subunits whilst recording from only two neurons. Our present aim has been to assess the effects of individual neurotransmitters on the subunits of this central pattern generator (CPG). Our preliminary data suggests that differential patterns result from bath application of various transmitters. Specifically, dopamine at relatively high concentrations ($2x10^{-6}$M) phase locks all three subunits; S1,S2 and S3 in the standard pattern. At a concentration of 10^{-8}M , serotonin phase locks S2 and S3 but S1 activity is variable. GABA most prominently turns on the S2 subunit and causes high rates of activity in S1 follower neurons whereas octopamine can activate S1 in the absence of S2 and S3. These neurotransmitters will be useful tools for manipulating the CPG to produce various motor patterns.

104.6

SLOW-WAVE AMPLITUDE AND PATTERN STABILITY IN GASTRIC MILL CIRCUIT OF LOBSTER STOMATOGASTRIC SYSTEM

Norman Herterich, Dept. of Biology, U.C. San Diego

The lobster gastric CPG produces a coordinated pattern of activity in efferent nerves. CPG cells undergo slow voltage oscillations and communicate via graded release and gap junctions. If they coordinate their activity by entraining each others' activity, one might ask if increasing the amplitude of oscillations in one cell, which could increase its postsynaptic effect, could make that cell more effective in determining the timing of the pattern. Here I report on experiments that alter the amplitude of oscillations in the LG cell (which synapses on all other cells in this CPG) by injecting current intracellularly. Injection is triggerred to the ongoing activity of the LG cell. Those manipulations which increase the oscillation amplitude make the pattern more regular, while manipulations which decrease amplitude destabilize the pattern. These manipulations must be affecting the timing cues which coordinate activity. These results are consistent which the hypothesis that the amplitude of a cells' activity is related to its ability to entrain the activity of other cells.

104.7

ALL NEURONS IN THE PYLORIC PATTERN GENERATOR OF THE LOBSTER ARE CONDITIONAL OSCILLATORS. T.Bal*, F.Nagy and M.Moulins. Lab. Neurobiol. CNRS. 33120 Arcachon, FRANCE.

The regenerative properties of the 14 pyloric neurons in the stomatogastric ganglion (STG) of the lobster, Jasus lalandii, were examined after isolating each neuron from its counterparts in the pyloric network (photoinactivation and pharmacological blockade of presynaptic neurons). Under these conditions, every pyloric neuron manifests voltage-dependent oscillations in membrane potential and rhythmic bursts of spikes. The temporal characteristics of oscillation are different for each type of isolated pyloric neuron. Two groups of these neurons can be recognized: 1) a group of relatively fast oscillators (the interneuron AB and the dilator motor neurons PD, VD), among which AB is the only one whose oscillations are not modified by isolation; 2) a group of slow oscillators (the constrictor motor neurons LP, PY, IC) whose oscillations are rhythmical plateaus with long-lasting and variable period and duration.

When the STG is disconnected from anterior ganglia (axonal conduction blockade of the input nerve), the oscillatory capability of all pyloric neurons is abolished. This capability is durably restored by brief electrical stimulation of the input nerve. In conclusion, the pyloric network is a set of conditional oscillators, requiring the appropriate modulatory inputs to express their rhythmic activity.

104.8

PHASE ENTRAINMENT OF A NEURONAL OSCILLATOR IN THE LOBSTER STOMATOGASTRIC GANGLION. T.Kiemel*(SPON: A.J.Tierney), Center for Applied Math, Cornell Univ., Ithaca, NY 14853.

The response of a neuronal oscillator in the stomatogastric ganglion (STG) of the spiny lobster, *Panulirus interruptus*, to periodic hyperpolarizations was studied and the results understood in terms of phase transition curves (PTCs).

With descending inputs from the oesophageal and commissural ganglia intact, 5μM picrotoxin was applied to the STG. Under these conditions, the anterior burster (AB) and two pyloric dilator (PD) neurons of the STG, which are strongly electrically coupled, function as a single neuronal oscillator with one active phase, undergoing synchronous rhythmic bursting (T~1sec) with little or no feedback from other cells in the ganglion. Steps of hyperpolarizing current (3-5nA, 100-600msec) were injected into one PD cell. PTCs and the response of the cell to periodic (T=.5-1.5sec) current injections were obtained.

Three types of responses to periodic current injections were seen in the PD cell: 1) 1:1 phase entrainment in which every hyperpolarization was followed by exactly one PD burst, 2) entrainment at a ratio other than 1:1, and 3) a chaotic response in which the pattern of PD bursts never repeated. The type of response observed varied depending on the duration and frequency of the current injection with the occurrence of 1:1 entrainment increasing as the duration of current pulses increased. Although current injection could produce either a phase advance or a phase delay, it was not possible to achieve 1:1 entrainment of the oscillator at a frequency much faster than its natural frequency. The above results were precictable based on the PTCs measured and the general properties of the PTCs were in turn consistent with the Fitzhugh-Nagumo model for a neuronal oscillator.

Work was supported by a NSF graduate fellowship. Special thanks to Ron Harris-Warrick for the use of lab facilities and for advice along the way.

104.9

MODULATION OF TRIGEMINAL MESENCEPHALIC NEURONS DURING FICTIVE MASTICATION. A. Kolta*, J.P. Lund and S. Rossignol. Cent. Res. Sci. Neurol., Univ. de Montréal, Montréal, Canada, H3C 3J7.

In most rhythmic motor systems, there appears to be phasic modulation of transmission in sensory pathways. Work on fictive locomotion indicates that presynaptic mechanisms may be implicated, because phase locked fluctuations of dorsal root potentials and rhythmic antidromic discharges of some afferents have frequently been observed. We have begun to look for similar phenomena in mastication. The experiments were conducted on anesthetized and paralysed rabbits. Cuff electrodes were placed around the XII nerves to monitor fictive mastication evoked by electrical stimulation of the masticatory area of the cerebral cortex. Neurons were recorded from the V mesencephalic nucleus (Vmes.) and V ganglion with glass-coated tungsten microelectrodes. The Vmes. neurons were identified by their brisk responses to stretch of the jaw closing muscles and by pressure on the masseter and temporalis muscles. The majority could be made to fire tonically by fixing the jaw in an open position. Fictive mastication was then induced and changes in firing rate were calculated. The activity of the majority of Vmes neurons was phasically modulated during fictive mastication, but approximately 25% were unaffected. In contrast, skin and hair afferents from the face did not appear to fire antidromically during mastication.

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104.10

PHASE COUPLING DURING ENTRAINMENT OF FICTIVE LOCOMOTION IN LAMPREY SPINAL CORD. K.A. Sigvardt and T.L. Williams* (SPON: A. Davies). Univ. of California at Davis, VAMC, Martinez, CA 94553 and Physiology Dept., St George's Hospital Medical School, London SW17 0RE, UK.

In the isolated spinal cord/notochord of the lamprey, fictive locomotion can be entrained by rhythmic lateral bending. This is mediated by intraspinal mechanoreceptors (edge cells). We have investigated the phase relations between the movement and the ventral root activity near the point of bending and up to 40 segments more rostral or caudal. We show that if the point of bending is in the caudal half of the cord then the mechanical-neural phase coupling at that point is near the value seen in the intact swimming animal, when the feedback loop is unbroken. If, however, the bending is in the rostral half, the phase relationship is more labile. We have also investigated the effects of length of spinal cord, rostral vs. caudal segments, and rostral vs. caudal bending upon the neural-mechanical coupling, the frequency range of entrainment, and the intersegmental phase coupling in order to test predictions of coupled-oscillator theory.

104.11

THE ROLE OF PUTATIVE NEUROTRANSMITTERS OR MODULATORS ON JAW OPENER MOTONEURONAL DISCHARGE DURING CORTICALLY INDUCED RHYTHMIC JAW MOVEMENTS. S.H. Chandler and N. Katakura*. Dept. of Kinesiology and the Brain Research Institute, UCLA, L.A. CA, 90024

Repetitive stimulation of the masticatory area of the cortex induces rhythmical jaw movements (RJMs) in the anesthetized guinea pig. Underlying these RJMs are rhythmical synaptic and spike potentials in both jaw opener and closer motoneurons. At the present time there is no information as to the nature of the neurotransmitters(NT) or modulators(NM) contributing to the rhythmical activity of these cells during RJMs. The purpose of the present study was to elucidate the role(s) of selected putative NTs/NMs in the production of cortically induced RJMs utilizing classical extracellular single unit recording and microiontophoretic techniques.

In ketamine/urethane anesthesized, and paralyzed guinea pigs, repetitive stimulation (30-40Hz) of the masticatory area of the cortex was used to evoke RJMs. Extracellular recordings of digastric motoneurons (DIG MNs) were made through the central barrel of seven-barreled microelectrodes. Responses of neurons during RJMs were challenged with either L-glutamate, N-methyl-D-aspartate (NMDA), D-2-amino-5-phosphonovalerate (APV), kynurenate (KYN), noradrenalin (NA) or 5-Hydroxytryptamine (5HT). Rhythmical field potential and spike activity were monitored by metal microelectrodes inserted into the contralateral DIG MN pool.

The results are summarized as follows: 1) Application of glutamate or NMDA facilitated spike discharge during RJMs whereas application of the specific NMDA antagonist APV or the broad spectrum amino acid antagonist KYN suppressed rhythmical spike activity. KYN was more effective than APV. Application of NA or 5-HT (<20nA) produced a predominate facilitation of DIG MNs discharge during RJMs which lasted up to 10min following termination of the ejection current. The effects of NA and 5-HT antagonists are currently being examined. The results suggest that excitatory amino acids, 5-HT, and NA may be involved in activation and/ or modulation of rhythmical discharge of DIG MNs during RJMs. Supported by NIH grant DE 06193.

104.12

GRASP FORCE RESPONSE IN MAN MATCHES THE CHARACTERISTICS OF PULLING LOADS. R.R. Riso, C. Hägar*, R.S. Johansson and G. Westling*. Dept. of Physiology, University of Umeå, S-901 87 Umeå, Sweden.

Subjects were seated with there arm abducted and supported up to wrist. The S's were instructed to use their thumb and index fingers to grasp an instrumented object that consisted of two parallel 30mm dia. disks spaced 25mm apart and connected to a servo controlled puller. Strain gages mounted on the object allowed independent registration of the grip (normal) and load (tangential) forces imposed on the finger tip skin. The studies utilized two different sets of load force stimuli each consisting of up to 34 consecutive "ramp and hold" trials. In one series the rate of rise of the load force was pseudorandomly varied and the amplitude was held constant. In the other series, the rate was held constant but the amplitude of the load force was varied.

Analysis of the grip responses to the ramp pull showed that the latency (typically 80-120ms) was shorter for the trials involving the higher rate load forces, and the rate of development of the grip force was clearly graded from its onset to match the rate of rise of the load force stimulus. The subject's automatically modulated the grasp force in response to the rises and decays of the load force ramps. Changing the direction of application of the tangential load forces showed no qualitative differences in the grip responses. It was concluded that somatosensory information acquired during the initial onset of the stimulus (ca. 50-100ms) was utilized in programming the grip force response. When the fingers were anesthetized, the modulation of the grip force was drastically reduced, implying that the "automatic" regulation of the grip response is dependent on cutaneous input.

Signals in tactile afferent units are now being analyzed using the technique of human microneurography.

104.13

NEURAL NETWORK MODEL WITH APPLICATIONS TO PLEUROBRANCHIA. V. Bedian*, F. Zhang*, J.F. Lynch* and M.H. Roberts (SPON: G. Felsten) Depts of Biology, and Math and Computer Science, Clarkson Univ., Potsdam, NY 13676

A computer model of neural networks was formulated to simulate behavior and learning in simple systems. Each neuron has a firing threshold, time constant, absolute and relative refractory periods, and reversal potentials for excitatory and inhibitory inputs. The network is defined by connection strengths between neurons, and neurons and external inputs. Connections have associated delays to represent variable transmission times.

Feedback circuits in the nervous system of the carnivorous gastropod Pleurobranchia produce a typical bursting strike-bite feeding response, and competition between feeding and withdrawal responses. We constructed an 18 neuron model of this system. Simulation results reproduce the bursting strike-bite response to chemo-sensory stimuli, and withdrawal response to tactile stimuli. Mixed stimuli show transition from strike-bite to withdrawal separated by a narrow range of no response. Inhibiting peripheral feedback from the feeding circuit does not abolish the oscillatory behavior between feeding command and central inhibitory neurons. The network model should be useful in simulating behavior in this and other organisms, providing testable predictions, and simulating learning by addition of modification rules for time constants, connections, and/or thresholds.

104.14

A CONSTANT-NOISE MECHANISM FOR ENHANCING LEARNING IN NEURAL NETWORKS AND ITS COMPARISON TO SIMULATED ANNEALING. R. M. Burton and G. J. Mpitsos. Mathematics Department, Oregon State Univ., Corvallis, OR 97331, and the Hatfield Marine Science Center, Oregon State Univ., Newport OR 97365.

Variability in neural and behavioral responses can arise from sources that follow some deterministic law such as chaos, and from nondeterministic processes such as random noise. There is evidence for the first type in the sea slug Pleurobranchaea (e.g., Mpitsos et al., In: Dynamic Patterns in Complex Systems, J. A. S. Kelso, A. J. Mandell, & M. F. Shlesinger, eds., World Scientific Pub., Singapore, 1988). Using simulated "temperature" annealing procedures to avoid local minima in searching for optimal solutions, the role of random noise has been shown in computational systems. Biological systems, however, do not necessarily seek optimizations as long as success is achieved using responses arising from local minima, and it is quite unlikely that temperature-dependent annealing occurs in brain function. Using backpropagating networks (Mpitsos et al., these proceedings), we have devised noise-enhancement methods (NE) that are simpler than simulated annealing (SA), do not require control of temperature schedules, and whereas SA requires constants for both the noise level and for the rate of temperature reduction, NE requires only one constant relating to noise level. In one method, learning is a function of a Hebbian rule plus a noise term proportional to the amount of error in the backpropagating algorithm. In behavioral terms the network "anneals" itself by making the constant noise appear smaller when the response is "successful", but larger when the response is less "successful". This noise could arise from random processes or from variability in chaos. SA is as effective as NE during the acquisition of learning to the first of a sequence of different situations. But having learned one task, SA has used up its temperature range, and must be reset by another process that must perform the difficult problem of distinguishing between simple variations in executing the same task and variations relating to the appearance of a new task. In contrast, by requiring no temperature schedules, NE automatically adjusts to all errors and does not have to distinguish between normal variations in doing a particular task or those that appear when a new task has to be learned. NE may occur naturally in brain function during learning, in selection of unlearned responses, and may be useful in generating robotic adaptive responses. Supported by AFOSR-86-0076 to G. J. M. (SPON: M.I. Schimerlik)

104.15

CAN CHAOS PROVIDE READABLE INFORMATION SIGNALS IN NEURAL NETWORKS? G. J. Mpitsos, R. M. Burton, H. C. Creech, and S. O. Soinila. Mark O. Hatfield Marine Science Center, Oregon State University, Newport OR 97365.

Our neurophysiological studies provide evidence showing that patterned neural activity relating to behavior may be generated by dynamical processes attributable to chaos (Mpitsos et al., In: Dynamic Patterns in Complex Systems, J. A. S. Kelso, A. J. Mandell, & M. F. Shlesinger, eds., World Scientific Pub., Singapore, 1988). The inherent unpredictability of chaos, however, could pose difficulties in information transfer in brain function. Because biological systems are not sufficiently controllable, we used a simple connectionist, error-backpropagating network, consisting of 1 input, 1 output, and 4 intermediate units, to determine whether a chaotic signal originating in one part of the network could be identified and transmitted by another part. We used the Rössler attractor, and the 3.60 and 3.95 logistic equations as inputs to train the network, and compared the output of the network to each input. We found that: (1) The network could learn to distinguish different chaotic signals. (2) The parametric values of the outputs were significantly different from the input values, but (3) the circuit accurately transmitted the dynamical qualities of the inputs. (4) Having learned a particular dynamical quality, the circuit could immediately identify other, previously unlearned signals, using the same set of connections and synaptic weights; i.e., the circuit was multifunctional by generalization through dynamical qualities common to different test stimuli. Thus, chaos can provide an appropriate information signal through transmission of dynamical qualities. This is consistent with our findings of variability and chaos in neural and behavioral activity in our experimental animal, the marine mollusc Pleurobranchaea.

This research was supported by AFOSR-86-0076 to G. J. M.

104.16

HOW SYNAPTIC NOISE MAY AFFECT CROSS CORRELATIONS. G. Midroni and P. Ashby. (SPON: B. Nixon) Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, Ont. M5T 2S8.

Cross-correlations between input and output spike trains of repetitively firing neurons are used to derive the characteristics of the underlying postsynaptic potentials (PSP).

In the present study a computer simulation was used to explore the effects of synaptic noise on the PSP cross-correlation relationship.

We conclude: 1) In the presence of synaptic noise the cross-correlation profile tends toward representing PSP shape directly but will never do so under physiological conditions. 2) There is no reason to view the cross-correlogram as the sum of 2 terms, proportional to the PSP and to it's derivative. 3) Peak area provides the best estimate of PSP amplitude. 4) Synaptic noise decreases peak area and lead to an underestimate of PSP amplitude. The results of this study correlate with experimental findings.

104.17

WHY "GRANDMOTHER'S FACE" AND "COMMAND" NEURONS ARE RARE (Answer: the fireworks finale). William H. Calvin, University of Washington, Biology NJ-15, Seattle WA 98195.

Thomas Young's 1802 patterning principle (for trichromaticity) has been called many things by its reinventors: population codes, parallel processing, distributed functions, ensemble coding, and across-fiber pattern. Whether something tastes salty, bitter, acid, or sweet seems to be a matter of irreducible combinations; there are no labeled-line specialists in one taste or another in your tongue. And there need not be any later in the brain.

The most fundamental reason why labeled lines aren't needed in sensory processing is that all actions require a motor program that orchestrates **many** motorneurons. Triggering this ensemble into action need not require a single "command neuron"; it only requires a committee of interneurons active in some characteristic pattern. A **many-to-many transformation** from sensory-to-motor should suffice without the bottleneck of **many-to-one-to-many**. Perhaps only motor programs with the simplest spatiotemporal patterns can get by with the single command neuron approach to orchestration, e.g., Mauthner cells. The appropriate trigger for most motor programs is likely a key-like combination; indeed, it will probably be just as important which cells are inactive as which are active. And unlike a spatial pattern such as key notches, it will be a spatiotemporal pattern like the fireworks finale, the order in which various neurons are activated, as well as which neurons are activated, being the adequate stimulus.

104.18

IDENTIFICATION OF FIRING PATTERNS IN MULTIPLE SPIKE TRAINS USING A NEURAL NETWORK EMPLOYING THE BACK-PROPAGATION ERROR CORRECTION LEARNING ALGORITHM. D. C. Tam and D. H. Perkel. Theoretical Neurobiology Facility, Department of Psychobiology, University of California, Irvine, CA 92717 USA.

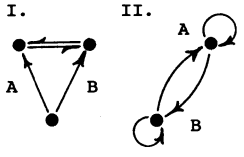
A parallel-distributed processing (PDP) network using the back-propagating error-correction algorithm is used to compute the temporal correlation of firing patterns among many simultaneously recorded spike trains. The spike trains are analyzed by a multi-layer PDP network with each hidden layer computing the time-shifted pattern of activity between the input and output. The activity of each of the N input neurons is treated as a separate input channel to the PDP network and the activity of each of the M output neurons is treated as the expected output of the PDP network during training. After training, the n-th hidden layer computes the correlation of input patterns with the n time-step shifted output patterns. Once the PDP network is trained to produce the observed set of M output spike trains in response to the N input spike trains, the correlations among neurons at different lag times can be represented by the connection weights among the network units at different hidden layers. These weights may be displayed graphically in a 3-dimensional histogram form, providing an easily interpretable form of the input/output relationship. Simulation results show that the temporal correlation characteristics among different neurons are revealed by this technique.

104.19

EMERGENCE OF MULTICOMPONENT RHYTHMS, T.K. Leen and G. McCollum, Robert Dow Neurol. Sciences Institute, Portland, OR 97209.

Different rhythmic patterns of biological activity are distinguished primarily by the relative timing of marker events within each pattern (e.g. motor neuron bursts). The relative timing between markers of different interacting components determines a spatio-temporal pattern among the components.

A minimal description of such patterns can reveal the principles of connected components participating in a rhythmic process. Connectivity of the components is linked to the stability of the particular patterns. This link is explored mathematically through coupled circle maps. These maps possess graphical counterparts depicting connectivity (I) and the corresponding flow of timing influence (II). This flow, along with the connection strengths, determines stability of the patterns formed.



The approach illuminates the fundamental relation between component connectivity and the emergence of temporal patterns.

104.20

A COMPARISON BETWEEN DIFFERENT NEUROPHYSIOLOGICAL MODELS OF RHYTHMIC MOTOR CONTROL USED TO DRIVE A SIMPLE DAMPED PENDULUM. S. F. Giszter* (SPON: E. Bizzi) Dept. Brain and Cognitive Sciences, M. I. T., Cambridge MA 02139.

Planning and control of movement may be thought to reside in both the central neural and in the peripheral mechanical systems. The neural portion may be thought of as comprising three components of (1) central pattern generator (CPG), (2) reflex corrections, (3) phaselocking properties of the reflexes. What are the roles of these in determining the range of frequencies and amplitudes in which a simple rhythmic movement is successfully generated? To examine this question, models utilizing some or all of these different components were compared when connected to a simple damped pendulum.

It is known that 'chaotic' non-linear behavior can occur in the sinusoidally forced simple damped pendulum. In fact a pure central pattern generator control scheme fails to generate predictable rhythmic behavior in the simple damped pendulum for some parameters and types of forcing functions. I tested the hypothesis that 'reflex' interactions increase the range of controlled cyclic motions of the pendulum which are possible and the parameter regions in which these can be generated, by shaping and phase adjusting the forcing function in response to pendulum behavior.

Simulations show that closing one type of first order feedback loop (which gives a phaselocking response of the forcing function to the pendulum motion) generates a control operating in limit cycles over a wider range than the open loop forcing. For appropriate parameters this phaselocking controller can operate at the frequencies and amplitudes of forcing where chaotic behavior was found in the pure central pattern generator model. A more flexible control is therefore possible using this scheme. However pure CPG control is adequate over a smaller range of values.

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104.21

A GENERAL PURPOSE NEURAL NETWORK SIMULATOR FOR IMPLEMENTING REALISTIC MODELS OF NEURAL CIRCUITS. M.A.Wilson*, U.S.Bhalla*, J.D.Uhley*, J.M.Bower, Division of Biology, Caltech, Pasadena CA 91125 (SPON: P. Monaghan)

Rapid improvements in the speed and power of computers readily available to neurobiologists has made possible the construction of computer simulations of neural networks that are complex enough to provide data relevant to the organization and function of real neural networks (c.f. Wilson and Bower, NIPS, AIP Press 1988). To facilitate the design of such detailed, realistic biologically-based models we have developed a general-purpose network simulator, written in C, running under the Unix operating system. A graphics based front end using the X windowing system allows rapid interactive specification of network circuitry and parameters. The simulator is capable of generating output in the form of spike activity, EEGs, intracellular potentials and field potentials to facilitate the comparison of modeled results to physiological measurements. The simulator currently runs on conventional serial machines but has been designed for use on parallel computers to provide greater computational power. The hierarchical representation of components used by the simulator is designed to be sufficiently general to support simulations ranging from detailed single cell models to large networks of simple or complex cells. Current models developed under this system include mammalian olfactory cortex, invertebrate neural structures, and high end cognitive/connectionist level simulations. A library of simulations performed on the system is being established, and several of these will be presented. (work supported by NSF grant EET-8700064, and the Lockheed corporation)

104.22

MODEL OF RHYTHMIC NEURONAL POPULATION OSCILLATIONS IN THE LONGITUDINAL CA3 HIPPOCAMPAL SLICE. R.D. Traub, R. Miles and R.K.S. Wong. IBM Research Div., T.J. Watson Res. Ctr., Yorktown Heights, NY 10598 and Dept. of Neurology, Columbia Univ., New York, NY 10032.

A long-standing question in cortical neurophysiology and EEG concerns how a population of interconnected neurons can generate rhythmical extracellular waves (derived from correlated synaptic currents in the population), even when the correlation is small between the actual firing of any given neuron and the extracellular potential. How do the functional parameters, such as synaptic strengths and cellular excitability, determine the amplitude and frequency of rhythmic activity? We propose a solution to this problem, presented as a 9900-cell computer model, based on our physiological studies of the longitudinal CA3 slice. Critical features of this system are these: (1) excitatory cells generate intrinsic bursts with long hyperpolarizing afterpotentials; (2) excitatory cells are interconnected with each other by a sparse network of powerful excitatory synapses; (3) the outputs of any particular excitatory cell are spatially restricted; (4) both fast and slow IPSPs are activated by local recurrent circuitry. Even with locally random connections, and with a random distribution of firing properties for the individual cells, this model produces rhythmic waves of activity, reflected as periodicity in time in the number of cells firing and in average synaptic currents; but the firing of individual cells appears random. The amplitude and period of population activity are determined, in part, by the strength of inhibition (in a way that agrees with experiment, i.e. less inhibition leads to larger amplitude and lower frequency), by excitatory synaptic strength, and by cellular excitability. Oscillations are coherent over distances long compared with the spatial distribution of synapses from any given neuron. This model provides considerable insight into the rhythmic behaviors available to isolated cortex.

105.1

KINEMATIC ANALYSIS OF SCRATCHING IN CHICKS. M.B. Smith and A. Bekoff. Dept. EPO Biology, University of Colorado, Boulder, CO 80309.

We have previously analyzed EMG recordings obtained during scratching (Smith, M.B. et al, Soc. Neurosci. Abstr. 13: 355, 1987). The current investigation extends the study by characterizing the limb movements that occur during scratching. Kinematic analysis was carried out in 1- to 7-day old chicks. Black dots were placed on the lateral aspect of the right hip, knee, ankle and mid-tarsus. A stimulus was then applied near the right external ear and the resulting behavior was videotaped. Frame-by-frame analysis of the right limb trajectory was accomplished using a computer program to digitize the joint markers.

A typical scratch sequence consists of an initial positioning movement of the limb, followed by several cycles of rhythmic flexion and extension, and then a return to baseline. During the rhythmic flexion and extension cycles, the knee joint motion preceded the ankle. Both knee and ankle joints showed greater excursions than the hip. Supported by NIH grant NS 20310.

105.2

CHARACTERIZATION OF SCRATCHING IN NORMAL CATS. P. Carlson-Kuhta and J. L. Smith. Laboratory of Neuromotor Control, Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

Hindlimb scratching at 2-5 cycles/s in the decerebrate cat has asymmetrical flexor and extensor muscle activities, with the flexor phase occupying about 85% of a scratch cycle (Deliagina *et. al., Brain Res.* 100: 297, 1975). This muscle synergy has been used to model the central pattern generator (CPG) for scratching, but studies of kittens (Bradley & Smith, *Dev. Brain Res.* 38: 69, 1988) suggest that the neuromuscular patterns in normal cats may differ from those described for decerebrate cats.

The purpose of this study was to characterize the muscle activity during scratching in normal cats. Fine-wire bipolar electrodes were surgically implanted under anesthesia and aseptic conditions in uniarticular flexor (TA, IP) and extensor (LG, SOL, VL, ABF, GM) hindlimb muscles in three cats. Scratching was elicited by water or a small tapeball placed in the ear canal.

Cats varied their posture greatly during different scratch responses, ranging from lying to standing. The majority of responses had cycle periods between 5 and 7 cycles/s. There were symmetrical flexor and extensor phases. Some muscles varied in activity during different scratch responses. A muscle had either rhythmical bursting, tonic activity, or no activity. These differences may be due to the variations in postures for scratching which change the orientation of the scratching hindlimb with respect to the head.

The differences in cycle period and the flexor-extensor muscle symmetry between decerebrate and normal cats suggests that the decerebrate preparation may not be an appropriate model to study the CPG for scratching. Funded by NIH NS19864.

105.3

KINEMATICS OF BACKWARD AND FORWARD TREADMILL WALKING IN NORMAL CATS. J. A. Buford, J. L. Smith, and R. F. Zernicke. Laboratory of Neuromotor Control, Department of Kinesiology, UCLA, Los Angeles, CA. 90024-1568 (USA).

Grillner (1981) cited backward walking (BWD) to illustrate the facultative nature of a unit burst generator, since BWD purportedly resulted from a 0.5 phase shift between the output of hip and knee extensor units. However, EMG data from bipeds (Thorstensson, 1986; Bekoff, 1987) do not support this mechanism for BWD.

Three cats (Felis domesticus) were filmed for four speeds BWD (0.3-0.6 m/s) and one forward (FWD) (0.6 m/s). As BWD speed increased, stride length increased 24% (27 to 34 cm), and cycle period (650 to 460 ms) as well as stance duration (460 to 300 ms) decreased 44%. At 0.6 m/s, stride length (49 cm) was 44% greater and cycle period (750 ms) was 75% greater for FWD. The cats crouched with spinal ventroflexion during BWD, with hindlimb trajectories limited to a smaller range and shifted rostrally under the center of mass as compared to FWD.

During BWD stance, the knee extended greatly while the hip flexed slightly. But during FWD stance, the hip extended greatly while the knee slightly yielded and then extended. With a slightly greater range during FWD, the ankle moderately yielded and then extended during stance. For both directions, swing began with flexion at all three joints. During BWD swing knee flexion dominated, but during FWD swing hip flexion dominated. During BWD swing, each joint reversed from flexion into extension: first the hip, then the ankle, and finally the knee. But during FWD swing the hip flexed only, and the knee extended before the ankle.

Thus, to explain differences between FWD and BWD, we suggest different functional roles: propulsion from knee extension during BWD *vs.* propulsion from hip extension during FWD. We expect further analyses to show that the BWD movement patterns optimized BWD propulsion and minimized changes needed to produce BWD from the FWD motor program. Preliminary EMG supports this with extensors active during stance and flexors during swing at all three joints. Funded by NIH NS19864.

105.4

IMMUTABILITY OF ANKLE MUSCLE ACTIVITY DURING PAW-SHAKE RESPONSES. G.F. Koshland and J.L. Smith. Dept. of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

Our previous studies of kinematic and kinetic analyses of paw-shake responses suggest that ankle muscles are preprogramed without regard to limb dynamics, predicting that ankle muscles are not influenced by motion-dependent feedback. In this study, details of the EMG pattern of ankle muscles (lateral gastrocnemius, LG and tibialis anterior, TA) were examined in 4 chronic spinal cats after motion-dependent feedback was perturbed by casting the ankle (115°) and by wrapping a lead cuff (46 gm) around the paw segment. Under all conditions, responses were elicited by applying tape to the paw, and a total of 225 cycles from 25 responses were analyzed for each condition.

During control responses, cycle period averaged 90 ms with 9 cycles/response, and cycle periods increased linearily over consecutive cycles within individual responses. Burst durations of the LG were short (33 ms) and remained constant over consecutive cycles. In contrast, TA burst durations were longer (59 ms), and onsets and durations of the TA increased over consecutive cycles. During all cycles, a constant period of cocontraction (15 ms) occurred between the TA offset and LG onset of the subsequent cycle. After ankle-casting and paw-weighting, cycle characteristics, as well as the durations and timing of TA and LG muscle activities were unaltered, supporting the prediction that recruitment of ankle muscles is immutable with altered motion-dependent feedback.

In another study of responses after partial deafferentation of the hindlimb (L3-S1), however, the timing and durations of the TA were altered, while LG recruitment did not change. These results suggest that although recruitment of the LG appears fixed, recruitment of the TA may be modulated by temporal proprioceptive information that was available during casting and weighting of the limb, but eliminated after deafferentation. Supported by NS 19864.

105.5

MODULATION OF THE FLEXION REFLEX IN HUMAN LOCOMOTION. J. Duysens, V. Dietz and G.A. Horstmann. Dept. Med. Physics, K.U.N., Geert Grooteplein N. 21, 6525 EZ Nijmegen, The Netherlands and Dept. Clin. Neurology & Neurophysiology, Univ. of Freiburg, Hansastr. 9, D-7800 Freiburg, F.R.G.

The sural nerve of a series of human volunteers was stimulated electrically at two intensities which were 20 and 40% above the flexion reflex threshold respectively. These shocks were given either at 4 fixed points in the step cycle while the subjects walked on a treatmill at 4 km/hr or when they were at rest and imitated the postures at these 4 phases of the qait. The EMG responses were compared with the pain ratings of the subjects.

Ipsilaterally a response with a latency of 70-90 ms appeared in biceps femoris (BF) for stimuli in early and mid stance. For each subject the modulation patterns were identical during real and simulated stepping and for the two stimulus intensity levels. The changes in reflex amplitudes were largely independent of the pain ratings which exhibited little variation throughout the step cycle.

It is concluded that the modulation of the flexion reflex during locomotion reflects a type of gating which has no perceptual equivalent and which depends on limb position information.

105.6

WHAT ARE THE RELATIONSHIPS BETWEEN WALKING AND AIRSTEPPING IN CHICKS? R.M. Johnston* and A. Bekoff. Dept. of EPO Biology, University of Colorado, Boulder, CO 80309-0334.

A previous kinematic study showed many differences between walking and airstepping in chicks (Johnston, R.M. and A. Bekoff, *Soc. Neurosci. Abstr.*, 13:356, 1987). To determine if the differences were due to differences in cycle periods we compared kinematic data taken from cycles which fell within the range of overlapping cycle periods. In addition, we compared airstepping to data based only on the swing phase of walking to determine if the previously observed differences result from the presence of stance in walking.

We videotaped walking and airstepping in 1- to 7-day old chicks. Recordings were made when the chick walked on a runway and airstepped while suspended. Hip, knee and ankle joint markers on the lateral aspect of the limb were digitized. A cycle was defined from peak ankle flexion to peak ankle flexion.

Many differences between walking and airstepping remain when data from overlapping cycle periods are compared. However, when airstepping is compared to data based only on the swing phase of walking, several of the differences between the behaviors are decreased or eliminated. That is, duration versus period relationships for flexion and extension at each joint approach those observed for airstepping. In addition, the percent swing flexion and extension at the knee or the ankle in walking do not differ from airstepping. Thus the presence of stance in walking accounts for some of the major differences between walking and airstepping. This supports the idea that airstepping may be generated using a less modulated version of the output of a basic pattern generating circuit for leg movements. Supported by NIH grant 20310.

105.7

VISUAL CONTROL OF STEP LENGTH DURING OVERGROUND LOCOMOTION. A.E. Patla, M. Samways, C. Robinson, *C.J. Armstrong*
Neural Control Laboratory, Dept. of Kinesiology, Univ. of Waterloo, Waterloo, Ontario, N2L 3G1,

In this study, we have examined how the step length changes made during overground locomotion to avoid obstacles or provide proper placement of the foot occur, by directly measuring the ground reaction forces and temporal information. Subjects (n=20) were required either to walk or run on a long walkway. Lights placed at distances corresponding to short, normal, and long step were selectively triggered by trigger mats placed before or over the force plate. Subjects were required to place their contralateral foot on the appropriately lighted mat. The results demonstrate that depending on when the cue to alter step length is given, the strategies adopted were different. Overall to modulate step length, subjects increased or decreased the pushoff and/or the braking impulse. In late cue subjects did not alter the impulse, rather they made changes during the free flight phase. During the experiment subjects slowed down: although in the early cue conditions they were able to better maintain the average velocity of progression. In contrast to results of Warren et al. (1986), the results of this study suggests that adapting the locomotor synergy, to accomodate different step lengths, is a complex phenomena requiring more than just changes in the vertical impulse.

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105.8

FOOT TRAJECTORIES OF DIFFERENT GAIT POPULATIONS: GROSS OR FINE MOTOR CONTROL? B.J. McFadyen and D.A. Winter*, Gait Laboratory, University of Waterloo, Waterloo, Ontario, N2L 3G1.

The swing phase of walking involves a number of degrees of freedom within the linked system of the contralateral support limb, pelvis and ipsilateral swing limb. Yet, during normal walking, the average minimum ground clearance is less than a centimeter (x=.88 cms.) at a point in time when the toe is at its peak horizontal velocity of approximately 4.5 m/s. During continued forward progression, the foot re-establishes gentle contact with the ground by reducing heel velocities to near zero. These end-point trajectories during level gait can only be the result of finely tuned motor control in spite of the involvement of gross musculature and larger limbs.

Different populations from the gait laboratory data base, including normals at different cadences, normals jogging, the elderly (between the ages of 61 and 82) and below-knee amputees, were compared with respect to toe and heel trajectories during level walking. The results showed differences which were dependent upon age, cadence and degree of disability, but all suggested the existence of fine motor control. The lowest toe clearances occurred during natural cadences in normals. The results are discussed with respect to joint kinetics.

105.9

TENDON STRETCH IN CAT GAIT ESTIMATED FROM MUSCLE SPINDLE FIRING IN ACTIVE AND PASSIVE MOVEMENTS. A. PROCHAZKA, J. ELEK, M. HULLIGER, S. VINCENT & V. WALDON. Dept. of Physiol. University of Alberta, Edmonton, AB, CANADA.

Tendons undergo large tension variations in normal movement. The resultant stretch must contribute to external muscle length (l_{ext}), but there is debate over how large this effect is. The issue is important, as muscle spindles might "see" length changes very different from l_{ext}. The CNS would be faced with controlling movements of inertial loads through compliant "springs".

In the cat step cycle, E_2 phase, ultrasonic data suggested that while l_{ext} increased, active muscle fibres actually shortened at the expense of tendon (Griffiths & Hoffer, Soc.Neurosci.Abstr.13,1214, 1987). Inexplicably, fibre shortening was also registered at swing onset: increasing l_{ext}, muscle silent. Ultimately spindles themselves best monitor local length change. In anesthetized cats, we recorded the responses of l.gastroc. spindles to step cycle length variations with distributed ventral root stimulation (fusimotor action avoided) modulated to replicate step cycle tensions: 20N peak. Spindle firing in trials without stimulation was then matched to active trials by iterative changes of the length profile. Small differences in active and passive length profiles suggested reduced muscle fibre lengthening in active E_2, and slightly advanced lengthening at swing onset. Thus tendon compliance effects were detectable but modest.

105.10

LOCOMOTOR NEUROCONTROL PATTERNS IN SPINAL CORD INJURY SUBJECTS. A. M. Sherwood, M. R. Dimitrijevic, W. J. Eaton* and L. Verhagen Metman*. Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030.

Gait is characterized by a high degree of automaticity, integrating many complex tasks in a seemingly effortless fashion. In contrast to fictive locomotion in spinal cat, thus far it has been impossible to elicit stepping in primates with cord transection. Ambulatory spinal cord injury (SCI) subjects with incomplete recovery walk with diminished supraspinal influence and hence with altered neurocontrol patterns. We will present data on the patterns of neurocontrol of ambulation in 16 traumatic SCI subjects, all spastic, all incomplete. A brain motor control assessment (BMCA) protocol was employed to study segmental reflexes and suprasegmental control of single and multi-joint movements in a supine position, and gait. The BMCA employed multiple pairs of surface electrodes over the lower extremities. For gait, footswitches were used to denote foot position. We found that gait patterns were different from those in healthy subjects, were dependent on suprasegmental control, and were idiosyncratic though stable patterns. The residual volitional control was both directly expressed and through influence on segmental reflexes. Features of altered gait will be presented, and their implications for restorative intervention discussed.

105.11

KINEMATIC GAIT ANALYSIS IN THE QUANTIFICATION AND ELUCIDATION OF GERIATRIC GAIT DISTURBANCES. R.J. Elble, S. Sienko Thomas* and C. Moody*. Southern Illinois Univ. School of Medicine, Springfield, IL 62794-9230.

Gait kinematics and lower extremity EMG patterns were recorded from five elderly patients (ages 72-87) with symmetrical gait disturbance of central nervous system (CNS) origin ("senile gait"). Two patients exhibited the clinical triad of normal pressure hydrocephalus (NPH), and one fulfilled the clinical criteria of multi-infarct dementia. Both patients with NPH responded to ventriculoperitoneal cerebrospinal fluid (CSF) shunting. The etiology in two patients remained obscure despite exhaustive clinical evaluation. The abnormal kinematic and EMG data of each patient were compared with control data obtained from 14 healthy elders, ages 70-84 (mean 74.4, S.D. 4.7). In general, the kinematic parameters of the five patients were 3 to 10 standard deviations from the mean control values. CSF shunting in the two NPH patients resulted in normalization or near-normalization of most kinematic parameters. However, the gait characteristics of our patients were qualitatively similar, regardless of etiology. Thus, standard kinematic gait analysis is useful for quantifying disability in elderly patients with symmetrical gait disturbances of CNS origin but provides little if any insight into the underlying pathophysiology.
(Supported by the Whitaker Foundation.)

105.12

THE EFFECTS OF TREADMILL SPEED ON SPASTIC PARAPARETIC GAIT. M. Visintin* and H.Barbeau. School of Physical and Occupational Therapy, McGill Univ., Montreal, PQ, H3G 1Y5

The aim of this preliminary study is to investigate the effects of speed on the EMG, kinematic and temporal distance parameters of spastic paraparetic gait. Three spinal cord lesioned subjects walked on a treadmill at their relative minimal and maximal speeds (LR:0.1 & 0.3 ms^{-1}; MB:0.2 & 0.6 ms^{-1}; BP:0.2 & 0.6 ms^{-1}). EMG activity of trunk and lower limb muscles were recorded simultaneously with kinematic and temporal distance data. A decrease in % stance (LR:69.4→66.4%; MB:75.5→64.3% & BP:83.5→67.7%) was noted in all subjects at the higher speeds. The kinematic changes seen were an increase in trunk flexion (LR:7°→14° & BP:10°→14°) and total hip angular excursion (LR:22°→34° & MB:36°→43°) throughout the gait cycle. LR and MB demonstrated an increase in knee flexion during initial ground contact (9°→16° & 22°→32° respectively). BP walked with a straighter knee (18°→6°) at foot-floor contact, however a 10° yield was seen during initial loading of the limb. Plantarflexion range during push-off increased for MB (4°→12°) and BP (6°→10°). EMG data on the muscles investigated demonstrated an increase in peak burst amplitude at the higher speeds in the 3 subjects. LR and MB showed an increase in co-activation for Vastus Lateralis (VL) and Medial Hamstrings during the loading phase. BP showed a stretch induced activation in VL following knee flexion at initial loading at 0.6 ms^{-1}. The important changes evidenced in the distal muscles for the three subjects were an increase in duration and amplitude of clonus in Gastrocnemius at the higher speeds. All 3 subjects demonstrated an increase in amplitude of the early stretch activation in Soleus (SOL) following initial foot floor contact. An increase in the burst amplitude was seen in SOL for MB and BP and was related to an increase in plantarflexion at the ankle during push-off. The prolonged duration of activity during stance in the proximal muscles, increase in clonus and early stretch activation seen at the higher speeds support the concept that walking speed is an important factor contributing to the spastic features of paraparetic gait. (Supported by MRC and FRSQ).

105.13

CONTROL OF LOWER LIMB INTERSEGMENTAL DYNAMICS DURING SPONTANEOUS KICKING IN 3-MONTH-OLD HUMAN INFANTS. K. Schneider*, R. F. Zernicke, B. D. Ulrich*, J. L. Jensen*, and E. Thelen. Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568 and Department of Psychology, Indiana University, Bloomington, IN 47405.

One important component of skill acquisition is the harnessing of joint forces which may be generated not only by muscle actions, but by passive reactions related to movements of the limb segments or by gravity. Here we ask about the control of these intersegmental dynamics in involuntary, cyclic leg movements in young infants (n=3; age=0.21 + 0.02 years) while in a supine position. To calculate joint torque for infants, accurate estimates are needed of the limb's anthropometric parameters, which we determined for upper and lower extremities (n=18; age=0.18-1.5 years) by modifying a 17-segment model of the human body (Hatze, J. Biomech., 13, 1980). Using inverse dynamics (Hoy & Zernicke, J. Biomech., 19, 1986), we calculated the contributions of muscular, interactive, and gravitational torque components to the net joint torque at the hip, knee, and ankle from three-dimensional limb kinematics. The analysis of the intersegmental dynamics of spontaneous supine infant kicking revealed a counterbalancing effect of muscular and passive forces for controlling coordinated movement. For example, the kicking action was initiated at the hip joint by a muscle flexor torque. In the course of the movement a motion-dependent torque related to leg angular acceleration generated knee flexion, which was counteracted by a knee muscle extensor torque. Supported by grant NIH HD22830.

105.14

POSTURE-RELATED CHANGES IN LOWER-LIMB INTERSEGMENTAL DYNAMICS IN SPONTANEOUS KICKING IN 3-MONTH-OLD INFANTS. J. L. Jensen*, B. D. Ulrich*, E. Thelen, K. Schneider*, and R. F. Zernicke. Department of Psychology, Indiana University, Bloomington, IN 47405 and Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

A systems-functionalist approach to early skill development (Thelen, Kelso, & Fogel, Devel. Rev., 7, 1987) views coordinated behavior as emerging from the self-organizing properties of the neuromuscular system acting within a task context and the maturational constraints of the organism. Analysis of the intersegmental dynamics of spontaneous supine infant kicking (Schneider, et al., Soc. Neurosci. Abstr., 18, 1968) showed an interplay of active and passive forces to control movement. Here we ask about the task-sensitivity of these self-organizing processes by calculating joint torque components as a function of infant posture. Although lower limb movements in 3-month-olds are spontaneous and involuntary, our analysis revealed that they are sensitive to intersegmental dynamics and self-organize to control forces generated by movement and gravity. In all postures (supine, upright, 45°), at the hip, flexion was initiated by a muscle flexor torque. In the course of the movement the hip muscle flexor torque counterbalanced extensor torques related to gravity and leg and thigh angular acceleration. As the posture became more upright, the torque related to the thigh angular acceleration became more important. At the knee as the body's posture became more upright, gravitational torques became less significant and motion dependent torques became more pronounced. Supported by NIH grant HD22830.

105.15

MYOTATIC REFLEXES AND GAIT: RELATIVE ROLES IN EARLY DEVELOPMENT AND SUCCESSFUL AGING. BM Myklebust, GL Gottlieb, MA Tomski, JB Myklebust. Laboratory of Sensory-Motor Performance, Zablocki VA Medical Center & Medical College of Wisconsin, Milwaukee, WI 53295 & Rush Medical College, Chicago, IL 60612.

Our current understanding about normal human gait fails to define an explicit role for the myotatic reflex. The stretch reflex is not evoked in rthythmic locomotion. The implicit role of the monosynatpic reflex in error correction (*i.e.*, stepping over objects and avoidance of trips and falls) may operate in concert with polysynaptic pathways and central pattern generators of the spinal cord.

Studies of myotatic reflexes and gait in early childhood, aging, and neurologic disease have identified the following: [1] Locomotor skills in normal children do not develop until the normal adult-like pattern of reciprocal inhibition is seen in the tendon jerk reflex, and reciprocal excitation is absent. [2] A child, whose stretch reflexes did not disappear until age 4, has a gait pattern which is grossly unimpaired. A second child, whose stretch reflexes were absent from the time of birth, has failed to develop normal rthythmic locomotor skills by the age of 6. [3] Children and adults with hyperexcitable stretch reflexes and/or reciprocal excitation have aberrent gait patterns (*i.e.*, abnormal joint angle and velocity profiles). [4] "Successfully aging" adults (*e.g.*, those individuals who are physically active and who are not known to have neurologic or orthopedic disease) do not have definitive gait abnormalities. Tendon jerk reflex studies evoke a range of responses, from absence of the myotatic reflex, to reflex EMGs like those in normal young adults.

These results suggest that the development of spinal cord connections which allow the normal adult-like myotatic reflex pattern may be a fundamental requirement for the acquisition of normal locomotor skills. The presence of the myotatic reflex may not be a necessary requirement for the persistence of normal gait patterns in childhood and adulthood. Excessive levels of reflexly-evoked agonist and/or antagonist EMG activity may play a contributing role in modulating the patterns of gait with respect to the available joint range of motion, speed of movement, or performance of complex flexion-extension synergies.

This work has been supported by funds from VA Rehabilitation R&D and VA Medical Research.

105.16

THE COMBINED EFFECT OF CLONIDINE AND CYPROHEPTADINE WITH INTERACTIVE LOCOMOTOR TRAINING ON SPASTIC PARAPARETIC GAIT. J.Fung, J.E.Stewart* and H.Barbeau, Sch. of P.& O.T., McGill University, Montreal, Quebec H3C 1Y5

The locomotor pattern of incomplete spinal cord injured (SCI) subjects is often impaired by spasticity as manifested by clonus, hyperactive stretch reflexes and spasms. An integrated drug and locomotor training approach, combining the action of the noradrenergic agonist clonidine and the serotonergic antagonist cyproheptadine with an interactive gait training program, incorporating both progressive body weight support (BWS) and treadmill stimulation, has been proposed. This study examined the effectiveness of such an approach in 2 chronic, wheelchair-bound SCI subjects (SQ: T_{4-7}, SH: C_{7-8}). The experimental design consisted of a double-blind crossover drug trial, followed by the gait training program. Kinematic, temporal distance and EMG data were collected during treadmill locomotion before and after each intervention. Functionally, neither subject was capable of independent ambulation in the placebo period due to spasticity. They required at least 50% BWS and maximal manual assistance to advance the limbs on the treadmill. With medication, SQ could walk unassisted at 20% BWS, and SH at 0% BWS. After locomotor training, both could walk independently at 0% BWS with greater endurance. SQ also achieved a 30% increase in speed. Kinematically, the excessive flexion of the hip (SQ:90°, SH:70°) and knee (SQ:104°, SH:92°) in midswing due to flexor spasms was diminished by medication (hip:(SQ)90°→22°, (SH)70°→17°; knee: (SQ)104°→56°, (SH)92°→52°). A more normal midstance position of the hip (SQ:20°→0°, SH:-10°→-3°) and knee (SQ:20°→0°, SH:4°→0°) were further achieved by training. The EMG activity, initially characterized by clonic discharge and abnormal timing, became phasically more appropriate with less clonus following medication. This improvement was retained with training. Thus, the combined medications modulated the neural output at the spinal level, as reflected by EMG and kinematic changes. The subsequent interactive gait training with progressive BWS further optimized the locomotor pattern. (Supported by MRC, FRSQ, Boehringer-Ingelheim and Merck Frosst)

105.17

INFLUENCE OF HEAD TILT ON THE REGULATION OF STANCE AND GAIT. V. Dietz*, A. Horstmann* and W. Berger*. (SPON: F.B. Horak) Dept. of Clinical Neurology and Neurophysiol., Univ. of Freiburg, D7800 Freiburg.

Sudden tilts of the head to the front or rear were induced during stance, balancing, gait and during perturbations of gait. The most prominent repsonse in the leg muscle electromyogram (e.m.g.) to head tilt occurred in the tibialis anterior muscle (latency about 55 ms) following a backwards head tilt induced during balancing. During stance and gait, the e.m.g. activity related to head tilt was only a minor component of the leg muscle activity normally occurring during gait. When the head tilt was induced shortly after a perturbation of gait (treadmill acceleration impulse), the compensatory reaction in the leg muscles did not significantly differ from that seen after the gait perturbation alone. In addition the rate of acceleration of the head was tested against the compensatory e.m.g. responses: No correlation or influence could be discerned.

The results indicate that sudden head tilts and the resulting head acceleration have little influence on the e.m.g. pattern that occurs during gait and perturbations of gait. It is assumed that these patterns are regulated by central programs, and that the compensation for leg perturbation is achieved mainly by spinal reflex mechanisms. It is discussed whether the lack of head tilt responses is the result of an antagonistic vestibular-neck interaction, or whether its indicates a reduced effectiveness of vestibulo- and cervico-spinal reflexes during gait.

105.18

VENTRAL MIDBRAIN TRAJECTORY OF FIBERS REQUIRED FOR LOCOMOTION ELICITED BY LATERAL HYPOTHALAMIC STIMULATION IN THE ANESTHETIZED RAT. D. I. Levy* and H. M. Sinnamon. Neuropsych. Lab., Wesleyan University, Middletown, CT 06457.

Unilateral procaine injections (70 sites in 46 rats were tested for their ability to reversibly block stepping elicited by 75-uA pulses (10 to 160 Hz) to the lateral hypothalamus. In Differential blocks, procaine (0.5 ul or less) blocked stepping elicited by ipsilateral but generally not contralateral stimulation. In Nondifferential blocks, locomotion initiated by ipsi and contra stimulation was impaired equally. Procaine into the posterior dorsal tegmentum was ineffective. Differential blocks were characteristic of injections into the anterior ventral tegmentum. Both Differential and Nondifferential blocks were produced by injections into the posterior ventral tegmentum as dorsal as the superior cerebellar peduncle. In the posterior ventral region, both types of sites were widespread and they were intermixed with each other and ineffective sites. Locomotor initiation involves interdependent midbrain tegmental systems that, in some cases, must integrate bilateral information to produce a patterned output.

105.19

EFFECT OF NEMBUTAL ON LOCOMOTION ELICITED BY HYPOTHALAMIC STIMULATION IN UNRESTRAINED VS. RESTRAINED RATS. (SPON: D.B. Adams) B. Sklow* and H.M. Sinnamon. Laboratory of Neuropsychology, Wesleyan Univ., Middletown, CT 06457.

Locomotor stepping elicited by hypothalamic stimulation can be studied in rats anesthetized by Nembutal and restrained in a stereotaxic apparatus. We determined if this method selected against Nembutal-sensitive locomotion by comparing the effects of Nembutal on locomotion of chronically prepared rats tested either awake in a runway or anesthetized in a stereotaxic apparatus. For 27 hypothalamic sites, hourly maintenance anesthetic injections (7 mg/kg) transiently slowed or in some cases blocked locomotion initiation of restrained rats. The depression was less for sites that in the runway had low predrug latencies which were lowered even more by 14 mg/kg. The lowest latencies in the stereotaxic apparatus were similar to predrug latencies in the runway. If head movement was completely restrained by the use of earbars (accompanied by Xylocaine injections), the lowest latencies in the stereotaxic apparatus were actually less than runway latencies. The factors responsible for this facilitation are not yet known. The anesthetized and restrained rat seems to provide a method at least as sensitive as the awake and unrestrained rat.

105.20

ANALYSIS OF PREMOVEMENT EMGS OF THE SPINAL FROG DURING WIPE AND FLEXION REFLEXES. J.L.Schotland and W.Z. Rymer. Neuroscience Program and Dept. of Physiology, Northwestern University Medical School, Chicago, Il. 60611.

In the wipe reflex of the spinal frog, information about target location in sensory coordinates is transformed into motor output resulting in a coordinated movement of the multijoint hindlimb to the target location in space. It has been reported that the complexity of this task is reduced by the hindlimb assuming an initial flexion posture common to all target locations (Berkinblitt, et al., 1986). If, as suggested by Easton (1972), spinal circuitry underlying simple reflexes is used during more complex tasks, this common initial flexion posture should utilize the same pathways involved in the flexion reflex.

We investigated these possibilities by recording intramuscular EMGs in the spinal frog during flexion and during wipe to two distinct target locations -- one on the forelimb (FW) and one on the hindlimb (HW). We focused our analysis on the premovement EMGs to reduce feedback related effects. EMGs were sampled at 200 Hz, band-pass filtered, and stored on computer. Normalized EMGs were characterized by 1) peak amplitude and 2) areas for the 100 (Area3), 350-250 (Area2), and 750-650 (Area1) msec prior to movement onset (MVON).

Different EMG patterns were evident for each of the three behaviors. During the flexion reflex, all muscles were activated simultaneously. In contrast, onset times were staggered for different muscles during both forms of the wipe. During FW, tensor fascia latae (TFL) peaked pre-MVON, while all other muscles were still increasing in amplitude. Cruralis, peroneus, and plantaris were usually coactivated, and in any one frog showed a consistent temporal relation to the activity of other muscles.

Many muscles exhibited significantly different magnitudes between behaviors ($P<.006$). The amplitude of TFL was smaller during flexion than FW or HW and smaller during HW than FW. The amplitude of sartorius, which was similar during flexion and FW, was much smaller during HW. Iliofibularis is activated more strongly in flexion and the HW than in the FW. The magnitude of gracilis is much greater in flexion than in the FW or HW, but does not differ significantly between the two forms of wipe.

These results suggest that different neural organizations underly the initial flexion for different target locations, and furthermore that this circuitry may not be uniformly shared with the flexion reflex.

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MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: POSTURE AND MOVEMENT IV

106.1

ENDOGENOUS RELEASE OF 5-HT MODULATES FICTIVE LOCOMOTION IN LAMPREY SPINAL CORD BY ALTERING THE INPUT-OUTPUT RELATION OF INDIVIDUAL NEURONS. P. Wallén*, J. Christenson*, S. Cullheim* and S. Grillner. Depts. of Neurophysiology and Anatomy, Karolinska Institute, S-104 01 Stockholm, Sweden.

The lamprey spinal cord contains serotonergic (5-HT) neurons forming a dense plexus, as well as descending 5-HT fibers. Exogenously applied 5-HT markedly affects fictive locomotion induced in the in vitro spinal cord preparation, - burst duration and intensity increases and burst rate decreases. We have investigated (1) effects of endogenously released 5-HT, (2) a specific effect of 5-HT on the afterhyperpolarization (AHP) in motoneurons and interneurons, and (3) the ultrastructural correlate of the 5-HT action: (1) L-Baclofen (500 μM) in combination with the 5-HT uptake blocker Zimelidine (10-20 μM) induces changes in the fictive locomotor pattern analogous to those evoked by 5-HT application. That this is due to an endogenous release of 5-HT was confirmed using HPLC analysis of the perfusate. (2) Local application of 5-HT selectively reduces the late phase of the AHP caused by Ca^{2+}-dependent K^+-channels, without affecting resting membrane properties or action potential shape. This potentiates the response of the neurons to a given excitatory input, and may explain the effects on the locomotor pattern. (3) A combined immunohistochemical and ultrastructural analysis revealed 5-HT varicosities close to dendrites and cell bodies, but no synaptic specializations, suggesting that the 5-HT systems exert their action by volume transmission.

106.2

EXCITATORY AMINO ACID AND GABA TRANSMISSION TO RETICULOSPINAL NEURONS IN THE LAMPREY. R. Dubuc, Y. Ohta* and S. Grillner. The Nobel Institute for Neurophysiology, Karolinska Institutet, S-104 01 Stockholm, Sweden.

During fictive locomotion, reticulospinal (RS) neurons within the posterior rhombencephalic reticular nucleus (PRRN) show membrane potential fluctuations which depend upon spinal cord inputs (Dubuc, R.and Grillner, S. Acta Physiol.Scand., 1988, 132, 34A). RS neurons receive polysynaptic EPSPs from the ipsilateral spinal cord, IPSPs from the contralateral side, and EPSPs and/or IPSPs from several cranial nerves. This study describes the putative transmitters involved in mediating these responses. In the in vitro brain-spinal cord preparation, kynurenic acid markedly depresses intracellularly recorded EPSPs evoked by microstimulation of spinal cord fasciculi and cranial nerves. NMDA transmission is involved since an AP5-reversible prolongation of the responses occurs in Mg-free ringer. Moreover, RS neurons have NMDA receptors since they are depolarized by NMDA in TTX. Bicuculline markedly depresses inhibitory responses evoked by contralateral spinal cord stimulation. Moreover, an AP5-sensitive inhibitory response is unveiled in Mg-free ringer, suggesting the involvement of NMDA in the initial part of the polysynaptic inhibitory pathway. It is concluded that excitatory amino acids and GABA are important in the transmission of spinal cord and cranial nerve inputs to RS neurons. R.D. receives a fellowship from FRSQ.

106.3

STRYCHNINE-SENSITIVE COMPONENTS OF THE SPINAL MODULES FOR HINDLIMB LOCOMOTION IN THE CAT. D.J. Kriellaars*, P. Fortier, L.M. Jordan. Dept. of Physiology, University of Manitoba, Winnipeg, Canada, R3E 0W3.

The hyperpolarized phase of motoneurnal locomotor drive potentials is due to inhibitory synaptic input as evidenced by the fact that it is blocked by strychnine (Pratt & Jordan, J. Neurophysiol., 57:50-71, 1987) and is reversed by chloride injection (Orsal et al, Exp. Brain Res., 64:217-224, 1986). Quantitative analysis of the phase relationship between the onset and termination of the hyperpolarized phase with the excitation of antagonist motoneurones reveals a strong correlation (Kriellaars et al, Soc. Neurosci. Abstr., 13:875, 1987). These observations have allowed the derivation of a neural circuit responsible for strict alternation between flexor and extensor motoneurones, termed the locomotor reciprocity module. This module is composed of last order excitatory interneurons which projects to agonist motoneurones and interneurons which inhibit antagonist motoneurones and last order antagonist excitatory interneurons. Previous studies indicate that the inhibitory interneurons should be blocked by strychnine which should result in disorganization of locomotor activity. During fictive locomotion in the mescencephalic cat we administered strychnine intravenously and observed overlapping flexor and extensor activity. This observation may represent a strychnine-induced failure of the inhibitory components of the reciprocity module. The failure could conceivably occur at the inhibitory interneurons directly projecting to the motoneurones or at inhibitory interneurons projecting to the antagonist excitatory interneurons.

Supported by the Canadian MRC.

106.4

MAPPING OF LUMBAR CORD POTENTIALS EVOKED BY STIMULATION OF THE MESENCEPHALIC LOCOMOTOR REGION (MLR) IN THE CAT. P. Fortier, D. Kriellaars and L. Jordan. Dept. Physiology, Univ. Manitoba, Winnipeg, CANADA, R3E 0W3.

This study was based on the hypothesis that an examination of the distribution of lumbar cord potentials, evoked by stimulation of the MLR, would permit the localization of the interneurons that have been postulated (Shefchyk & Jordan, J. Neurophysiol. 53:1345, 1985) to be interposed between the volley descending from the brainstem to lumbar motoneurons involved in locomotion. Monopolar potentials were recorded along the 4th to 7th lumbar segments (L4-L7) during stimulation of an MLR-site that could produce fictive stepping in the mesencephalic cat. Potentials recorded over the dorsal surface of the cord typically showed 4 positive waves (P1-P4) which varied in amplitude along the lumbar segments. P1 may reflect the descending volley from the brainstem and P2 could result from the action of this volley on lumbar interneurons with subsequent waves resulting from the chain of events leading to rhythmical locomotor activity. The largest P2 wave was found in the region of L5. Isopotential mapping within this segment revealed steep gradient fields localized, among other regions, in the dorsal part of the ventral horn. This region overlaps the distribution of transneuronally labelled interneurons active during MLR-evoked locomotion (Noga et al., Soc. Neurosci. 13:826, 1987). These results suggest that the interneurons involved in the generation of locomotion may extend along the caudal lumbar segments with an important assembly within L5 since locomotor activity is abolished following transections in this segment (Grillner & Zangger, Exp. Brain Res. 34:241, 1979). Research funded by Canadian MRC.

106.5

TAIL-PINCH-INDUCED AKINESIA IN MEDIAN RAPHE'-LESIONED RATS. Rebecca M. Chesire. Psychology Dept., Univ. Hawaii-Manoa, Honolulu, HI 96822.

Electrolytic lesions of the median raphe' nucleus (MRN) of rats produced excessive, rapid relatively straightforward locomotion and loss of lateral head orienting in the absence of a stimulus applied to the head, snout, or encountered during unrestrained locomotion; 1). Undamaged animals showed normal forward locomotion and head orienting. When tail-pinch or a tail clamp was applied (30s-1m), MRN-damaged rats immediately became completely akinetic, stopping in a forward locomotion posture (1, cf.2) until the clamp was removed or the pinch ceased. They then resumed forward locomotion. Undamaged rats ran forward, but within 20s oriented toward the tail, often twisting their forequarters arear toward the spine and over the opposite hindquarter. They kicked using the rearquarters, boxed and scratched using the forepaws, bit the clamp or the tail and vocalized.

Tail-pinch can inhibit the excessive release of forward locomotion produced by MRN damage. Thus, the arousing effects of tail-pinch are not limited to those that release behaviors inhibited by damage (3,4). Such arousal might serve to release any of several excitatory or inhibitory behaviors that reduce or are incompatible with the predominant symptom produced by the particular form of neurological damage.

106.6

ACTIVITY OF MIDLUMBAR GROUP II INTERNEURONS DURING BRAINSTEM EVOKED FICTIVE LOCOMOTION IN THE MESENCEPHALIC CAT. S. Shefchyk, D. McCrea, D. Kriellaars, L. Jordan, and P. Fortier. Depts. Med. & Physiol., U. Manitoba, Winnipeg, CANADA R3E 0W3.

Edgley and Jankowska (J. Physiol. 389:647, 1987) have described a population of interneurons (INs) in the midlumbar segments which receive group II afferent input and project monosynaptically to lower lumbar motoneurons. This study describes the activity of a subpopulation of these midlumbar group II neurons which are rhythmically active during fictive locomotion produced by electrical stimulation of the MLR (details in Shefchyk & Jordan, J. Neurophysiol. 53:1345, 1985). Midlumbar interneurons were examined using extra- or intracellular recordings and identified according to criteria described by Edgley and Jankowska (1987) which included their projection to caudal motonuclei. All rhythmically active INs examined to date displayed activity coincident with activity in ipsilateral tibialis anterior (flexor) muscle nerve or, in the case where the tibialis anterior neurogram was not available, out of phase with ipsilateral extensor neural activity. Experiments are in progress to determine the synaptic actions of rhythmically active group II neurons on motoneurons using the sucrose gap technique as well as confirming our initial observations that these cells are primarily active during the flexion phase of the fictive step cycle. This information will be important for an understanding of the contributions of the L4 group II INs to the pattern of activity of motoneurons during fictive stepping. Supported by the Canadian Paraplegia Association and the Medical Research Council of Canada.

106.7

LOCOMOTION ON AN INCLINED PLANE BEFORE AND AFTER SPINALISATION IN THE SAME CAT. M. Bélanger, T. Drew, J. Provencher* and S. Rossignol. Cent. Rech. Sci. Neuro., Univ. de Montréal, Qué, Canada.

This study compares the adaptation of the hindlimb walking pattern (EMG and Kinematics) to inclined planes (15deg uphill, 10deg downhill, 20deg roll) before and after spinalisation in a chronically prepared cat. Before spinalisation, walking uphill or downhill induced predictable changes in step length and EMG activity. The leg length changed appropriately to maintain the body close to the horizontal plane. After spinalisation, minor modifications were observed in the EMG activity during walking uphill or downhill as compared to level. The step length was increased for uphill walking. The leg length, on the other hand, did not change to compensate for the slopes. It should be mentioned, however, that here the cat walked with the forelimbs on a board so that the whole body was parallel to the treadmill. Before spinalisation, walking with the treadmill rolled to one side produced a restructuration of the walking cycle on both sides. After spinalisation, such changes were also seen when the cat was manually held vertically. Unaided, the cat could adapt only to a few degrees of roll. Thus, the spinal cat can partly adapt the locomotion of its hindlimbs provided the rest of the body is stabilized to compensate for the loss of vestibular function. (supported by MRC and NSCERC).

106.8

MODULATION OF EVOKED DORSAL ROOT POTENTIALS BY CUTANEOUS AND MUSCULAR STIMULATION DURING FICTIVE LOCOMOTION IN THE CAT. J.P. Gossard and S. Rossignol, Ctr. Rech. Sci. Neuro., Univ. de Montréal, Qué, Canada.

Previous studies on fictive locomotion in decorticated curarised cats have shown that the dorsal roots, at the lumbo-sacral cord level, are depolarised twice per locomotor cycle (Locomotor DRPs). The largest depolarisation occurs during flexor activity and terminates at the onset of extensor activity. To examine the possible functional role of these spontaneous locomotor DRPs, other DRPs or reflex responses were evoked by stimulating muscle nerves (flexor and extensor) or cutaneous nerves through cuff electrodes. For all nerves stimulated, the evoked DRPs were strongly modulated throughout the locomotor cycle, being maximally depressed during the flexor phase and enhanced during the extensor phase. The exact timing and depth of this modulation could vary depending on the rootlet recorded and the nerve stimulated. Moreover, the modulation of short latency excitatory responses evoked in Sartorius nerve by cutaneous stimulation paralleled the modulation of the evoked DRPs during flexion. These results suggest that, during locomotion, there is a centrally generated phasic modulation of the transmission in pathways involved in primary afferent depolarisation, as well as in other reflex pathways, and that the locomotor DRPs may partly reflect the underlying mechanisms. (Supported by the MRC)

106.9

INFLUENCE OF PROPRIOCEPTIVE INPUTS ON FICTIVE LOCOMOTION OF THE FORELIMB OF THE CAT. P.Saltiel and S. Rossignol. Cent. Rech. Sci. Neuro.,Univ. de Montréal, Qué, Canada.

This a study on the effects of manually imposed protractions or retractions of the shoulder on the fictive locomotor rhythm. Cats were decorticated, curarized and spinalised at T13. Locomotion was recorded with cuff electrodes placed on the nerves to the extensor, triceps (Tri), and the flexor, cleidobrachialis (ClB), on both sides (ipsi= i; contra= co). The elbow joint was fixed and the shoulder angle was monitored with a potentiometer. Sustained retractions had little effect on the overall locomotor frequency. However, the iTri and coClB bursts were shortened and decreased in amplitude while the coTri and iClB bursts were lengthened and increased in amplitude. Correspondingly, brief retractions applied during iTri reduced the burst duration and amplitude and shortened the corresponding contralateral silence. Brief retractions in the opposite phase prolonged the silence between iTri bursts and increased the duration and amplitude of the coTri burst. Protractions, brief or sustained, essentially had the opposite effect of retractions except for a variable change in the amplitude of the coTri burst. Thus proprioceptive inputs markedly influence the fictive locomotor pattern and may be expected to contribute significantly to the adaptation of real locomotion. (Supported by the MRC).

106.10

SEGMENTAL AND SUPRASEGMENTAL EFFECTS ON THE LOCOMOTOR RHYTHM IN INTACT CATS. T. Drew and S. Rossignol. Université de Montréal, Montréal, Québec, CANADA

To determine how different inputs may interact with the normal locomotor rhythm, trains of stimuli (300Hz, 200ms duration, 0.2ms pulse duration, 2-3XT for eliciting a muscle response) were applied either to the motor cortex (MC), the medullary reticular formation (MRF), or to the superficial radial nerve (SRn) of chronically implanted, intact cats walking on a treadmill.

Stimulation of all three pathways during the swing phase of locomotion caused large changes in limb trajectory and EMG activity. While stimulation of the MC and the MRF affected mainly the activity of flexor muscles, stimulation of the SRn evoked a complex pattern of activity in both flexor and extensor muscles.

During stance, stimulation of the MC frequently curtailed extensor activity and reinitiated the swing phase, while stimulation of the MRF more often prolonged the stance period. Phase changes from stimulation of the SRn in stance were infrequently seen but, when present, were similar to those evoked by MC stimulation.

These results show that different pathways may affect locomotion similarly in one phase but differently in another, suggesting that each pathway may interact very specifically with the locomotor generating networks. (Supported by the FRSQ and the Canadian MRC)

106.11

SELECTIVE RECOVERY OF DIFFERENT TYPES OF LOCOMOTION IN DEAFFERENTED CAT HINDLIMB. M. E. Goldberger and K.L. Ziegler.* Medical College of Pennsylvania, Phila, PA 19129.

Previous studies suggested that, after hindlimb deafferentation, some spinal pathways were more important than others for recovery of locomotor functions. In the present study,cats were trained preoperatively 1) to walk on a 30.5 cm-wide runway (conditioned unrestricted overground locomotion) 2) to walk on a 5 cm-wide runway (accurate locomotion) 3) to walk on a treadmill (reflex quadrupedal locomotion) 4) to stand on a platform with forelimbs while hindlimbs walked on a treadmill (reflex bi-pedal locomotion). Unilateral hindlimb deafferentation (L1-S2 dorsal rhizotomy/ganglionectomy) impairs all types of locomotion but to different extents. Recovery of unrestricted conditioned overground locomotion is rapid but recovery of accurate locomotion is delayed. Quadrupedal locomotion is also delayed and does not recover to the extent of overground locomotion. Bipedal locomotion does not recover; the control limb hops on the treadmill with little movement of the deafferented limb. This order of recovery is specific for deafferentation and is not seen after hemisection or partial deafferentation. Since, after deafferentation, descending systems are essential for overground but not reflex locomotion, (Goldberger, 88) the results suggest a differential or hierarchical access to spinal circuitry for locomotion by descending supraspinal (conditioned overground locomotion), propriospinal (quadrupedal locomotion) and crossed segmental (bipedal locomotion) systems that is established or released soon after deafferentation.

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106.12

HIP FLEXION IN HEMIPLEGIC GAIT IMPROVED BY FUNCTIONAL ELECTRICAL STIMULATION (FES) VIA IMPLANTED ELECTRODES J.Elek, M.G.A. Llewellyn, A.Prochazka, L.A.Davis, B.Dolphin and V.Waldon. (SPON: A. French) Dept. of Physiol., University of Alberta, Edmonton, Alta, CANADA.

Hip movements are often severely reduced in hemiplegia, and orthoses for this do not exist. We are assessing FES of hip flexor and abductor muscles as a possible solution. After pilot studies with surface stimulation, we implanted a percutaneous wire electrode into psoas muscle in a stroke patient (18 months post-CVA). The specialized coiled wire, with a nylon retaining barb, was inserted transcutaneously. Judging by muscle responses to stimulation, the wire has stayed in place in the muscle for 6 months without complications.

Goniometry and electromyography (EMG) were used to assess gait and optimize the FES control signals. Without stimulation, psoas EMG was deeply modulated, but delayed by about 500 ms on foot-off. FES of psoas, modulated by a signal from a force transducer under the sound foot, produced earlier, faster swing and increased walking speed. It did not elicit useful knee flexion reflexly as we had hoped. Knee control was improved by physiotherapy and on one occasion by FES of biceps femoris short head. We are optimistic that FES of proximal muscles will improve gait usefully in a small proportion of ambulatory stroke patients, but it is important that criteria for patient selection be adequately developed.

106.13

CHANGES IN MEMBRANE PROPERTIES OF CAT ANKLE EXTENSOR MOTONEURONS AFTER CHRONIC SPINALIZATION. S. Hochman* & D McCrea Dept Physiology, U. of Manitoba, Winnipeg CANADA, R3E 0W3.

The present study compares the electrical properties of medial (MG) & lateral gastrocnemius (LG), soleus (SOL) and plantaris (PL) motoneurons between unlesioned and chronic spinal barbiturate anesthetised cats (6 weeks, L1-L2 lesion). Only cells with action potentials greater than 80 mV were accepted. In the first analysis, data from all 4 motoneuron species were pooled. Amongst other changes, there was a 16% decrease in mean input resistance (Rin) and a 17% increase in rheobase current after chronic transection ($p > .025$). Although mean calculated rheobase voltages (Rin * rheobase current) were similar in the two preparations, measured rheobase voltage increased about 14% in the chronic spinal preparation ($p > .025$). However, the most notable change was a 20% decrease in membrane time constant ($p > .001$) without any corresponding change in equivalent cylinder electrotonic length.

These changes following spinalization were not uniform across the four motoneuron species examined. For example, the percentage decrease in Rin was greatest in LG (LG > PL > MG > SOL) whereas rheobase current increased the most in PL (PL > MG > LG > SOL). This illustrates that membrane properties of even close synergist motoneuron species may be affected differently by chronic cordotomy. Generally, no membrane property changes were found in chronic spinal animals that would promote a hyperreflexive state. This is in agreement with previous findings for SOL (Cope et al. J. Neurophysiol. 55:1202-1220, 1986) and MG motoneurons (Munson et al. J. Neurophysiol. 55:619-634, 1986). Supported by the MRC of Canada and the Manitoba Health Research Council.

106.14

CHANGES IN MUSCLE MECHANICAL PROPERTIES IN SPASTIC HEMIPARESIS. R.F. Kirsch and W.Z. Rymer Northwestern University Medical School and Rehab. Instit. of Chicago, Chicago, IL 60611.

Spastic hemiparesis due to stroke or head trauma is often accompanied by changes in muscle tissue, with normal contractile tissue being replaced by collagen-based tissue. In extreme cases, this tissue limits the range of motion of the joint. We have begun to compare the mechanical (compliance) properties of the elbow joints of spastic-paretic subjects to normal joints in an effort to determine the extent of this fibrosis when no loss of range of motion is present.

A torque motor was used to apply controlled angular perturbations to the elbow joint. Joint angle and joint torque were sampled by a computer during a perturbation using appropriate sensors, amplification, and filtering. The elbow joint was set to a mid-flexion (90°) position and a small (±1°), bandlimited random perturbation was applied while the subject maintained some degree of background flexion torque either voluntarily or augmented with primitive reflexes released in spastic hemiparesis. As large a range of torque levels as possible was collected from each elbow joint. The dynamic relationship between the torque and angle records was quantified by estimating the compliance impulse response (CIR) of the joint via a correlation-based identification algorithm. The form of the CIRs was quite similar to that of a second order mass (I)- spring (K)- damper (B) mechanical system, so a model of this form was fitted to the CIRs. The I parameter is not of interest since it reflects only the mass of the forearm. Thus, the K and B parameters succinctly summarize the mechanical state of the muscles of the joint.

Three hemiparetic spastic subjects with no loss of range of motion have been examined to date. In all three cases, the elastic stiffness K of the spastic elbow joint was significantly elevated above that of both the unaffected contralateral elbow joint and that of normal subjects, while the viscous stiffness B was essentially unchanged. It is unlikely that the increase in K is due to hyperactive stretch reflexes or co-contraction since these mechanisms would be expected to effect K and B in parallel. These results suggest significant, early, and hitherto unrecognized changes in muscle properties in spastic hemiparesis that may call for early therapeutic intervention.

Work supported by NIH grant NS-19331.

106.15

MOVEMENTS AND MUSCLE ACTIVATIONS DURING TRUNK FORWARD BENDING AND EXTENDING IN LOW BACK PAIN PATIENTS. N. Paquet*, F. Malouin, C.L. Richards, Lab. of Neurobiology and Dept. of Physiotherapy, Fac. of Med., Laval University, Québec, Canada.

We studied the relative contribution of dorso-lumbar (D-L) spine and hip movements (MVTS) and muscle activation patterns of trunk and leg muscles during sagittal trunk MVTS. D-L spine (T8-S1) and hip MVTS were measured with 2 electrogoniometers while surface EMG was recorded bilaterally from the external oblique (EO), erector spinae (ES) and hamstrings (H) in 6 low back pain (LBP) male patients and 4 normal men. In normals, the contribution of the D-L spine was greater in the first 2/3 of forward bending (FB) of the trunk while hip MVTS predominated at the end of the MVT. During EX of the trunk, the mirror image occurred with hip MVTS predominating at the beginning of the MVTS. Preliminary results indicate that 3 LBP patients displayed abnormal trunk FB MVT patterns, whereas abnormal EX MVT patterns were observed in only one patient who already had an abnormal FB MVT pattern. The activation of ES muscles in these 3 patients was maintained throughout the FB MVTS, while in the normals a relaxation of ES occurred before completion of trunk FB. This lack of ES relaxation was associated with a delayed H activation in the early phase of the trunk EX MVT. These abnormal muscle activation patterns, however, were also seen when MVT patterns were not markedly disturbed and this especially for trunk EX MVTS. These results suggest that disturbances in activations of ES and H muscles are usually associated with abnormal MVT patterns during FB and that EX MVT patterns may remain normal despite abnormal activation patterns in these two muscles. This work was supported by IRSST.

106.16

POTENTIAL MECHANISMS UNDERLYING THE VOLUNTARY SUPRESSION OF INVOLUNTARY MOVEMENTS IN TARDIVE DYSKINESIA M.P. Caligiuri and J.B. Lohr VA Medical Center, San Diego, CA 92161

It is often observed that patients with neuroleptic-induced tardive dyskinesia (TD) supress involuntary choreo-athetoid movements during voluntary actions involving the affected region. The goal of the present study was to evaluate voluntary control over two muscle variables: force and length. Patients with TD and normal controls were studied in order to gain insight into potential mechanisms which contribute to the supression of involuntary dyskinesia. Two tasks were employed to assess the ability to control steady-state force (isometric condition) and position (isotonic condition). It was hypothesized that patients with marked TD would perform better on the isotonic task than the isometric task on the basis that fusimotor reflex pathways may be utilized to gain control over disruptions in position. Because TD is primarily an orofacial phenomenon, all tasks involved jaw control. Strain gauge force and position transducers were used to record isometric and isotonic stability, respectively. Analyses were made of the magnitude of instability over multiple 2-second intervals. The standard deviation of the steady-state force or position array served as the index of instability. Results indicated that TD patients exhibited significantly greater instability on the isometric condition relative to the isotonic condition in comparison with normal controls. The findings support the hypothesis that TD patients utilize afferent information about muscle length and/or joint position during voluntary action to supress involuntary movements. These results suggest further that TD patients exhibit difficulty on the isometric task because the length servomechanism is disabled during isometric activity.

106.17

A CASE STUDY OF MOTOR OVERFLOW FOLLOWING RIGHT HEMISPHERE CVA. E. Roy, J.L. Starkes and J. Charlton* (SPON: C. Riach). Dept of Kinesiology, Univ. of Waterloo, Waterloo, Ontario N2L 3G1.

Motor overflow was examined in the contralesional left hand of a 62 year old, right-handed female, following right hemisphere CVA affecting posterior frontal and anterior-superior parietal areas. Force generation and tapping tasks were performed by the ipsilesional index finger and motor overflow recorded in the contralesional index finger. Force transducers recorded distance moved and simultaneous forces generated in both hands. In a series of force conditions, the subject generated maximum flexion force for 10 sec as well as forces at 25, 50, and 75% maximum. In a similar series of tapping conditions maximum speed tapping was performed for 10 sec, followed by 25, 50, and 75% maximum. The dependent measure for force trials was relative force generated by each hand. For tapping trials cycle time, flexion vs. extension time and forces were determined. The results indicated that even under direction to relax the contralesional hand, motor overflow was present when force was generated by the opposite hand. Degree of overflow was not related to target force. For tapping, a tonic as well as phasic (synchronous) component was seen in overflow. Fast Fourier transform analyses revealed that the exact frequencies of tapping by the ipsilesional hand were embedded within the overflow response by the contralesional hand. No such overflow was found in an age and gender-matched control subject.

106.18

LONG-LOOP RESPONSES IN PATIENTS ON CHRONIC NEUROLEPTIC MEDICATIONS. D.J. Beckley*, M.P. Remler, and J. Singh*. Lab of Neurophysiology. Dept. of Neurology-VAMC; Martinez, CA 94553.

Recent studies have demonstrated increased middle latency responses in the stretched gastrocnemius muscles in patients with Parkinson's disease. Primates rendered toxic on phenothiazine medication have also exhibited enlarged middle latency responses. The purpose of our study was to determine if patients on chronic neuroleptic medication, without clinical evidence of parkinsonism, could be shown to have abnormal long-loop responses. EMG recordings were obtained from 3 muscles in the right leg from 10 male patients on chronic moderate dosage phenothiazine therapy and from 10 age-matched male normal controls. Stimuli consisted of 15 trials of sudden toe-up ramp movements supplied by a moveable forceplate platform. The phenothiazine patients showed no increase in size of the middle latency response when compared to normal controls. We concluded that, in the absence of clinical signs of parkinsonism and at the usual therapeutic dosages, patients on phenothiazines cannot be differentiated from normals on the basis of the size of their middle latency responses.

106.19

CEREBRAL GLUCOSE UTILIZATION AND IMMUNOHISTOCHEMICAL EVALUATION OF IDPN-INDUCED CERVICAL DYSTONIA IN THE RAT. S. Caldecott-Hazard, J.S. Schneider, K. Thompson and S. Stopes. UCLA School of Medicine, Los Angeles, CA 90024.

Injections of 3-3-iminodipropionitrile (IDPN) into rats produce a permanent dystonic syndrome consisting of cervical dystonia, locomotor hyperactivity, and occasional choreothetoid head movements. Additional information about this syndrome was obtained by studying cerebral glucose metabolism and immunohistochemical profiles in these rats. Sprague Dawley rats were injected with 150 mg/kg of IDPN daily for 7-10 days or with saline. The frequency of dystonic head movements was quantified. Only animals in which neck dystonia was the most prominent feature of the syndrome were studied. IDPN and saline-treated rats were given 14C-2-deoxyglucose (2DG) (20 uCi/100 g, IV). Resultant autoradiograms revealed reduced glucose utilization in the medial caudate nucleus and locus coeruleus as compared to controls. However, immunohistochemical studies to date have not demonstrated any significant loss of dopaminergic substantia nigra neurons, serotonergic (5-HT) dorsal raphe neurons, or noradrenergic locus coeruleus (LC) neurons. Other putative transmitter and peptide systems are being assessed. While several lines of evidence may point to involvement of the LC and/or 5-HT system in dystonia, the present data suggests that at least in this dystonia model, the primary defect does not involve loss of these neurons. (Supported by the Dystonia Medical Research Foundation and grant #NS00979.

106.20

ABNORMAL rCBF RESPONSE TO VIBROTACTILE STIMULATION IN DYSTONICS. L.W. Tempel* and J.S. Perlmutter, Washington Univ. Sch. Med., St. Louis, MO 63110

The purpose of this study was to determine if patients with dystonia have an abnormal response to sensory stimulation. We studied 11 patients with predominantly unilateral dystonia and 13 normals. PET and $H_2{}^{15}O$ were used to measure rCBF at rest and during vibrotactile stimulation of one or both hands. The stimulation produced a consistent increase in rCBF in somatosensory cortex contralateral to hand stimulation in normal subjects (L brain response = 9.6 $\pm$ 2.3; R brain response = 12.0 $\pm$ 2.9 ml/(min $\cdot$ 100g)). In contrast, the comparable rCBF responses to vibration of the affected or unaffected hands in dystonic patients were significantly reduced (7.9 $\pm$ 2.0, $p<.05$; 8.4 $\pm$ 2.3, $p<.002$ respectively). Vibration induced a dystonic hand cramp in 6 patients, but not in any normals. To determine if co-contraction could attenuate the rCBF response, 5 of the normals were studied with vibration and voluntary hand co-contraction, as well as vibration alone. Vibration with voluntary co-contraction produced a greater response than vibration alone ($p<.05$). This differed from the diminished response seen in dystonics with vibration-induced co-contraction. These data suggest that patients with predominantly unilateral dystonia have an abnormal response to vibrotactile stimuli of either hand.

106.21

NEUROPEPTIDE-INDUCED POSTURAL DESTABILIZATION: A POTENTIAL STATE VARIABLE FOR RATE OF ONSET OF BARREL ROTATION. C. D. Balaban*, V.P. Starcevic* and W. B. Severs. Depts. of Anat., Surg. and Pharm., Penn State U., Hershey, PA 17033

Intracerebroventricular (i.c.v.) somatostatin (SRIF) and arg-vasopressin (AVP) can elicit barrel rotation (BR), convulsions and lethal pulmonary edema [Brain Res., 365 (1986) 21-41; 445 (1988) 117-129)]. This report summarizes BR latency data from 16 groups of rats given either i.c.v. SRIF or AVP alone or SRIF with either AVP or the AVP antagonist, Peninula Labs #8109 (mcAVP). Hazard plots of BR latencies were used to estimate the instantaneous rates of BR onset from a 2 parameter exponential model. ANOVA/Scheffe analysis of values of the hazard parameter (θ^{-1}) revealed four discrete states of risk of BR onset across 16 conditions ($p<0.05$): State 4 θ^{-1} (range, %/sec)=2.4-4.47; State 3 θ^{-1}=0.88-1.1; State 2 θ^{-1}=0.46-0.62; State 1 θ^{-1}=0.05-0.14; State 0 no BR. There are distinct patterns of state shifts after injections of SRIF versus AVP. Whereas i.c.v. SRIF (20 or 40 μg) pro-duced serial state transitions from 0 to 2 or 3 to 1 to 0, AVP (0.5 μg) in sensitized rats produced a transition series of 0 to 4 to 2 to 0. Combined doses of SRIF and either AVP or mcAVP produced transitions from 0 to 2-4 to 1 to 0. Thus, endogenous neuropeptides may affect discrete states of postural stability. As BR is not prevented by labyrinthectomy, it may serve as an animal model for central vestibular dysfunction. (RCDA NS00891 [C.D.B.] & Fogarty TW03743 [V.P.S.]).

106.22

EFFECTS OF ARGININE-VASOPRESSIN (AVP) ON SOMATOSTATIN (SRIF)-INDUCED BARREL ROTATION (BR) AND CONVULSIONS IN RATS. V. Starcevic, C.D. Balaban and W.B. Severs. (SPON: J.D. Connor). Depts. Anat., Surg. & Pharm., Penn State U., Hershey, PA 17033.

Intracerebroventricular (i.c.v.) injections of SRIF and AVP produce motor symptoms which include BR and convulsions associated with lethal pulmonary edema, via partially independent mechanisms [Brain Res. 365:21-41; 445:117-129]. Cerebellar Purkinje cells also degenerate in SRIF-treated rats. Alert, adult male SD rats received an i.c.v. bolus of a combined dose of 20 μg SRIF and either 1 μg AVP (SRIF+AVP-1) or 1 μg of a V1 antagonist, Peninsula Labs #8109 (SRIF+mcAVP). These experiments were compared with our cumulative data base for responses to 20 μg SRIF alone or 20 μg SRIF+0.5 μg AVP. BR incidence increased ($p<0.05$) in the SRIF+0.5 μg AVP group vs. SRIF-AVP-1; no other differences were significant. Mortality was elevated after SRIF+AVP-1 vs. SRIF or SRIF+mcAVP ($p<0.05$), but mortality after SRIF+0.5 μg AVP was greater than all other groups ($p<0.05$). Hazard analysis of BR latencies indicated that the initial phase instantaneous probability of BR onset (θ^{-1}) was lower for SRIF+mcAVP (0.49$\pm$.10) and SRIF 20 μg (0.62$\pm$.11) than for SRIF+AVP-1 (0.88$\pm$.12) or SRIF+AVP 0.5 μg (3.32$\pm$.23). Purkinje cell death appeared in all groups. These data imply that endogenous AVP contributes to motor symptoms elicited by i.c.v. SRIF. (NS00891 (CDB) & Fogarty TW03743 (VPS)).

107.1

EFFECTS OF CHRONIC SECOND MESSENGER AND CHOLINERGIC LIGAND EXPOSURE ON THE CELL SURFACE EXPRESSION OF NICOTINIC ACETYLCHOLINE RECEPTOR BINDING SITES BY THE TE671 HUMAN MEDULLOBLASTOMA CLONAL LINE. A. M. Joy*, M. Bencherif*, H. N. Siegel and R. J. Lukas (SPON: I. Westenberg) Division of Neurobiology, Barrow Neurological Institute, 350 W. Thomas Rd., Phoenix, AZ 85013.

The CNS-derived cell line, TE671, expresses a class of nicotinic acetylcholine receptors (nAchR) that are functionally sensitive to blockade by alpha-bungarotoxin (Bgt). Previous studies in this laboratory have demonstrated that chronic exposure (24-48 hr) of TE671 cells in situ to nicotinic agonists induces an up-regulation of binding sites for radiolabeled toxin (I-Bgt) on cellular membrane fractions, while chronic treatment with di-butyryl cyclic AMP induces a down-regulation. By contrast, chronic agonist exposure induces a loss of functional nAchR expressed on the surface of TE671 cells. This study concerns the effects of drug treatments on I-Bgt binding site expression on the cell surface of TE671 cells maintained in situ. Chronic exposure to carbamylcholine results in upregulation of I-Bgt binding sites at the cell surface, while the antagonist d-tubocurarine decreased the number of I-Bgt binding sites. The concentration dependence of the upregulation by agonist has a similar profile as receptor activation by agonist. Possible mechanisms for receptor upregulation will be discussed.

107.2

NICOTINIC ANTAGONISTS AND PHORBOL ESTERS UP-REGULATE α-BUNGAROTOXIN BINDING SITES VIA DISTINCT MECHANISMS. S. Geertsen*, R. Afar*, J.M. Trifaró* and M. Quik. (SPON: P. Braun). Depts. of Pharmacology, McGill Univ., Montreal, Que. and Univ. of Ottawa, Ottawa, Ont., CAN.

Incubation of adrenal chromaffin cells in culture with 4β-phorbol 12-myristate 13-acetate (PMA) for 2-3 days resulted in a marked increase in α-bungarotoxin (α-BGT) binding. The PMA induced increase in α-BGT binding occurred in a dose dependent manner, with 100 nM PMA resulting in a maximal 15 fold increase. Inactive phorbol esters, such as 4α-PMA and 4α-PDB (4α-phorbol 12,13-dibutyrate) resulted in only a minimal increase in binding. Additionally, the protein kinase C inhibitor, polymyxin B, completely inhibited the up-regulation of toxin sites produced by PMA. These results suggest that PMA increased α-BGT binding through the activation of protein kinase C. Previous results had shown that the nicotinic antagonist d-tubocurarine also resulted in a marked increase in α-BGT binding to adrenal chromaffin cells in culture (Quik, M. et al., Mol. Pharmacol. 31:385, 1987). However, polymyxin B did not affect the d-tubocurarine induced increase in α-BGT binding.

These results show that activation of protein kinase C can result in an up-regulation of the α-BGT sites in chromaffin cells in culture. Furthermore, this appears to be distinct from the nicotinic antagonist induced increase in the α-BGT sites.

107.3

EFFECTS OF PREGANGLIONIC DENERVATION AND POSTGANGLIONIC AXOTOMY ON ACH AND GABA RESPONSES OF CHICK CILIARY GANGLION NEURONS. A.E. McEachern[1], M.H. Jacob[2], and D.K. Berg[1]. [1]Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093 and [2]Worcester Foundation for Exptl. Biology, Shrewsbury, MA 01545.

Chick ciliary ganglion neurons have nicotinic acetylcholine receptors (AChRs) that mediate chemical transmission through the ganglion, and $GABA_A$ receptors of unknown significance. We report here that preganglionic denervation of the neurons produces a small decline in ACh sensitivity and a larger decline in GABA sensitivity. Postganglionic axotomy has a different effect, reducing ACh sensitivity dramatically while leaving GABA sensitivity unchanged. The results suggest that cell-cell interactions regulate the ACh and GABA responses of ciliary ganglion neurons, and the regulation involves different mechanisms for the two types of receptors.

Ciliary ganglia in newly hatched chicks were either denervated or axotomized by transecting the appropriate nerves, and later removed and enzymatically dissociated to yield single cells. Intracellular recording was used to measure changes in the membrane conductance of the cells caused by ACh or GABA pressure applied from nearby micropipettes under standard conditions. Five days after axotomy the mean ACh sensitivity of the neurons had fallen to 13 ± 3% (n=28) of that observed for neurons from unoperated contralateral ganglia from the same chicks. GABA responses were not altered by axotomy (n=32). Ten days after denervation mean ACh and GABA sensitivities had declined to 71 ± 12% (n=32) and 41 ± 12% (n=32), respectively, of the values observed for neurons from normal and unoperated contralateral ganglia. The declines could not be accounted for by changes in cell size or cholinesterase levels. The decline in ACh sensitivity after axotomy is similar in magnitude to the large decline in total ganglionic AChRs measured with an anti-AChR monoclonal antibody in detergent extracts. The decline after denervation, however, is not as great as that observed for total ganglionic AChRs with the antibody under the same conditions. (Supported by NS12601 and NS21725)

107.4

EFFECT OF CHRONIC DFP TREATMENT ON ACUTE NICOTINE RESPONSE IN MICE. J.L. van de Kamp* and A.C. Collins (SPON: W.E. Hahn). Instit. for Behav. Genetics, Univ. of Colorado, Boulder, CO 80309

Costa and Murphy (JPET 226: 392-397) have reported a decrease in [^{3}H]-nicotine binding and reduced sensitivity to the antinociceptive effect of nicotine in rats chronically treated with disulfoton. Previously, we demonstrated that chronic treatment with diisopropylfluorophosphate (DFP) for two weeks resulted in significant cross tolerance to nicotine on one measure (heart rate) in DBA mice and a decrease in the number of [^{3}H]-nicotine binding sites in cortex. In the present study, DBA male mice were injected with DFP or saline every other day for one month. The animals were subsequently challenged with one of several doses of nicotine. Heart rate, body temperature, Y-maze activity and rearing were recorded. DFP-treated mice exhibited more robust cross-tolerance to nicotine on only one measure (heart rate) and decreases in [^{3}H]-nicotine binding sites were observed in the cortex and midbrain. Some of the chronically treated mice were challenged with an acute dose of 1,1-dimethyl-4-phenylpiperazinium (DMPP), a nicotinic agonist that does not cross the blood-brain barrier. There was no significant cross-tolerance observed in the measures recorded (heart rate and body temperature). (Supported by DA-03194.)

107.5

EFFECTS OF COLD STRESS ON CHOLINERGIC RECEPTORS IN THE RAT ADRENAL GLAND. L.L. Miner*, A.P. Baruchin, and B.B. Kaplan (SPON: E.Weisberg). Molecular Neurobiology Program, Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Previously we demonstrated that chronic cold stress resulted in 2-to-4-fold increases in rat adrenomedullary tyrosine hydroxylase (TH) mRNA levels. These changes could be greatly attenuated by the administration of chlorisondamine, a cholinergic antagonist. Conversely, the chronic administration of cholinergic agonists elevated adrenomedullary TH mRNA levels. To further explore the involvement of the cholinergic system in the adaptive response to cold stress, we assessed the effects of cold exposure on adrenomedullary nicotinic and muscarinic receptors. Nicotinic and muscarinic receptors were estimated by radioligand binding assays using α-bungarotoxin (BTX) and quinuclidinylbenzilate (QNB), respectively. After 4 and 7 days of cold exposure BTX binding increased by 150-170%, whereas QNB binding was 25-35% of control levels. Scatchard analysis indicated that these differences were due to changes in the number of binding sites rather than changes in receptor affinity (BTX, .14 nM; QNB, 144 pM). No differences in receptor binding were observed prior to 4 days of cold exposure. These results indicated that the adaptive response to chronic cold stress involve reciprocal changes in adrenomedullary cholinergic receptors.

107.6

CONTRACTILE ACTIVITY REGULATES BLADDER MUSCARINIC RECEPTORS AND RESPONSE M.R.Ruggieri*, S.Kitada*, K.E.Whitmore*, A.J.Wein* and R.M.Levin* (SPON: K.Williams) Div. Urology, Univ of PA and Graduate Hospital, Phila., PA 19104.

Previous studies have shown that in the pineal gland, receptor density can be modulated within hours by neuronal activity. The current study investigates whether stimulation of the urinary bladder can alter muscarinic receptor density and response.

Transducer tipped catheters were used to monitor intraabdominal and intravesical pressure in male New Zealand white rabbits. Reflex bladder contractions were induced with a suture tied tightly around the catheterized penis. Control rabbits did not have the external tie. After 4 hours half the bladder was used for muscle bath study and half was used for muscarinic receptor assay. There was no relationship between intraabdominal and intravesical pressure. The bladders were not overdistended after 4 hr (mean volume = 35 ml). The contractile activity of the bladder in-vivo induced by penile ligation could be completely blocked by iv hexamethonium. Penile ligation reduced the maximal response to field stimulation and bethanechol to 45±5% and 46±3% respectively of the response of strips from control animals. Penile ligation reduced the muscarinic receptor density from 34±3 to 22±3 fmole/mg protein.

The effect of repetitive intermittent field stimulation on the contractile response and muscarinic receptor density of bladder strips from untreated rabbits was also investigated. Similar to in-vivo stimulation, intermittent in-vitro field stimulation (30 sec on, 30 sec off for 2 hr) resulted in a moderate (25%) reduction in contractile response to field stimulation and bethanechol and reduced the muscarinic receptor density from 29±2 to 18±2 fmol/mg protein.

In both cases, there was no change in the contractile response to KCl, indicating that there was no deficit in the contractile apparatus over the course of the experiment. Clinically, this process of downregulation may help explain the presence of voiding dysfunctions in patients showing a high degree of uninhibited bladder contractions.

107.7

REGULATION OF MUSCARINIC RECEPTORS BY INTRAHIPPOCAMPAL ADMINISTRATIONS OF GALLAMINE. W.S. Messer, Jr. and B.R. Ellerbrock*, Dept. of Medicinal and Biological Chemistry, College of Pharmacy, University of Toledo, Toledo, OH 43606

Long-term intrahippocampal injections of gallamine, a cholinergic antagonist, impair learning of a representational memory task. To determine if the behavioral deficits are due to an alteration in the binding properties of muscarinic receptors, we utilized quantitative autoradiographic techniques to measure the number of muscarinic receptors and the affinity for gallamine, pirenzepine and carbachol.

Animals were injected bilaterally with either saline or 5 µg of gallamine in 0.5 µl saline (10 µg total) daily for 22 days. Animals were sacrificed 24 h following the last injection by cardiac perfusion with 0.1 % formaldehyde in 40 mM phosphate buffer. Muscarinic receptors were localized in 12 µm rat brain sections using 0.06 - 4.0 nM [^{3}H]-l-quinuclidinyl benzilate ([^{3}H]-l-QNB) in phosphate buffer and the binding of gallamine, pirenzepine and carbachol was determined through inhibition of 0.2 nM [^{3}H]-l-QNB binding. Binding properties were examined in several brain regions through quantitative autoradiographic techniques.

The binding properties of muscarinic receptors did not differ when comparing whole slices, but regional analyses of autoradiograms revealed some striking differences. The number of muscarinic receptors was increased (13.5 to 17.4 % over saline controls) throughout all cortical layers in gallamine-treated animals, reaching significantly higher levels ($p < 0.05$) in the deep layers of the cerebral cortex. No differences were noted in the CA1 region of the hippocampus or the dentate gyrus. Significant increases (19.4 %, $p < 0.05$) in receptor number also were noted in the superior colliculus for gallamine-treated animals. Supported by NS 25765.

107.8

REGULATION OF MUSCARINIC RECEPTORS IN CULTURED EXPLANTS OF RAT HIPPOCAMPUS BY CHOLINERGIC INNERVATION, AGONISTS AND ANTAGONISTS. K. Rimvall*, F. Keller and P.G. Waser*, Inst. Pharmacol., Univ. Zurich, CH-8006 Zurich, Switzerland.

Agonist induced decreases in receptor number ("down-regulation") represent an in vivo mechanism for the regulation of receptor response to physiological stimuli. In the present study the relationship between the number of muscarinic acetylcholine receptors (mAChrs) in the hippocampus and its cholinergic innervation was investigated. Such studies are difficult to perform in vivo - hence, we used an in vitro approach. Explants of rat hippocampus and septum were cultured on cover slips in rotating test tubes. MAChrs were characterized in binding studies (Rimvall K. et al., Neuropharmacology, 25:221, 1986). Hippocampal explants were cultured either alone (Hal) or with septal explants (Hco). Hal is a deafferented structure devoid of cholinergic transmission whereas Hco is innervated by cholinergic fibers from co-cultured septal explants (Rimvall K. et al., Develop. Brain Res., 19:267, 1985). Septal but not hippocampal explants contain a high-affinity uptake system for choline and release ACh upon depolarization. Cultured explants were incubated with atropine or carbachol for 48 h before the binding experiment. Such pretreatment of Hal with carbachol caused a dose-dependent down-regulation of mAChr numbers (max. 30-40% of control) which was totally inhibited by the simultaneous addition of atropine. Atropine alone had no effect on the number of mAChrs in Hal. Receptor content in non-treated Hco was of the same magnitude as in Hal treated with carbachol. Carbachol pretreatment of Hco caused a down-regulation of mAChrs whereas atropine induced a up-regulation of mAChrs to levels comparable to the ones found in non-treated Hal. Thus, co-culture conditions, i.e. cholinergic (re)innervation of Hco and subsequent influence by septal acetylcholine, cause mAChrs to be sensitive to the down-regulating actions of atropine and also mimic the effects of pretreatment of Hal with carbachol. In conclusion, our findings definitely connect a cholinergic (re)innervation of hippocampal explants with a down-regulation of mAChr number. Our report also provides "reverse evidence" for a compensatory up-regulation of receptor number after cholinergic denervation.

107.9

ATROPINE-INDUCED UP-REGULATION OF MUSCARINIC BINDING SITES AND PHOSPHOINOSITIDE HYDROLYSIS IN RAT BRAIN: EFFECTS OF LITHIUM. R.H. Lenox, D.D. Hendley*, and J. Ellis. Neuroscience Research Unit, Dept. of Psychiatry, Univ. of VT Coll. of Med., Burlington, VT 05405.

The effects of chronic atropine administration on muscarinic systems of the rat CNS were studied with and without ongoing lithium treatment (achieving clinically relevant levels in the brain). Atropine was continuously infused via subcutaneously implanted osmotic pumps at the rate of 10 mg/kg/day for 6 days. Twenty-four hours following removal of the pumps, slices and membranes were prepared from the hippocampus and cortex. The binding of labeled quinuclidinyl benzilate, N-methylscopolamine, pirenzepine, and oxotremorine-M was determined. The muscarinic phosphoinositide response was assessed at concentrations of carbachol that produce maximal (1mM) and half-maximal (40µM) responses. Atropine treatment yielded an increase in Bmax of approximately 20% for each of the ligands. The degree of up-regulation was not diminished by concomitant administration of lithium. Atropine treatment also increased the muscarinic phosphoinositide response by about 20% at both carbachol concentrations. This effect of atropine was prevented by concomitant lithium administration. It therefore appears that the muscarinic phosphoinositide response is enhanced by up-regulation and that lithium may act beyond the receptor to prevent the enhancement of the response. Supported by NIMH PHS R01 41571.

107.10

MODULATION OF M1 AND M2 mAChR's IN WILD TYPE AND KIN 8 ADRENAL CARCINOMA Y1 CELLS. N.M. Scherer,* R.A. Shapiro,* and N.M. Nathanson. (SPON: S.D. Hauschka) Pharmacology Dept., SJ-30, Univ. of Washington, Seattle, WA 98195.

Wild type and a variant clone (Kin 8) of Y1 adrenal carcinoma cells were transfected with the mouse M1 and pig M2 mAChR's. Y1 cells lack endogenous mAChR's. The Kin 8 clone contains a mutant gene for the regulatory type 1 subunit of cAMP-dependent protein kinase A (PrK A) and is resistant to the effects of cAMP.

In Y1 cells both the M1 and M2 receptors are internalized as a result of exposure of the cells to the agonist, carbamylcholine. Treatment with phorbol 12-myristate 13-acetate (PMA) results in internalization of M1 receptors, suggesting the involvement of Prk C. The effect of PMA is not as great or as rapid as that seen with carbamylcholine, suggesting that activation of Prk C cannot fully replicate the agonist effect. In contrast, treatment with PMA does not result in internalization of M2 receptors. Increasing cAMP levels in Y1 cells does not alter the number of either receptor.

In Kin 8 cells carbamylcholine-induced internalization of the M1 receptor is greatly reduced; in contrast, loss of M2 receptors is greater than in wild type cells. Thus, Prk A is likely to be necessary but not sufficient for internalization of the M1, but not M2, receptor. Treatment of Kin 8 cells with PMA does not result in internalization of M1 or M2 receptors. The ability of PMA to induce internalization of M1 in wild type, but not Kin 8, cells suggests that both Prk C and Prk A are necessary for internalization of the M1 receptor. Neither Prk A nor Prk C appear to be involved in internalization of the M2 receptor. Supported by NIH grant HL30639 and HL07312. N.M.S. is a fellow of the American Heart Assoc., Wash. Affiliate.

107.11

REGULATION OF MUSCARINIC RECEPTOR EXPRESSION IN CULTURED SYMPATHETIC NEURONS. K.E. Smith, N.E. Kremer, V. Wong, and J.A. Kessler. Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Muscarinic receptor expression was examined in cultured sympathetic neurons of the neonatal rat superior cervical ganglion. Sympathetic neurons in culture contained abundant ^{3}H-QNB binding sites (2-3 pmoles/mg protein after 2 weeks in culture) and substantial levels of M2 muscarinic mRNA (detected by Northern blot analysis using an M2 muscarinic receptor cDNA provided by E. Peralta, Genentech). Muscarinic receptor numbers were decreased by treatment either with a 29 kD membrane associated molecule isolated from rat spinal cord (Wong and Kessler, PNAS, 84:8726, 1987) or with a soluble factor present in rat fibroblast conditioned medium. Similar decreases in ^{3}H-QNB binding were observed after chronic agonist (carbachol or muscarine) treatment or potassium-induced membrane depolarization. Despite the similar effects of MANS and RFCM on receptor numbers, effects on M2 muscarinic mRNA differed strikingly. MANS treatment decreased M2 mRNA levels whereas levels of the mRNA were unchanged after treatment with RFCM. Thus these two factors, which have similar effects on receptor numbers, act at least in part through different mechanisms. Both factors also promote cholinergic and peptidergic neurotransmitter expression by cultured sympathetic neurons, suggesting that transmitter and receptor expression may be regulated by the same environmental signals. Although mechanisms underlying the effects of MANS and RFCM are unknown, chronic treatment with phorbol myristate acetate (PMA) also decreased both ^{3}H-QNB binding and M2 receptor mRNA. Thus PMA mimicked the effects of MANS, raising the possibility that protein kinase C mediates effects of the membrane associated factor.

107.12

POSTNATAL DEVELOPMENT AND SEX DIFFERENCES OF MUSCARINIC ACETYLCHOLINE RECEPTOR CHARACTERISTICS IN RAT CEREBRAL CORTEX SLICES. F. van Huizen [1]*, C. Shaw [1]*, M. Wilkinson [2] and M. Cynader [1] (SPON: R.M. Douglas) [1] Dept. of Ophthalmology, University of British Columbia, Vancouver, B.C., Canada V5Z 3N9 and [2] Dept. of Physiology and Biophysics, Dalhousie University, Halifax, N.S., Canada.

We have shown previously that muscarinic acetylcholine receptor characteristics could be assessed in 'living' slices of rat cerebral cortex (*Soc. Neurosci. Abst. 13, 727*). In this study we examined the possible changes in these receptor characteristics during postnatal development. Fresh cerebral cortex slices of male and female rats (4-100 days old) were incubated in increasing concentrations of [^{3}H]-NMS to yield saturation binding curves. From these curves, K_d and B_{max} values could be calculated. A sharp increase in both Kd and Bmax was found for both males and females, peaking at about 30 days postnatal. Following this peak (12.8 $\pm$ 1.4 nM and 2148 $\pm$ 187 fmol/mg protein, resp.) , Kd and Bmax dropped to a lower level in adult male rats, but in female rats the values were quite varied. This appears to be due to the stage of the ovarian cycle. The peak in Kd and Bmax values at 30 days postnatal coincides with the peak in synapse number. Displacement studies revealed sharp increases in the IC_{50} for pirenzepine and oxotremorine (M_1 and M_2 mAChR) but only a slight increase for NMS during development with a consecutive drop to adult values. Downregulation of the receptor after agonist stimulation is greater in adult rats (-23.8 $\pm$ 2.7%) than in rats younger than 30 days (-8.0 $\pm$ 4.1%).

107.13

EFFECTS OF TESTOSTERONE AND ESTRADIOL ON MUSCARINIC RECEPTOR BINDING IN RAT CEREBRAL CORTEX SLICES. D. March[1*], J. Simmons[2*], F. van Huizen[1*], and C. Shaw[1*]. (SPON: P. Bawa), [1]Dept. of Ophthalmology, UBC, Vancouver, Canada; [2]Dept. of Biology, Trinity College, Hartford, CT 06106

Recent studies have characterized the muscarinic acetylcholine receptor (mAChR) in living slices of rat cerebral cortex (van Huizen et al. 1987). In the present study the effects of testosterone (T) and estradiol (E_2) on [^{3}H]-N-methyl-scopolamine ([^{3}H]-NMS) binding to the mAChR was investigated. Prepubescent rats (25 days old) were gonadectomized then sacrificed after 96 hours. Littermates were sham-operated and similiarly sacrificed, creating four distinct groups: orchiectomized males (ORM), sham males (SHM), ovariectomized females (OVF), and sham females (SHF). Cortical slices (400μm) were preincubated for 3 hours at 37°C alone, with 10^{-5}M T or 10^{-7}M E_2. Slices were incubated for 2 hours at 4°C with [^{3}H]-NMS. Bmax and Kd values were determined for each condition (see Table).

Both Bmax and Kd decrease in ORM and return to normal levels with the addition of T. Preliminary evidence in females suggest that increases are seen in Bmax and Kd in OVF, but no significant changes occur when E_2 is added. The present results support a role for gonadal hormones in the development of mAChR characteristics in rat cerebral cortex. Further, hormonal regulation of the mAChRs may be very rapid (hours) as shown by the recovery to normal values after the addition of T.

Group	Control(Bmax*,Kd ^)		+T (Bmax,Kd)		+E_2 (Bmax,Kd)	
SHM(n=3)	2159±132	11.7±2.2	2362±10.1	12.2±3.6	-	
ORM(n=3)	1333±103	7.52±1.4	2045±18.5	15.4±2.6	-	
SHF(n=1)	1557	11.8	-		1369	7.33
OVF(n=1)	3771	17.7	-		3761	20.5

*Bmax: Fmol/mg protein ^Kd: nM

107.14

PHORBOL 12,13-DIBUTYRATE REGULATES MUSCARINIC RECEPTOR BINDING IN RAT CORTICAL SLICES BY ACTIVATING PROTEIN KINASE C. W.-G. Jia, C. Shaw*, F. van Huizen*, M. Cynader. Dept. of Ophthalmology, UBC, Vancouver, Canada, V5Z 3N9.

Stimulation of muscarinic receptors (mAChR) elicits phosphatidyl-inositol turnover , which yields inositol phosphates (InsP) and diacyl-glycerol (DG); the latter activates a second messenger, protein kinase C (PKC). Activating PKC with phorbol esters inhibit mAChR agonist stimulated phosphoinositide hydrolysis and InsP production (Labarca,R. et al. Biochem. Biophys. Res. Commun. 123:707-709,1984). A possible mechanism of this inhibition may be down- regulation of mAChR by PKC. In the present work, rat cortical slices were preincubated with phorbol 12,13-dibutyrate (PDBu) followed by binding assays for [^{3}H]-quinuclidinyl benzilate (QNB) or [^{3}H]N-methyl scopolamine (NMS). Binding of both ligands was reduced after pretreatment with PDBu. This binding loss was dose-dependent in the range of 10^{-9} to 10^{-7}M PDBu and started within 5 min of treatment. After 60 min of pretreatment with 10^{-7}M PDBu, binding of [H^3]-NMS and [H^3]-QNB was reduced by 18.8% ($P<0.01$) and 19.9% ($P<0.02$) respectively. In addition, binding of [^{3}H]-pirenzepine (PZ) or [^{3}H]-oxotremorine-m (OX-m) ($<10^{-9}$M), which selectively bind to M_1 or M_2 mAChR subtypes, was reduced after the PDBu pretreatment (-16.8% for PZ , $P<0.01$ and -20.9% for OX-m, $P<0.001$). Moreover, pretreatment with 1-oleoyl-2-acetyl-rac-glycerol, a synthetic DG, caused the same reduction (-17.8% at 10^{-6}M DG) in [H^3]-QNB binding. These results suggest that activation of PKC may cause "down-regulation" of mAChR and mimic a negative feedback mechanism that exists in mAChR stimulated phosphoinositide hydrolysis.

107.15

EVIDENCE AGAINST THE INVOLVEMENT OF PROTEIN KINASE C IN AGONIST-INDUCED DESENSITIZATION OF MUSCARINIC RECEPTORS. W.S. Lai and E.E. El-Fakahany. Dept. of Pharmacology and Toxicology, Univ. of Maryland. Sch. of Pharmacy, Baltimore, MD 21201.

Preincubation of mouse neuroblastoma N1E-115 cells with muscarinic agonists results in rapid down regulation of cell surface muscarinic receptors and in desensitization of receptor-mediated cyclic GMP (cGMP) synthesis. In contrast, short-term exposure of these cells to phorbol esters diminished carbamylcholine (CBC)-induced cGMP and inositol phosphates accumulation without affecting binding of the muscarinic antagonist [^{3}H]N-methylscopolamine ([^{3}H]NMS). CBC, however, added after short-term incubation with phorbol ester still caused down-regulation of [^{3}H]NMS bindings. In addition, long-term (24 hrs) pretreatment of cells with phorbol-12, 13-dibutyrate (PDBu) abolished [^{3}H]PDBu bindings without altering the dose-response curve of CBC-induced down-regulation of [^{3}H]NMS binding. Under the latter condition where protein kinase C is desensitized, CBC preincubation caused a time-dependent desensitization of cGMP response similar to that observed in control cells. These results suggest that protein kinase C may not play an important role in agonist-mediated desensitization of muscarinic receptors. (Supported in part by NIH grants NS-24158, AG-07118 and AG-00344.)

107.16

PHOSPHORYLATION OF PURIFIED CHICK BRAIN MUSCARINIC CHOLINERGIC RECEPTOR BY Ca/CALMODULIN (TYPE II) PROTEIN KINASE. **J.S. Aguilar*, J.P. Sullivan*, A.F. Skorupa*, S. Chan*, A. Routtenberg and W.L. Klein. Neuroscience Program. Northwestern University. Evanston IL. 60308.**

Receptor phosphorylation may play an important role in the regulation of acetylcholine muscarinic receptors (mAChRs) as is the case for the nicotinic and adrenergic receptors. The muscarinic agonist carbamylcholine reduces the number of mAChRs and stimulates their phosphorylation in the heart. Also, phosphorylation of brain and heart mAChRs by cyclic AMP dependent protein kinase causes receptor inactivation. To assess the role of phosporylation in receptor regulation, we evaluated mAChRs as substrates for other key protein kinases. For this, we purified the receptor from chick brain to about 2000 pmol 3H-QNB binding sites per mg of protein by affinity chromatography in ABT-agarose. These prepartions of mAChRs were used as substrate for phosphorylation by protein kinases. Ca++/CM dependent protein kinase (Type II) phosphorylates a polypeptide with the same Mr as the 3HPrBCM labelled mAChR as determined by SDS-polyacrylamide electrophoresis. This observation suggests that Ca++/CM dependent protein kinase may phosphorylate mAChRs in vivo. In contrast, Protein kinase C causes no phosphorylation of purified mAChRs. We are now evaluating the role of Ca++/CM in the regulation by studying the binding properties of the phosphorylated mAChRs.

PROCESS OUTGROWTH, GROWTH CONES, AND GUIDANCE MECHANISMS I

108.1

CELL ADHESION MOLECULES IN THE DEVELOPING EIGHTH NERVE OF CHICKEN EMBRYOS. Sharon G. Hemond[1], Urs Rutishauser[2] and D. Kent Morest[1]. [1]Ctr. Neurological Sciences, Anatomy Dept., Univ. of Conn. Health Ctr., Farmington, CT 06032; [2]Case Western Reserve University, Cleveland, OH 44106.

In the maturation of the cochleovestibular ganglion, cellular interactions may play a role in the generation of specific innervation patterns of different auditory and vestibular endorgans. To address this, we are studying CAMs at the time of ganglion formation and early central and peripheral innervation, E2-E7, and report on two studies. First, immunocytochemistry with antibodies directed against NCAM and AxCAM(G4) shows that NCAM is found in the ganglion and its processes during early innervation and G4 is observed in the forming ganglion associated with initial process outgrowth and is maintained in processes during innervation. Second, we have developed an in vitro system in which neuroblasts, associated with the immature otocyst, extend processes which fasciculate. Aggregation of neurons is apparent. Immunocytochemistry of 2-3 day cultures shows that G4 is expressed by neurons; NCAM data are also suggestive of its expression on neurons.

The temporal and spatial expression of CAMs in situ suggests a functional role in the generation of specific innervation. The in vitro system is being used to assess this possibility. (Supported by NIH grants NS14354 and T32 NS07299)

108.2

NCAM POLYSIALIC ACID CAN REGULATE CELL-SUBSTRATE ADHESIVITY **J.L. Sunshine* and U. Rutishauser Case Western Reserve Univ. School of Medicine, Cleveland, OH**

Explants from embryonic day 7 chick spinal cord produce a fasciculated pattern of neurite outgrowth on a laminin substrate. The NCAM on the outgrowing neurites has a high content of polysialic acid (PSA), a unique carbohydrate moiety associated with modulation of NCAM function. Surprisingly, specific enzymatic removal of the PSA from NCAM during outgrowth resulted in substantial defasciculation of the neurites, although this had been expected to enhance NCAM-mediated fasciculation based on previous studies using DRG explant cultures. Furthermore, the defasciculation was unaffected by anti-NCAM Fab fragments, and was reversed by antibodies to the laminin substrate. Taken together, these data suggest that PSA can influence growth cone-to-substrate adhesion independently of NCAM function.

108.3

REGULATION OF L1 FUNCTION BY NCAM POLYSIALIC ACID ON DRG X NEUROBLASTOMA HYBRID CELLS A. Acheson, J. Dodd, T. Jessell and U. Rutishauser Case Western Reserve University School of Medicine, Cleveland, OH and Columbia University, New York, NY.

E17 rat DRG neurons X murine N18TG2 neuroblastoma hybrid cells express NCAM with a high content of polysialic acid (PSA) as well as the L1 cell adhesion molecule. Treatment of cells with endoneuraminidase N specifically removes PSA from NCAM without affecting L1, as assessed by immunostaining and immunoblot analysis. The presence of both cell adhesion molecules and PSA on the cell surface allows direct assessment of a potential hierarchy of function among these components. Untreated cells show little aggregation after 30 min at 37 °C. However, after specific enzymatic removal of PSA from NCAM, the aggregation rate increased (55% decrease in particle number), and half of the resultant aggregation was inhibited by anti-L1 Fab fragments. These data support the hypothesis that NCAM's PSA moiety can independently regulate the function of other cell adhesion molecules.

108.4

EXPRESSION OF THE CYTOPLASMIC DOMAIN OF 5B4/NCAM-180, VIA ITS cDNA, IN HETEROLOGOUS CELLS. Leland Ellis and Purita Ramos, HHMI and Dept.of Biochemistry, UTSWMC, Dallas, Tx.

Monoclonal antibody 5B4 recognizes a large (~185-255 kd) developmentally regulated membrane glycoprotein, whose expression on fetal rat neurons is coincident with neuronal sprouting. The primary sequence of the transmembrane and cytoplasmic domains of the 5B4 antigen, deduced from the nucleotide sequence of λgt11 cDNA clones which encode 5B4, reveals significant identity (75%) with the sequence reported by Edelman and collegues for chick NCAM-180. The cytoplasmic domain (391 aa; M_r 39284) has an unusual amino acid composition (52% proline, alanine, serine and threonine) with little predicted α or β secondary structure, and is of unknown function. To test the feasibility of expressing this domain independently as a soluble protein, with the goal of ultimately exploring the physical properties and interactions of this unusual molecule, we have engineered an expression plasmid designed to encode such a derivative. In Cos cells transfected with this 5B4 cDNA under the transcriptional control of a viral promotor, mAb 5B4 specifically recognizes a protein which migrates anomolously (M_r ~80000) on SDS-PAGE under denaturing and reducing conditions. The successful expression of the 5B4 cytoplasmic domain in heterologous cells now provides an experimental approach with which to assess the phenotype(s) of cells and animals which express this domain autonomously.

108.5

MONOCLONAL 12F8 ANTIBODY IDENTIFIES A SUBCLASS OF NCAM ACTIVE IN PROMOTION OF NEURITE OUTGROWTH. C. Lagenaur, J. Yip, and V. Lemmon. Depts. of Neurobiology, Anatomy & Cell Science and Physiology, Sch. of Med., Ctr. for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Rat Mab 12F8 recognizes a neuraminidase sensitive epitope on mouse and chick NCAM. Purified 12F8 antigen is an effective substrate in the nitrocellulose based neurite outgrowth assay described by Lagenaur and Lemmon, PNAS, 84:7753-7757, 1987. 12F8 attaches neurons from P6 mouse cerebellum and promotes fasciculated neurite outgrowth. We have compared the distribution of the 12F8 epitope with total NCAM recognized by Mab 1A6 in developing chick. The distribution of 1A6 and 12F8 are initially similar but by stage 44 12F8 becomes most prominently expressed in sympathetic ganglia while 1A6 is widespread throughout the nervous system. Unlike 1A6, 12F8 is never found in notochord. The developmental distribution of 12F8 epitope containing NCAM may represent the appearance of sites favorable for axon outgrowth. Supported by: Basil O'Connor Grant from March of Dimes 5-518; NIH EY05285 and NS23916.

108.6

cDNA SEQUENCE ANALYSIS OF GRASSHOPPER FASCICLIN II PROTEIN: HOMOLOGY WITH IMMUNOGLOBULIN SUPERFAMILY. A.L. Harrelson and C.S. Goodman, Dept. of Biochem., Univ. of California, Berkeley, CA, 94720.

To elucidate the mechanisms that control cell recognition during neuronal development, we have been studying the developing nervous system of insects. Previous studies identified three different surface glycoproteins, called fasciclins, which are expressed on subsets of axon fascicles and whose distribution suggests a possible role in labeling developing axon pathways. One of these proteins, fasciclin II, is expressed on the surfaces of longitudinal axon fascicles in the grasshopper embryo. We isolated and sequenced a 3.5 kb cDNA which encodes for nearly the complete fasciclin II protein. The deduced amino acid sequence of the protein contains a putative transmembrane domain with a C-terminal cytoplasmic domain of about 100 amino acids. The most interesting feature of fasciclin II is the presence of five repeated domains in the extracellular region, each containing cysteine and other amino acid residues in a precise spacing similar to that seen in the immunoglobulin superfamily. In particular, fasciclin II has highest amino acid homology to the neural cell adhesion molecules in this family, including N-CAM, MAG, and L1/Ng-CAM/NILE (Schachner et al., in press). Seeger and Kaufman (unpublished) have shown that the amalgam gene in Drosophila encodes a protein with IgG-like domains which is expressed in the developing CNS. The finding of this 2nd IgG-like molecule in the developing insect CNS raises the issue of whether the initial evolutionary role for such proteins was in adhesion and cell recognition, and moreover, raises the question of how many such proteins are present in the developing insect nervous system. To begin a genetic analysis of these proteins, we are presently searching for the Drosophila homologue of fasciclin II.

108.7

TEMPORAL EXPRESSION OF TAG-1 AND L1 GLYCOPROTEINS ON CENTRAL AND PERIPHERAL NEURONS IN VITRO. D. Karagogeos*[1,2], J. Dodd[1] and T.M.Jessell[1,2] (SPON: J. Wood). Center for Neurobiology[1] and Howard Hughes Medical Institute[2] Columbia University, New York, NY 10032

The axons of developing vertebrate neurons express several surface glycoproteins that have been implicated in axon extension and fasciculation. Studies in the developing rat spinal cord have shown that two of these proteins, TAG-1 and L1, are segregated spatially and temporally on sensory, motor and commissural (C) neurons (Dodd et al. 1988, Neuron 2). The initial expression of TAG-1 precedes that of L1 on these neurons. TAG-1 expression is transient whereas L1 expression persists throughout development but appears on restricted domains of motor and C axons.

The regulation of TAG-1 and L1 expression on spinal axons may be cell autonomous or influenced by the environment of the developing axon. To investigate the factors that regulate TAG-1 and L1 we have examined their expression on sensory and spinal neurons in dissociated cell culture. About 40-50% of E13 dorsal spinal cord neurons express TAG-1 on their surface when plated on astrocyte monolayers or on laminin. Expression of TAG-1 decreases with time in culture and by 72 hrs in vitro TAG-1 is not detectable. In contrast,most of E13 dorsal spinal cord neurons do not express L1 for the first 24 hrs in vitro although the protein is detectable on the majority of neurons by 72 hrs and expression is maintained with time in culture. In vivo, C neurons stop expressing TAG-1 by E16-17. Neurons dissociated from the dorsal spinal cord of E19-P2 rats regenerate processes in vitro but do not appear to express TAG-1 although L1 is expressed soon after plating. TAG-1 expression by C neurons therefore appears to be confined to the phase of initial axon extension and does not reappear on re generating axons. In contrast, DRG neurons that have stopped expressing TAG-1 in vivo re-express the protein on regenerating axons in vitro, suggesting that there are differences in the regulation of TAG-1 expression on central and peripheral neurons .

108.8

DISRUPTION OF COMMISSURAL AXON GUIDANCE IN THE ABSENCE OF THE MIDLINE FLOOR PLATE. P. Bovolenta, T. M. Jessell[1] and J. Dodd. Center for Neurobiology and [1]Howard Hughes Medical Institute, Columbia University, New York, N.Y. 10032.

Recent studies have suggested that floor plate epithelial cells of the embryonic rodent spinal cord can influence the guidance of commissural axons by: 1. releasing a diffusible factor(s) that promotes directed growth of commissural axons (Tessier-Lavigne et al., Placzek et al., this meeting). 2. regulating glycoprotein expression on commissural axons (Dodd et al. 1988, Neuron 1). 3. providing an adhesive substrate for axons joining the ventral funiculus (Dodd et al., ibid).

To examine further the contribution of the floor plate to commissural axon guidance in vivo we have studied the trajectory of commissural axons in mouse mutants in which the floor plate is abnormal or absent. In one such mutant, Danforth's short tail (Sd), the first observable abnormality is the absence of the notochord in caudal regions. At segmental levels in which the notochord is not detected, the floor plate of the overlying spinal cord is also absent. In regions of the spinal cord where the floor plate is missing , commissural axons project ventrally via a lateral route that contrasts with the medially directed trajectory observed in unaffected segments of the spinal cord or in normal litter mates. The growth cones of commissural axons arriving at the ventral limit of the spinal cord do not appear to cross the midline but project out into the surrounding ventral mesenchyme. At later developmental stages, these axons form a large fascicle, which may also contain misrouted motor axons. Axons within the fascicle continue to express the glycoprotein TAG-1. In normal embryos TAG-1 expression ceases on commissural axons as they cross the midline and enter the ventral funiculus. In mutant embryos the ventral funiculus is perturbed in affected regions of the spinal cord. Immunocytochemical studies indicate that the basal lamina is also disrupted at the ventral midline of affected neural tube segments.

These results support the idea that floor plate epithelial cells contribute to the guidance of commissural axons.

108.9

ANTI-IDIOTYPE ANTIBODIES TO THE LIMBIC SYSTEM ASSOCIATED MEMBRANE PROTEIN (LAMP) RECOGNIZE SEVERAL PROTEINS IN THE DEVELOPING BRAIN.

P. Levitt, S. Fisher*, A. Zacco*. Dept. Anatomy, Med. Coll. Pa., Philadelphia, Pa. 19129

The limbic system associated membrane protein (LAMP) is a developmentally regulated 64kd protein that mediates, in part, the ability of axons from limbic neurons to recognize and innervate appropriate targets. The molecular components that may interact with LAMP to perform this function are unknown. In order to identify potential LAMP receptors or interactive molecules, we used an anti-idiotype strategy to generate a series of monoclonal antibodies against anti-LAMP that theoretically could be used to identify such receptor molecules. Three of 45 anti-LAMP positive clones cross-reacted with brain. Western blot analysis, performed on fresh preparations in the presence of protease inhibitors, revealed that one antibody recognized many protein bands ranging from 15-200kd. The other monoclonals recognize only 2 of these protein bands, at approximately 75 and 65kd. Immunocytochemical staining of rat hippocampal cultures revealed that cells with neuronal morphology (including their processes) are immunoreactive. Double staining with anti-LAMP demonstrated that a subset of cells expressed both proteins. Ongoing immunoaffinity purification of these newly identified proteins will allow direct testing of binding between these molecules and LAMP. Supported by NSF grant BNS8519647, March of Dimes Research Grant 1-919.

108.10

CHARACTERIZATION OF THE P8-4 ANTIGEN, A NOVEL NEURAL CELL ADHESION MOLECULE.

W. Chuang and C. Lagenaur. Dept. of Neurobiology, Anatomy and Cell Science, Ctr. for Neuroscience, Univ. of Pittsburgh, Sch. of Med., Pittsburgh PA 15261.

Adhesive properties of isolated neural cell surface molecules can be assessed using a recently described technique that immobilizes proteins on nitrocellulose coated petri plates. (Lagenaur, C. and Lemmon, V., PNAS, 84:7753-7757, 1987). Using this method, cell surface antigen P8-4, isolated from mouse brain membranes has been identified as a new neural cell adhesion molecule. Immunoaffinity purified P8-4 contains three proteins of 200, 80, & 60 kD. P8-4 is not cross reactive with N-CAM, L1/NILE, and does not bear the HNK-1 epitope. This antigen is found on cultured cerebellar neurons and a subclass of unidentified flat cells. P8-4 is not found on oligodendrocytes or GFAP positive astrocytes. In the cell adhesion assay, P8-4 attached neurons and promoted neurite outgrowth. Glial cell attachment was also observed. Monoclonal anti-P8-4 detects antigen only in the floor plate of mouse embryonic spinal cord from E9 to P1. Beginning at P2, P8-4 becomes widely expressed throughout the CNS and is retained throughout adulthood. Our data suggest that P8-4 is a novel adhesion molecule that may be important in the early CNS development. Supported by: Basil O'Connor grant, March of Dimes; Culpepper Fellowship.

108.11

MONOCLONAL ANTIBODY 8A2 RECOGNIZES A CLASS OF *O*-ACETYLATED GANGLIOSIDES IN THE CHICK RETINA THAT PARTICIPATE IN AXON OUTGROWTH.

M. Pierce*, J. Drazba* and V. Lemmon. Dept. of Anatomy and Cell Biology, Univ. of Miami, Miami, FL 33101 and Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Monoclonal antibody 8A2 binds to axons in the chick nervous system (Soc. for Neurosci. Abs. 385.2, 1987). Using TLC-immunostaining techniques, the antigens in E10 retina display solubilities similar to those of gangliosides, and upon DEAE-Sephadex chromatography, elute almost entirely in the disialylated fraction. TLC-immunostaining of this fraction after HPLC using a NH_2-LiChrosorb column demonstrates binding to 1 major band. Treatment of the fraction with alkali abolished binding to all bands while periodate did not. This suggests that a common structural feature of these gangliosides is a sialic acid residue which is *O*-acetylated, probably at the 9-position. We are using high resolution NMR to complete structural analysis of the epitope. Using a cell culture system described in another abstract (Drazba and Lemmon) we have found that the addition of this antibody to culture medium inhibits neurite outgrowth on laminin and glia. Supported by NIH EY06968 and EY05285 and MOD 1-979.

108.12

ALTERED NEURITE OUTGROWTH IN MUTANT PC12 CELLS WITH REDUCED LEVELS OF SPECIFIC NEURONAL SURFACE GLYCOPROTEINS.

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As an alternative to the antibody perturbation approach in the study of the function of neuronal surface proteins, we have used specific mutagenesis to produce mutant cell lines deficient in NILE (also known as L1 and Ng-CAM). Mutagenized PC12 cells were selected for NILE deficiency by using complement-mediated lysis in conjunction with an anti-NILE monoclonal antibody, ASCS4. 14 clonal cell lines isolated from this selection were examined for their levels of NILE and several other surface proteins by immunocytochemistry and immunoprecipitation and SDS-PAGE. Three classes of mutants with low levels of ASCS4 binding were found: i) 1 mutant with an altered form of NILE, present at normal levels, ii) 8 mutants with low levels of NILE and normal levels of other proteins, and iii) 5 mutants with low levels of NILE and altered levels of N-CAM. Eight of 9 mutants examined grow processes in response to NGF or FGF. The mutant of the first class grows long, thin neurites similar to normal PC12 cells. Mutants of the second and third classes, however, grow neurites that are much shorter and thicker than wild type cells. These results suggest that NILE may be important in determining morphology. It is also interesting that alterations in N-CAM levels frequently occur when NILE is reduced.

108.13

LAMININ PROMOTES AXONAL BUT NOT DENDRITIC GROWTH IN RAT SYMPATHETIC NEURONS IN VITRO.

P.J. Lein and D. Higgins. Dept. Pharm., State Univ. of N.Y., Buffalo, NY 14214

We have characterized the effects of laminin on the morphology of neurons from embryonic rat superior cervical ganglia. Neurons were grown on polylysine-coated coverslips with or without substrate-bound laminin in the absence of serum and nonneuronal cells. In short-term ($\leq$24 hr) culture, laminin has a potent neurite-promoting effect, inducing increases in the number of neurites/cell and in the length and branching of processes. Data from long-term studies ($\geq$2 wk) indicate that these laminin-induced increases in the number of processes/cell and in branching are maintained.

The identity of the neuronal processes induced by laminin was determined in long-term cultures. Axons were distinguished from dendrites on the basis of intracellular dye injections, ^{3}H-norepinephrine uptake, and the immunocytochemical localization of dendrite-specific (microtubule associated protein 2, dephosphorylated M subunit of neurofilaments) and axon-specific antigens (synaptophysin, phosphorylated form of the M and H subunits of neurofilaments). The majority (> 75%) of neurons grown on laminin extend only axons. In contrast, when sympathetic neurons are grown for the same time period in the presence of a basal lamina extract, all neurons (100%) form both axons and dendrites. Thus, the neurite-promoting activity of laminin appears to be axon-specific. (Supported by March of Dimes Predoctoral Fellowship and NSF grant BNS 851960).

108.14

NEURITOGENESIS ON DIFFERENT COLLAGEN SUBSTRATA.

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We have earlier demonstrated that soluble macromolecules extracted from the visual areas are essential for trophic support of retinal neuritogenesis. In the present experiments we studied collagen types I-IV in combination with full trophic support stimulation. Neurite outgrowth from retinal explants (White Leghorn, E6) grown on collagenous substrates was studied. The substrata were prepared (native or denatured in 100 µl drop, overnight at 4°C) in the centre of the plastic dishes. Collagen types I, II and III were also studied as hydrated matrices in the centre of the dish. The explants were cultured with optic lobe at a protein concentration of 500 µg/ml. The cultures were then incubated for 4 days in a CO_2 incubator at high humidity and outgrowth controlled every~24 h in phase contrast in an inverted microscope. The behavior shown by explants was different in each case. Attachment and survival(studied by Trypan Blue exclusion) of the retinal explants were good on all types of collagen including the denatured (30 minutes at 56°C) forms. However, neurite formation occurred only on layer of collagen I and III and profound elongation occurred on these types of collagen in their hydrated forms. No outgrowth was present on collagen types II and IV. From the behavior observed on collagen types I and II it can be suggested that a receptor mechanism is involved here.

108.15

MENINGEAL CELLS FROM EMBRYONIC RAT SECRETE AN AUTONOMOUS PATTERN OF COMPLEXED OR FREE LAMININS AND FIBRONECTINS WITH DIFFERENT NEURITE PROMOTING ACTIVITIES. H.P. Matthiessen*, C. Schmalenbach*, and H.W. Müller. Molecular Neurobiology Laboratory, Department of Neurology, University of Düsseldorf, F.R.G. (SPON:S. Thanos)

In order to characterize the vigorous neurite outgrowth promoting activities (NPA) in meningeal cell conditioned media (M-CM) from embryonic rat we separated serum free hormone supplemented M-CM by FPLC on the anion exchange column Mono Q and tested the NPA of the polylysine binding fraction in a quantitative bioassay using hippocampal neurons from embryonic rat. We could determine two main activity peaks. Using antibodies against the substrate adhesion molecules laminin (LN) and fibronectin (FN) we could correlate a smaller NPA eluted at 250-350 mM NaCl with the highest immunoreactivity for free FN and a smaller one for free LN. The major activity peak, however, always coincided with the highest anti-LN and a smaller anti-FN immunoreactivity. This material which was highly sulfated was eluted at about 1 M NaCl and possibly consisted of proteoglycan complexes with LN or FN, respectively. It is noticeable that we could exclusively detect the ß-chains of LN within both peaks of NPA. The distribution of LN and FN in free or complexed forms is different in CM of meningeal cells and astrocytes, respectively. Supported by the DFG (Mu 630/3-1). C.S. is recipient of a fellowship from BMFT.

108.16

ANTIBODIES TO LAMININ AND THE 140KD FIBRONECTIN RECEPTOR UNCOUPLE NEURITE OUTGROWTH FROM CLOCKWISE GROWTH. Y. Tseng* and P. Grant, Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403.

As Xenopus retinal ganglion cell axons mature during development, they begin to express clockwise growth between stages 37 and 50, when grown in serum free media containing laminin or fibronectin. To explore the neurite-substrate interactions responsible for the development of neurite growth patterns, a commercially available monoclonal antibody to laminin and a polyclonal antibody to a putative 140kd fibronectin receptor (generously supplied by Dr. K. Yamada) were employed. Dilutions of the antibodies were added to wells in culture dishes coated with polylysine, in a medium containing laminin or fibronectin (20-30 ug/ml). As controls, dilutions of mouse ascites fluid or rabbit Igg were used. Fragments of stage 25 optic vesicles (whose neurites never express clockwise growth) and stage 50 tadpole retinas were explanted and the patterns of neurite outgrowth and clockwise growth were assayed over six days. For both antibodies, dilutions were obtained which exhibited no, or only a modest inhibition of neurite outgrowth at both stages, while completely inhibiting clockwise bundle patterns in stage 50 neurites. The results suggest that more than one domain on ECM substrate molecules may be involved in retinal neurite outgrowth. (Supported by NSF grant BNS-85 16317).

108.17

LAMININ MEDIATES AGGREGATION OF PC12 CELLS ON A 3-DIMENSIONAL RECONSTITUTED BASEMENT MEMBRANE. J. Keshmirian* and S. Carbonetto, McGill Univ. Centre for Research in Neuroscience, 1650 Cedar Ave., Montreal, Canada H3G 1A4.

During development neural crest cells migrate through an extracellular matrix which strongly influences their aggregation into dorsal root and sympathetic ganglia. PC12 cells (a neural crest derivative) seeded on a reconstituted basement membrane (RBM) gel form aggregates interconnected by process-bearing cells. By 3 days these networks coalesce into large aggregates, which in the presence of NGF, extend a halo of nerve fibers and resemble dorsal root ganglia in culture. PC12 cells, when seeded onto gel of LAM alone behave essentially the same, whereas crude COL gels fail to induce aggregation. The extent of aggregation depends on cell density and is enhanced by NGF. Antisera to LAM or 3A3, a monoclonal antibody against a LAM/COL receptor (Turner et. al, J. Cell Biol. 105:137a, 1987) inhibit the REM gel-mediated aggregation in a dose-dependent fashion. Time-lapse studies indicate that cell movement leading to aggregation on RBM gel is accompanied by extensive blebbing as well as extension of processes (5-10 µm) that attach and pull together neighbouring cells.

Our results suggest that polymerized LAM in RBM is responsible for aggregation of PC12 cells. This homogenous cell line may prove a useful model for analyzing the cellular and molecular events in neural cell aggregation.

108.18

GLIAL-ASSOCIATED PROTEOGLYCAN 5B12 IS EXPRESSED UPON SUBSETS OF NERVE PROCESSES IN THE EMBRYONIC AND ADULT INSECT. M.R. Meyer*, P. Brunner*, and J.S. Edwards (SPON: R.M. Longley). Department of Zoology, NJ-15, University of Washington, Seattle, WA 98195.

Monoclonal antibody (MAb) 5B12 recognizes a M_r 185 kD chondroitin sulfate-like proteoglycan in the adult cricket that is associated with glial cells of the glial lacunar system (Meyer et al., *Soc. Neurosci. Abstr.* 13:1143, 1987) and glia which juxtapose sensory neurons in the periphery. In the embryo, 5B12 is expressed peripherally as a component of the basal lamina and upon processes of glial cells located in cercal sensory appendages. In both the embryonic periphery and adult glial lacunar system, antigen 5B12 is localized within the extracellular matrix (ECM).

In addition to the glial and ECM distribution of the antigen, we observe 5B12 in close association with distinctly neuronal elements in the embryonic and adult nervous system. In the early embryonic CNS, MAb 5B12 binds in a segmentally repeated fashion to both anterior and posterior commissural tracts. However, by ca. 50% development, expression is highly restricted to only a small subset of nerve fibers located at the ventro-anterior margin of the anterior commissures where 5B12 may be localized on the neuronal cell surface.

The adult nervous system contains distinct peripheral and central 5B12-immunoreactive fiber tracts. In the terminal abdominal ganglion, 5B12 is expressed in sensory, but not motor nerves, and labelling is associated with discrete neural elements within ganglionic commissures. We are correlating 5B12 expression with identity of specific embryonic and adult neurons in order to determine the role of the proteoglycan in the organization of the nervous system. Supported by NIH #NS-07778.

108.19

SCHWANNOMA CELL DERIVED INHIBITOR OF THE NEURITE-PROMOTING ACTIVITY OF LAMININ. D.Muir*, E. Engvall*, S. Varon, M. Manthorpe. (Spon: C. Wiley) Dept. of Biology, M-001, Univ. of Calif. at San Diego, La Jolla, CA. 92093

We have previously reported (J. Neurochem. 37:759-767,1981) that medium conditioned by rat RN22 schwannoma cells contains a polyornithine-binding neurite promoting activity (NPA) as well as another activity that interfered with neuritic growth. We and several other groups have demonstrated that the NPA of conditioned media is due to the glycoprotein laminin (LN). Here we have undertaken a study of the inhibitor of neuritic growth. Medium from radiosulfate-labeled RN22 cultures was fractionated by ion-exchange chromatography and the fractions and their western blots examined for NPA, LN and entactin immunoreactivities and for radioactivity. NPA was always associated with LN which co-eluted with entactin. We found fractions containing significant amounts of LN and entactin that were devoid of NPA. These inactive fractions also were inhibitory when mixed with isolated rat RN22 LN or yolk sac LN. The peak inhibitory fractions are highly anionic, heavily sulfated, have a high buoyant density and their inhibitory activity is destroyed by heparitinase. The inhibitor is also prevented from inactivating LN by; a) a monoclonal anti-LN antibody (2E8) which binds to the cross region of the LN molecule and does not block NPA,and b) polyclonal antibodies against entactin (a protein known to bind near the cross region of LN) also which do not block NPA. We propose that the NPA of LN is subject to regulation through association with entactin and proteoglycan. Supported by NSF BNS86-17034.

108.20

A *XENOPUS* GLIAL CELL LINE PRODUCES AN EXTRACELLULAR MATRIX WITH NEURITE OUTGROWTH PROMOTING ACTIVITY. D.S. Sakaguchi, C.R. Coffman*, and W.A. Harris, Dept. of Biology, B-022, UCSD, La Jolla, CA 92093

A glial cell line (XR1 cell line) isolated from *Xenopus* retinal neuroepithelium serves as an excellent substrate for neurite outgrowth from embryonic retinal explants. XR1 conditioned cell-free substrates were prepared to examine whether outgrowth-promoting activity was also associated with the extracellular matrix (ECM). Substrates of XR1 cells grown on collagen were still capable of promoting outgrowth following hypoosmotic shock and chemical extraction with Triton X-100. Collagenase and trypsin were used to examine the proteolytic sensitivity of components of the ECM and the promotion of neurite outgrowth. Both enzymes were effective in eliminating the outgrowth promoting activity of the XR1 conditioned substrates. Thus, although collagen alone was ineffective in supporting outgrowth, it may play an indirect role by acting as an effective support for attachment of other ECM components. Immunological and biochemical studies suggest that neither fibronectin nor laminin plays a role in this ECM promoted outgrowth. To identify molecules which mediate growth cone-glial cell interactions, polyclonal antibodies were generated against membrane components of the XR1 cells. An antiserum (NOB1) blocked neurite outgrowth on conditioned XR1 substrates in a dose dependent fashion. Identification of the active components associated with the neurite outgrowth promoting activity is currently being attempted using affinity depletion of the NOB1 antiserum and the production of monoclonal antibodies.

109.1

PATTERNS OF 2-DEOXYGLUCOSE UPTAKE REFLECT THE NEURAL PROCESSING OF LORDOSIS-INDUCING TACTILE STIMULI IN HAMSTERS. O. R. Floody and R. D. Lisk*. Dept. of Psychology, Bucknell Univ., Lewisburg, PA 17837 and Dept. of Biology, Princeton Univ., Princeton, NJ 08544.

Semi-quantitative [^{14}C]2-deoxyglucose (2DG) autoradiography was used to map the neural responses of female hamsters to lordosis-inducing flank stimuli. Specifically, the manual stimulation of one flank was used to maintain estrous females in lordosis for 20 min after an i.v. injection of 200 μ Ci/kg of 2DG. Hemispheric differences in 2DG uptake then were sought in brain nuclei previously implicated in the programming of lordosis, or in the processing of somatosensory or hormonal influences on this response.

The responses to lateralized flank stimulation included reliable contralateral elevations in 2DG uptake in the tectum, the dorsal mesencephalic central gray (dCG), and the ventral posterior lateral nucleus of the thalamus (VPL). The first two of these effects are consistent with much previous evidence implicating these areas in the mediation of somatosensory influences on lordosis and suggesting that these influences are strongly lateralized until at least this stage of neural processing. In contrast, elevated activity on the part of the VPL probably is not crucial for lordosis. Nevertheless, this effect, together with the absence of lateralized 2DG uptake by the gracile nuclei, suggests that lordosis-controlling somatosensory inputs to the brain traverse the anterolateral, not dorsal, spinal columns. On the other hand, the ability of dorsal column lesions to disrupt lordosis (Rose et al., Physiol. Behav., 31: 801, 1983) suggests that lordosis draws upon both of these systems, perhaps shifting from a reliance on dorsal column to anterolateral projections as lordosis duration increases. (Supported by MH-08070 and MH-33191 from the N.I.H. and by PCM-7812582 from the N.S.F.).

109.2

INHIBITION OF LORDOSIS IN INTACT FEMALE RATS FOLLOWING ADMINISTRATION OF SCOPOLAMINE. C. S. Menard and G. P. Dohanich. Tulane Univ., New Orleans, LA 70118.

Cholinergic antagonists, such as scopolamine (SCOP) and atropine, have been found previously to inhibit lordosis in ovariectomized rats, primed with estrogen and progesterone. The present study further examines this effect using intact cycling female rats. Cycling was determined by daily monitoring of sexual behavior and vaginal cytology. In Experiment 1, 19 female rats received, during natural estrous, bilateral ventricular infusions of either .5 ul saline solution, 10 ug SCOP/.5 ul saline solution, or 20 ug SCOP/.5 ul saline solution. Administration of both levels of SCOP was found to significantly inhibit lordosis behavior ($p < .0001$). In Experiment 2, 14 animals received, during natural estrous, IP administration of either .2 ml saline solution or 1 mg SCOP/.2 ml saline solution. Likewise, systemic administration of SCOP was found to significantly inhibit lordosis behavior ($p < .0001$). These results lend further support for the cholinergic regulation of sexual behavior. They also suggest that lordosis behavior induced in ovariectomized rats may be equivalent to that seen in intact females.

109.3

LORDOSIS RELEVANT PATHWAYS NEAR THE VENTRAL TEGMENTUM IN FEMALE HAMSTERS. E. Kouri* and J.F. DeBold. Department of Psychology, Tufts University, Medford, MA 02155.

In addition to the ventromedial nucleus of the hypothalamus, the ventral tegmental area (VTA) is an important site of progesterone action in the control of sexual receptivity in estrogen-primed female hamsters.

We investigated the connections of the VTA that are critical for progesterone's behavioral action by disrupting neural pathways with stereotaxically placed 4mm knife cuts. We used three different types of knife cuts: 90° horizontal cuts dorsal to the VTA, coronal cuts anterior to the VTA and cuts bi-lateral to the VTA in the sagittal plane. After an eight-day recovery period ovx. animals that did not show motor side-effects or weight loss were injected with 10 ug EB and then 500 ug P. The total lordosis duration (TLD) of experimental animals was compared to that of animals with appropriate sham cuts.

Knife cuts placed just dorsal to the VTA had the greatest inhibitory effect on TLD. The coronal cuts anterior to the VTA caused a moderate decrease in TLD, but there was no significant effect of the sagittal bi-lateral cuts.

The dorsal cuts disrupted dorsal afferents and efferents including those from the habenula and deep tectum. These connections may be required for the integration of sensory and hormonal stimuli that lead to the lordosis response.

109.4

Differential Roles of Alpha-1 and Alpha-2 Noradrenergic Receptors in Regulation of Lordosis Behavior in Guinea Pigs. P. A. Vincent, J. E. Thornton, H. H. Feder. Inst of Animal Behav, Rutgers Univ, Newark, NJ 07102

We examined whether blockade of alpha-1 and alpha-2 noradrenergic receptor subtypes has differential effects on hormone-facilitated lordosis behavior. All animals were given free estradiol (E2) at hr 0 and hr 28, followed by progesterone (P) at hr 39. Experiment 1: Animals were given a single injection of either the alpha-1 antagonist prazosin or the alpha-2 antagonist idazoxan (5 mg/kg, s.c.) 30 min before the E2 at hr 0, 30 min before the E2 at hr 28 or 30 min before E2 injection at hr 0 and hr 28. Prazosin, but not idazoxan, significantly blocked lordosis under each condition. Experiment 2: When idazoxan was given as repeated injections (i.e., 30 min prior to and 60 and 120 min after both of the E2 injections) blockade of lordosis still did not occur. Experiment 3: When idazoxan was given as a single injection to estrogen-primed guinea pigs at hr 38 (1 hr prior to the P) or at hr 44 (5 hr after P) lordosis was blocked only in those animals that received idazoxan at hr 44 (hr 44 coincides with the usual time of onset of lordosis). These data suggest that noradrenergic transmission through alpha-1 receptors mediates the hormone-priming process leading to lordosis facilitation. Apparently, transmission through alpha-2 receptors is not involved in the hormone-priming process but may mediate sensory input to hormone-primed neurons.

109.5

Identification of Cholinergic Cell Groups that Facilitate Sexual Behavior in Female Rats

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Estrogen facilitation of sexual receptivity in the female rat depends, in part, upon cholinergic stimulation of muscarinic receptors in the hypothalamus and/or the midbrain central gray. In the present study we provide evidence to suggest that the cells which produce the endogenous acetylcholine for this neuro-behavioral system are located in the laterodorsal tegmental nucleus (LDT) and in the pedunculopontine tegmental nucleus (PPT). Electrolytic lesions in either region completely abolished estrogen-progesterone induced receptivity. However, cholinergic agonists (eserine or oxotremorine), microinjected into the lateral ventricles, restored lordosis behavior in lesion females given low doses of estrogen. Both the LDT and PPT have been shown to contain choline acetyl transferase (CAT) staining cells (Satoh, et.al. 1983, Brain Res. Bull.). It is possible that these cell groups provide the cholinergic input necessary for estrogen facilitation of sexual behavior.

109.6

HYSTERECTOMY COUPLED WITH CERVIECTOMY SUPPRESSES RECEPTIVITY FOLLOWING ESTRADIOL BENZOATE PLUS PROGESTERONE IN RATS. J.A. Witcher*, C.F. Donahue*, W. Michener*, and N.T. Adler. Dept Psych, Univ Pennsylvania, Philadelphia, PA 19104

This study investigates how the female reproductive tract may interact with the dose-dependent, facilitatory actions of the ovarian steroids to permit receptivity. The female reproductive tract was altered in three surgical groups; (1) ovaries were removed sparing the uterine horns and cervix (OVX), (2) ovaries and the uterine horns were removed while sparing the cervix (HYS), and (3) the ovaries, the uterus, and the cervix were removed (CVX). Three weeks after surgery, rats were treated with a range of estradiol benzoate (EB) doses plus progesterone (P4). For 5 consecutive weeks females were treated with a 4-day regimen: EB on days 1-3, plus P4, on day 4. During weeks 1-5; 0.175, 0.175, 0.35, .5 and 1.0 ug EB respectively, were administered. On day 4, P4 (0.5 mg) was injected 4-6 hr before testing for receptivity. During behavioral testing a male was allowed 10 mounts while incidence of lordosis was observed, and a lordosis quotient (LQ) was calculated. All groups displayed a dose-dependent response in LQ to the range of EB doses used. CVX rats displayed a lower LQ than OVX and HYS rats following 0.35 ug EB priming. Since all groups displayed an EB dose-dependent response of receptivity, the suppressed LQ in CVX rats indicates a possible regulatory role for the cervix. Following ovarian hormone exposure the cervix may act as a "filter" for the CNS by modulating reproductively relevant somatosensory stimulation. (Support NIH HD04522 to NTA)

109.7

ELECTRICAL STIMULATION OF THE MIDBRAIN ELICITS SHORT- AND LONG-LATENCY PROCEPTIVE PRESENTING IN THE FEMALE MONKEY. S. Aou*, Y. Oomura, A. Takaki*, E. Okada* and Y. KOYAMA* (SPON: K. Yamaguchi). Natl. Inst. Physiol. Sci., Okazaki 444, Japan.

Recently we have reported that single train electrical stimulation (50 Hz, 2.5 sec) of the ventromedial nucleus of the hypothalamus elicits proceptive presenting (active solicitation to a male partner) with latency ranged from 1.3 to 17.7 s in the female monkey (Koyama, Y. et al. Brain Res., 446: 199, 1988). The midbrain central gray and the adjacent tegmentum are known to have close connection with the hypothalamus. Since they are involved in regulation of sexual behavior in various mammalian species, we studied effects of midbrain electrical stimulation (0.2 ms, 50 Hz, 2.5 sec, 50-500 μA) on sexual behavior. Two ovariectomized, estrogen-treated female rhesus monkeys and their male partners were kept and used in accordance with the NIH Guide (1985). Two types of presenting responses were evoked after stimulation of the ventral part of the central gray and the adjacent tegmentum. One was elicited with short-latency (≤15 s) and was similar to that in case of hypothalamic stimulation. The other, with longer latency (>15 s), included long-lasting period of repetitive presenting. The present study suggests that at least two neural components are involved in regulation of proceptive presenting in the midbrain of the female monkey.

109.8

FACILITATION OF FEMALE SEXUAL BEHAVIOR BY THE PROGESTIN RECEPTOR ANTAGONIST RU 38486. E.T. Pleim*, P.J. Cailliau*, M.A. Weinstein*, A.M. Etgen[1], and R.J. Barfield. Dept. Biol. Sci., Rutgers Univ., Piscataway NJ and [1]Albert Einstein Coll. Med. Bronx NY

The progestin receptor antagonist RU 38486 (RU 486) was tested for facilitative effects on female receptive behavior in 44 ovariectomized Long-Evans rats primed (sc) with a low dose (2 μg) of estradiol benzoate. 0, 0.5, 1.6, or 5.0 mg of RU 486 (sc) was administered 48 hours after estrogen priming. Lordosis quotient (LQ) and lordosis score (LS) were assessed 4 hours after RU 486 administration in a 10 mount test with stud male rats. 5 mg RU 486 acted as a weak facilitator of female sexual behavior. Lordosis measures were elevated above control levels in a dose-dependent manner, but subjects did not display the high levels of proceptive behavior seen with progesterone (P) facilitated receptivity.

Because in previous studies of RU 486 inhibition of P facilitated receptivity RU 486 facilitation was not seen, we tested 5 mg RU 486 *vs* vehicle pretreatment followed after one hour by 500 μg P or sesame oil (replicating RU 486 inhibition studies) or a no-injection, no handling condition (as in the dose-response study). In all RU 486 pretreatment conditions, considerable receptivity was seen, though lordosis measures were lower in the RU 486 + P group than in the vehicle + P condition, and little proceptive behavior was seen.

These studies indicate that although an excess of RU 486 diminishes P facilitation, the progestin receptor antagonist has significant facilitating properties of its own. Further study of P *vs* RU 486 induced receptivity and proceptivity, and the interaction of these steroids with neuronal steroid receptors, may help elucidate the mechanism(s) through which progesterone acts to affect female sexual receptivity.

109.9

GABA MEDIATION OF LORDOSIS IN THE RAT. M.M. McCarthy, K.F. Malik (SPON: Jennifer Swann) Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.

The role of GABA in mediating lordosis in the rat remains unclear. Conflicting reports suggest that enhanced GABAergic activity in the HYP may increase, decrease or have no effect on lordosis. We report here that GABA does play a role in mediating lordosis and has differential effects in the VMN and POA. Adult, ovx'd female Sprague-Dawley rats were bilaterally cannulated into the VMN or POA. After priming with EB (10ug;48hr) and P (1.0mg;4hr) females were pretested with a male for 10 mounts. Females with an LQ>60% were then infused with saline or bicuculline (BI) in the VMN (10ng/.25ul/cannula) or POA (10ng/.5ul/cannula). There was a significant decrease in LQ in females receiving BI into the VMN at 10 and 30min post-inj. (LQ=0% at 10', LQ=40% at 30', t-test;$p<.01$). Females receiving BI in the POA did not show a significant decrease in LQ post-inj.In a second experiment, females in EB-induced heats (30ug/day for 5 days) received BI in the VMN (10ng/.25ul/cannula) and showed a similar pattern of inhibition (LQ at 10'=8.8%; 30'=48%, $p<.01$). In a third experiment, females in EB+P heats received muscimol in the POA (25ng/.25ul/cannula) resulting in significant inhibition of lordosis (LQ at 10'=34%;$p<.01$:LQ at 30'=73%;$p<.05$). These results indicate that high GABAergic activity in the VMN facilitates lordosis, whereas high GABAergic activity in the POA inhibits this behavior. Supported by grant NIH-HD04467 to Harvey H.Feder

109.10

INHIBITION OF LORDOSIS IN RATS AND HAMSTERS BY MUSCARINIC ANTAGONISTS. G. Dohanich, R. Holland*, M. McMullan*, A. Piazza*, and D. Cada*. Dept. of Psychology, Tulane Univ., New Orleans, LA 70118.

Hormonal control of lordosis may be mediated by central neurotransmitter systems including acetylcholine. In a series of experiments, muscarinic receptor blockers were infused bilaterally in 0.5 ul volumes of saline into the lateral ventricles of ovariectomized rats and hamsters that had been induced into behavioral receptivity by treatment with estradiol benzoate and progesterone.

Scopolamine, a lipophilic muscarinic antagonist, inhibited the frequency of lordosis in rats (5, 10, and 20 ug/cannula) and reduced the duration of lordosis in hamsters (10 and 20 ug/cannula) 15 min. after infusion. Pirenzepine, a hydrophilic muscarinic M1 antagonist, did not inhibit the frequency of lordosis in rats (5, 10, and 20 ug/cannula). Methylscopolamine, a hydrophilic muscarinic antagonist, inhibited lordosis as effectively as the lipophilic scopolamine following intraventricular infusion (5 and 10 ug/cannula) despite differences in the lipid solubility and receptor affinity of the compounds.

Results indicate that muscarinic antagonists inhibit the display of lordosis in female rats and hamsters. The effectiveness of muscarinic antagonists appear to be related to subtype specificity but not lipid solubility.

109.11

INTRACRANIAL IMPLANTS OF DILUTED ESTRADIOL: INDEPENDENT EFFECTS ON FEEDING AND REPRODUCTIVE BEHAVIORS P.C. Butera & R.J. Beikirch*. Dept. of Psychology Niagara University, NY 14109

This study examined the effects of central implants of diluted estradiol (E2) on feeding, body weight, and sexual behavior in ovariectomized rats. Thirty-three adult females were implanted with bilateral double-walled cannulae in either the paraventricular nucleus (PVN), medial preoptic area (MPOA), or posterior hypothalamus (PH) and stimulated unilaterally with cholesterol (CHOL) and E2. Females that received PVN implants were stimulated with either undiluted E2, a 3:1, or 10:1 mixture of CHOL and E2. Animals in the other groups received undiluted E2.

Compared to CHOL implants, undiluted E2 in the PVN reduced food intake and body weight. More importantly, diluted E2 implants in the PVN significantly lowered food intake and body weight. In contrast, undiluted E2 in the MPOA, PH, or VMH had no significant effects on feeding or body weight. Analysis of variance revealed a significant main effect of implant location on lordosis quotients. A Newman-Keuls test indicated that diluted E2 implants in the PVN produced lordosis quotients that were significantly lower than those obtained with undiluted E2 in the VMH.

These findings suggest that the effects of E2 on food intake are mediated by its actions in the PVN, whereas effects on reproductive behavior involve actions of E2 in the VMH. (Supported by NIMH MH42127)

109.12

RAPID, LOCAL EFFECTS OF PROGESTERONE ON THE ACTIVITY AND SENSORIMOTOR PROPERTIES OF SUPERIOR COLLICULUS NEURONS RECORDED IN BEHAVING HAMSTERS DURING THE HORMONAL INDUCTION OF LORDOSIS. J. D. Rose. Department of Psychology, University of Wyoming, Laramie, WY 82071.

Research in this laboratory has shown that systemic progesterone (P) administration to estrogen-primed golden hamsters initiates cumulative changes in the activity level and sensorimotor function of superior colliculus (SC) neurons over the 2-4 h prior to the emergence of lordosis responses. In the present study, crystalline P was applied directly to the SC with a removeable cannula, near an array of chronically-implanted microwire electrodes. This method was used to identify direct, as opposed to anatomically remote, actions of P on the dorsal midbrain. P implantation produced changes, within seconds or minutes, in the activity level, movement-related firing and somatosensory responsiveness of SC neurons near the implant. P effects on these neurons entailed either suppression or enhancement of activity and sensorimotor function and were thus similar to effects previously seen with systemic P injection. However, the local effects of P were sometimes antagonized by subsequent systemic P. These results demonstrate that P can act locally on midbrain neurons to alter their sensorimotor function. The rapidity of some of the P implant effects suggests a non-genomic mechanism. Supported by NIH Grant NS13748.

110.1

IODOACETAMIDE ENHANCES TRANSMISSION AT PERFORANT PATH AND SCHAFFER COLLATERAL-COMMISSURAL SYNAPSES IN THE GUINEA-PIG HIPPOCAMPAL SLICE BY A POST-SYNAPTIC MECHANISM. J. Waalen* & P. Lipton (SPON: D. Gilboe). Dept. of Physiol., Univ. of Wisc., Madison, WI 53706.

The sulfhydryl reagent iodoacetamide (IAM) profoundly enhances synaptic transmission in hippocampal dentate granule cells and CA1 pyramidal cells prior to inhibiting glycolysis.

Paired pulse inhibition studies show IAM's excitatory effect is not due to blockade of inhibitory transmission. Paired stimuli were applied to the perforant path and population spikes were measured in the dentate granule cell layer. IAM increased the size of the first spike but decreased the size of the second spike; this contrasts with the effect of GABA blockade by 10μM bicuculline which causes a large increase in the size of the second spike.

Extracellular and intracellular recordings show that IAM increases excitability of the post-synaptic cells. Extracellularly, IAM enhances the evoked population spike without changing the size of the field EPSP. Intracellularly, IAM increases the probability of evoked spike generation in CA1 pyramidal cells while decreasing the associated EPSP; it decreases both input resistance and the ability of injected depolarizing current to generate a spike. Thus IAM specifically enhances the spike-generating capability of synaptically evoked EPSP's.

110.2

GENERATION OF A NOVEL SLOW EPSP IN THE FROG SYMPATHETIC GANGLIA. Parviz Yavari* (SPON: M. Shelanski). Dept. Physiol & Cell. Biophys., Columbia Univ. CPS, New York, NY 10032.

Several electrophysiologically interesting postsynaptic potentials (PSP) can be elicited in the frog sympathetic ganglion by suitable stimulation of the preganglionic inpu ts (Weight, F. Autonomic Ganglia:309, 1983). These include the nicotinic fast excitatory PSP (EPSP), muscarinic slow EPSP and slow inhibitory (IPSP), and the peptidergic late slow EPSP. Here I describe a slow synaptic excitation in the bullfrog Rana catesbeiana 9th or 10th paravertebral sy mpathetic ganglia which appears to be distinct from the pre viously known PSPs in this ganglion (recorded by the sucro se-gap method). It is generated by tetanic stimulation (w/ trains at as low a freq. as 7 Hz) of the sympathetic chain between the 6th & 7th ganglia with stimuli supramaximal (normally at twice the strength) for the B fiber pathway activation. The macroscopic slow EPSP generated by such sti muli has an early fast-rising component (the muscarinic sl ow EPSP) which overlaps with a slowly-rising, long-lasting component-- a second hump (a non-cholinergic slow EPSP sin ce it persists in Ringer containing curare and high doses of atropine or pirenzepine). The transmitter/pathway for the novel EPSP is not known at present, but it may involve the Substance P- or CGRP-containing fibers seen in the frog S. chain recently (Jan&Jan, J. Physiol 327:219,1982; Horn & Stofer, SN 13:297,1987). The pathway and neurotransmitter for novel slow EPSP is being studied by intracellular reco rding and the use of peptidergic agents.

110.3

CHARACTERISTICS OF MINIMAL FEED-FORWARD IPSPs IN GUINEA PIG CA1 PYRAMIDAL NEURONS. D.A. Turner. Neurosurgery, Univ. of Minnesota and VAMC, Minneapolis, MN 55455.

Stimulation of stratum radiatum (SR) afferents evoked intracellular synaptic responses (<1 mV), many of which were hyperpolarizing and inhibitory. Response ensembles showing clear separation of the hyperpolarizing IPSPs from EPSPs were analyzed for time to onset, waveform parameters and stationarity (n=39 ensembles in n=69 CA1 neurons).

Time to onset of the SR IPSP following the stimulus artifact ranged from 1.4 to 7.5 ms and averaged 3.20±1.53 ms (mean±SD). Responses evoked from proximal SR, distal SR or antidromic stimulation (DA Turner, J. Physiol. 295:419) were not different in terms of amplitude (0.49±0.27 mV) or 10-90% risetime (7.88±6.40 ms). The distal halfwidth was longer than the proximal: 52.0±36.0 ms and 38.1±21.3 ms, compared to a typical antidromic response with halfwidth of 28.9 ms. Response ensembles less than 1.0 mV in magnitude showed no trends in peak amplitude or waveform parameters at stimulation rates of 1.0 or 2.0 Hz.

The minimal stimulation intensity (no detectable field potential), rapid time to onset, hyperpolarizing polarity and long time course all strongly suggest that these responses are direct, feed-forward IPSPs onto CA1 pyramidal neurons. The divergence of proximal SR, distal SR and antidromic evoked waveforms indicates the probable recruitment of a different group of feed-forward interneurons for each site. Supported by a VA Research Service award and a B.S. Turner Foundation award.

110.4

LTP AND PAIRED-PULSE FACILITATION AFFECT DIFFERENTIALLY THE NMDA RECEPTOR MEDIATED COMPONENT OF SYNAPTIC RESPONSES. D. Muller and G. Lynch. Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA 92717.

A significant component of the synaptic responses evoked in field CA_1 of hippocampal slices can be identified as reflecting currents through NMDA receptor associated channels when using low magnesium concentrations (10-50 μM), the selective antagonist D-2-amino-5-phosphonovalerate (D-AP5), and a "priming" paradigm to suppress the fast IPSPs that usually truncate synaptic responses in hippocampus. "Priming" was accomplished by stimulating one of two groups of afferents to a common dendritic field 200 ms prior to stimulating the second test input. Under these conditions application of D-AP5 (50-125 μM) caused in 19 experiments a 28 ± 1% reduction in area of field EPSPs. When two pulses separated by 50 ms were delivered to the test input, the size of the second response was 57% larger than that of the first response, an effect referred to as paired-pulse facilitation and that results from increased transmitter release. Interestingly, the NMDA receptor mediated component of the facilitated response then represented a greater percentage of the total area of the EPSP (32 ± 1%; n=13), thereby indicating that facilitation affected to a greater degree NMDA than non-NMDA components of synaptic responses. In contrast, LTP, which in 14 experiments resulted in a 51% increase in response area, was not accompanied by a similar change. The D-AP5 sensitive component represented only 22 ± 1% of the area of the potentiated response, indicating that potentiation affected to a greater degree non-NMDA than NMDA components of synaptic responses. These results considerably restrict the number of possibilities concerning the mechanism of long-term potentiation and strongly argue against the idea that the potentiation effect might be related to increased transmitter release.

Work supported by AFOSR #86-0099 and FNRS #83.392.0.86.

110.5

EXTRACTION OF QUANTAL INHIBITORY EVENTS FROM SYNAPTIC NOISE IN HIPPOCAMPAL NEURONS. N. Ropert and H. Korn. Lab. Neurobiologie Cellulaire, INSERM, Institut Pasteur, Paris.

Spontaneous activity was recorded intracellularly from CA_1 pyramidal cells In Vitro in order to characterize inhibitory quantal events which contribute to build up synaptic noise in central neurons. In such conditions IPSPs were unlikely to be contaminated by excitatory potentials or large field effects. Fluctuating unitary IPSPs were easily distinguished; their frequency and amplitudes were reduced by TTX, which eliminated large responses, leaving only small depolarizing potentials of about equal size, presumed to represent inhibitory quantal events. Indeed these miniature potentials shared common properties with larger IPSPs, such as the unitary ones or the alveus-evoked population IPSP: 1) they had comparable time courses, 2) they were Cl^- dependent and abolished by picrotoxin, and 3) their apparent reversal potentials were the same. Using the latter to assess E, the driving force for Cl^-, the quantal conductance was calculated from the relation: $g_{ipsp}=(V/E)*G_m$, were V and G_m stand for the recorded potential and the input conductance of the cell. Values of 0.5 to 1 nS were found; if each open Cl^- channel has a conductance of 25 pS, no more than 20 to 40 channels should be involved in such a quantal response.

110.6

CONDITIONING-LIKE OPERATIONS ARISING FROM SIMULATION OF VOLTAGE-DEPENDENT ADAPTIVE PROPERTIES OF A NEURONAL MEMBRANE CURRENT. R.G. Pay and C.D. Woody, UCLA Medical Cntr, Los Angeles, CA 90024.

Some neuronal mechanisms underlying conditioning are supported by modifications of membrane ion currents (Alkon, Science, 1979). Earlier simulations of conditioning have emphasized operations of adaptive networks. We tested the hypothesis that conditioning-like operations could be accomplished by means of a single element with adaptive properties resembling those of an ionic conductance. Adaptation based on changes in a rapidly activated and inactivated, voltage-dependent current, much like an A-current, resulted in selective enhancement of signal transmission analogous to that found in cortical neurons following conditioning (Woody and Black-Cleworth, J. Neurophysiol., 1973;Woody et al., Soc. Neurosci. Abstr., 1987). An important feature of the simulation was that adaptation did not depend on modifying a passive resistance in the signal path but on varying a local current that responded to fluctuations about a reference potential. The current was activated by depolarization, inactivated by sustained depolarization, and potentiated by preceding hyperpolarization. The voltage-dependent operations were expressed by current-voltage curves of general exponential form. Simulated CS-US presentations yielded a learning curve analogous to that found with mammalian conditioning. Efficacy of conditioning varied with interstimulus interval with an optimum between 150-300 ms. Conditioning did not occur when the US preceded or coincided with the CS. Conditioning was more efficacious as CS amplitude increased. Outcomes of the simulation demonstrated enhancements of postsynaptic potentials elicited by the CS.

110.7

NMDA RECEPTOR-MEDIATED BURSTING IS INCREASED BY ELEVATED $[K^+]_O$ AND K-CHANNEL BLOCKADE. N.P. Poolos and J.D. Kocsis. Dept. of Neurology, Yale University and Center for Neuroscience and Regeneration, West Haven VA Hospital, West Haven, CT 06516.

We investigated the response of rat CA1 hippocampal pyramidal cells to single and paired orthodromic stimuli under conditions of elevated extracellular potassium ($[K^+]_O$) and K-channel blockade by TEA to assess the influence of NMDA receptor-mediated conductances on the EPSP and burst firing. EPSP amplitude was assessed before and after application of the selective NMDA blocker DL-APV (50-100 μM).

Elevated $[K^+]_O$ (4-9 mM) revealed an NMDA receptor-mediated component of the EPSP following a single stimulus which increased with higher $[K^+]_O$. EPSP facilitation and burst firing could be induced by paired stimuli. Facilitation and bursting became more prominent and increasingly sensitive to blockade by APV in elevated $[K^+]_O$. With normal $[K^+]_O$ (3 mM), TEA (1 mM) produced a facilitated EPSP following a single stimulus that was not sensitive to APV; however, following paired stimuli, the resultant burst firing was greater than in normal solution, and significantly reduced by APV. These results show that NMDA receptor-mediated postsynaptic activity in hippocampal pyramidal cells is sensitive to small changes in $[K^+]_O$ and TEA-sensitive repolarization, both of which may occur during repetitive activity.

110.8

MEMANTINE MIMICS THE EFFECTS OF PHENYTOIN, BUT NOT OF BACLOFEN IN SPINAL CORD NEURONS IN CULTURE. R.Netzer*, R.Koch*, H.Bigalke* (SPON: P. Sonderegger). Dept. Pharmacol. Toxicol., Med. School Hannover, 3000 Hannover 61, FRG, R.K. Merz & Co., 6000 Frankfurt 1, FRG.

The antiepileptic agent phenytoin blocks repetitive firing in spinal cord neurons by a use-dependent block of Na^+ channels (McLean, M.J. et al., J.of Pharmacol. and Experimental Therapeutics,Vol. 227: 779, 1983), whereas baclofen, an antispastic agent, inhibits pre- and postsynaptic events (Howe, J.R. et al., J.Physiol. 384:539, 1987). The mode of action of memantine, used in the treatment of spasticity from different sources, is widely unclear. We have compared effects of the three drugs on the bursting activities in cultured spinal cord neurons.

Convulsants, like strychnine, tetanus toxin and picrotoxin elicited burst- like activities in mouse spinal cord cultures similar to hyperactivities of seizure-producing nerve cells. The membrane potential depolarized by about 30 mV, accompanied by rapid firing of action potentials. Pressure perfusion of the cells with memantine (10 - 100 μM) decreased the duration of bursts from 1,3 s to 0,4 s and the firing rate from 30 Hz to 10 Hz. The onset of the effects were fast and reversible. The drug did not have any effects on the firing pattern and spontaneous synaptic activity in cultures not treated with convulsants. Phenytoin (1 - 10 μM) mimicked the block of action potentials in the bursting cells, whereas baclofen (10μM) decreased the frequency of the burst from 11/min to 4/min, leaving its duration and the frequency of action potentials within the bursts unaffected. Memantine, similar to phenytoin, thus appears to curb mainly hyperactive neurons, probably by an interference with the sodium channel.

110.9

ANALYSIS OF THE ANTIEPILEPTOGENIC ACTION OF BACLOFEN. ***B. Ault and C.M. Wang. Dept. of Pharmacology, Burroughs Wellcome Co., RTP, NC 27709, U.S.A.**

The $GABA_B$ receptor agonist baclofen inhibits bicuculline (BIC)-induced epileptiform activity in the CA3 region of the hippocampal slice at sub-micromolar levels. While micromolar doses of baclofen increase a potassium conductance and hyperpolarize hippocampal pyramidal cells, the mechanism of action at lower doses is unclear. We have now examined the effect of baclofen upon CA3 pyramidal cells at antiepileptogenic concentrations.

Submerged transverse hippocampal slices of 425 μ thickness were superfused with Elliott's medium (K^+ 5mM; Ca^{2+} 1.3 mM) containing 50 μM BIC. Intracellular and extracellular recordings were made of population burst discharges in area CA3. Application of 0.1 μM baclofen inhibited bursting by 68 ± 8% (N = 9) with no measurable change in V_m or R_m. As the frequency of bursting decreased, the burst duration increased: a similar effect was noted with varying rates of electrical stimulation. Baclofen (0.1 μM) did not significantly affect (i) the duration of stimulation-evoked bursts, (ii) the burst AHP or (iii) spike firing during a 250 ms depolarizing pulse. 1.0 μM Baclofen hyperpolarized CA3 neurons and reduced all of the above measures.

Since baclofen can inhibit burst discharges without altering passive or active pyramidal cell properties measured at the soma, the site of this action is likely to be the distal dendrites or presynaptic terminals.

110.10

EFFECTS INDUCED BY VALPROIC ACID ON THE IONIC CURRENTS IN CULTURED RAT CORTICAL NEURONS. C. Zona, G. Pirrone*, M. Avoli. Lab. Fisiologia Umana, Università "Tor Vergata", Roma, Italy and MNI, Montreal, Canada.

The effects induced by the antiepileptic drug valproic acid (VPA) were studied in rat neocortical neurons in culture by using the whole cell patch clamp technique. VPA was applied by pressure ejection. When the inward currents were selectively blocked by the addition of TTX (5 uM) and Cd (200 uM) in the bath, the application of VPA (1 - 5 mM) had no effect on the remaining outward currents. Instead, when K currents were blocked (Cs and TEA in the electrode, 4-AP (2 mM) in the bath), VPA (1 mM) modified the inward currents evoked by depolarization commands. The early component which was sensitive to TTX and therefore was carried by Na ions, was decreased by VPA. On the contrary, the late inward component which was blocked by Cd, was increased by VPA. These results demonstrate that the antiepileptic drug VPA affects selectively the inward currents in rat neocortical cells in culture.

110.11

SYNAPTIC POTENTIATION AND EPILEPTOGENESIS IN THE CA3 REGION OF THE IN VITRO HIPPOCAMPUS. W.H. Griffith and L.Taylor*. Dept of Medical Pharmacology and Toxicology, College of Medicine, Texas A & M University, College Station, TX 77843.

Short-term excitatory synaptic potentiation was studied in hippocampal CA3 neurons using single electrode voltage-clamp (SEVC) techniques in the rat in vitro slice preparation. Mossy fiber excitatory postsynaptic currents (EPSC) were studied in the presence of 10 μM picrotoxinin (2.5 mM Ca^{2+}, 4 mM Mg^{2+}). Repeated trains of electrical stimulation of the mossy fibers (100 Hz for 0.5 - 1 sec) produced episodes of posttetanic potentiation (PTP) that eventually led to paroxysmal depolarizing shift (PDS) firing. Characteristics of PTP of the mossy fiber synapses included potentiation of 100-150% with a time constant of decay (τ_d) between 50-70 sec. The τ_d of individual EPSCs did not change after PTP and were 90-110% of the control value (n = 5). After high frequency stimulation spontaneous EPSCs were sometimes observed that were not recorded before PDS development. The range of τ_d of spontaneous EPSCs was 1.5 - 3 ms at membrane potentials near rest (-60 to -80 mV). Conversion of single synaptic responses to network-driven synchronization may involve, in part, presynaptic mechanisms that recruit populations of cells to fire in unison.

(Supported by NIH Grant NS22456)

110.12

FUNCTIONAL IMPLICATIONS OF BURST FIRING IN MAMMALIAN NEURONS. Hilarey R. Feeser and David A. McCormick. Section of Neuroanatomy, Yale Univ. School of Medicine.

Neurons in the mammalian brain often display two basic firing patterns: single spike or burst activity. The functional consequences of neuronal burst firing on the responsiveness of neurons to synaptic inputs was investigated through intracellular recordings of relay neurons in the guinea pig lateral geniculate slice, maintained in vitro. Intracellular injection of short (2.5-5 msec) depolarizing current pulses or the local activation of EPSPs with the cell at Vms negative to -70 mV resulted in bursts of 3-6 action potentials (Jahnsen and Llinas, J. Physiol. 349:205-247). The amplitude and duration of the low threshold Ca^{++} spike underlying these bursts declined in response to increased frequency of activation such that the output of the neuron fell to near zero with inputs of greater than 6-10 Hz. In addition, during periods of artificially imposed rhythm generation, activation of EPSPs was relatively ineffective in generating additional neuronal action potential output. These limitations appear to be due to the kinetics of the major currents (e.g. t-current) underlying burst firing. In contrast, at Vms positive to -60 mV, the same inputs could generate action potentials faithfully at frequencies of up to 200 Hz. These results help to explain why rhythmic burst firing in vivo (such as during slow wave sleep) is associated with a decrease in the faithfulness with which synaptic inputs are transferred to target neurons.

110.13

A SLOW REGENERATIVE DEPOLARIZING POTENTIAL IN CELLS IN DEEP LAYERS OF RAT PIRIFORM CORTEX. G.-F. Tseng and L.B. Haberly, Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706

Cells in layer III of piriform cortex and the underlying endopiriform nucleus were studied in 500μm thick slices cut perpendicular to the surface. When deep cells were held at depolarized membrane potentials, a slow depolarizing potential could be triggered by subthreshold depolarizing current pulses. When current pulses were of brief duration, the depolarizing potential grew in amplitude after current offset, thus revealing a regenerative nature. Addition of 2mM Ba^{2+} with 1μM TTX to the bathing medium greatly accentuated the slow depolarizing potential. In the presence of Ba^{2+} and TTX, the slow depolarizing potential triggered long duration spikes. Co^{2+} blocked the slow regenerative potential in both normal bathing medium and Ba^{2+}. In cells with a weak Ca^{2+} activated K^+ conductance, the depolarizing potential was sustained for up to 20s. These results suggest that the regenerative potential is mediated by a high threshold, non-inactivating Ca^{2+} conductance (see Constanti et al, Pflügers Arch.404:259). Injection with Lucifer Yellow revealed that this conductance is present in both deep pyramidal and multipolar cells. Slow regenerative potentials were not observed in layer II pyramidal cells (n=25). In recordings with Lucifer Yellow-containing pipettes, the regenerative potential was increased in amplitude and duration, apparently as a consequence of an increase in input resistance and membrane depolarization that were consistently observed following leakage of the dye and Li_2SO_4 electrolyte into the cell. In these recordings, the regenerative potential could evoke action potentials at a latency up to 250ms following the triggering current pulse or 'SP.

The slow regenerative potential may explain, in part, the long latency, very high amplitude paired shock facilitation displayed by deep cells (Tseng & Haberly, Soc. Neurosci. Abs. 13:156) and the long latency, all-or-none EPSP induced by bursting (W.H. Hoffman & L.B. Haberly, this vol.). Supported by grant NS19865 to LBH.

110.14

THE DEEP PIRIFORM CORTEX IS THE SITE OF GENERATION FOR LONG-LATENCY EPSPS INDUCED BY BURSTING ACTIVITY. W.H. Hoffman* and L.B. Haberly (SPON: A. Berman) Neurosci. Training Prog. and Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

Bursting activity in slices of rat piriform cortex induces long-latency EPSPs in layer II pyramidal cells that can persist for the duration of experiments (up to 10 hr) (W.H. Hoffman & L.B. Haberly, Soc. Neurosci. Abs. 13:1101). These EPSPs occur in all-or-none fashion with a discrete threshold, but are variable in amplitude within a narrow range. Studies of properties of layer II pyramidal cells failed to reveal changes that could underlie development of late EPSPs. The hypothesis was therefore tested that cells in the deep layers of piriform cortex (layer III and the endopiriform nucleus) contribute to generation of bursting-induced, late EPSPs. Standard intracellular recording methods were employed. Bursting was induced by 10-25 min of perfusion with 0 Mg^{2+}; experiments were begun 5-15 min after return to normal bathing medium. Findings in support of the hypothesis include the following: 1) Late EPSPs still occurred after isolation of piriform cortex from adjacent cortical and subcortical structures. 2) Late EPSPs occurred in deep cells, were higher in amplitude than in layer II cells, and consistently evoked action potentials. 3) In small vertical segments of cortex, late EPSPs could occur in deep cells, but not in layer II cells. 4) Microinjection of 10mM Co^{2+} into deep layers could block late EPSPs in layer II cells without affecting the strength of monosynaptic EPSPs evoked by association fiber stimulation. This evidence indicates that bursting-induced late EPSPs are generated by deep cells and synaptically transmitted to layer II pyramidal cells. It can be postulated that the delay in the late EPSP is a consequence of the slow regenerative depolarizing potential in deep cells (G.-F. Tseng and L.B. Haberly, this volume). Supported by NINCDS grant NS19865 to LBH.

POSTSYNAPTIC MECHANISMS II

111.1

ACTIONS OF PUTATIVE NEUROTRANSMITTERS IN THE HUMAN CEREBRAL CORTEX, IN VITRO. Anne Williamson, Dennis Spencer and David A. McCormick. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

The ionic actions of some putative neurotransmitters were investigated using the in vitro slice technique on cerebral cortical tissue removed from patients for the treatment of intractable epileptic seizures. Local stimulation of synaptic inputs to presumed pyramidal neurons resulted in an EPSP followed by two phases of IPSP. The early IPSP was brief in duration, displayed a substantial increase in membrane conductance (65 nS), was mimicked by application of GABA, reversed polarity at -69 mV (which was very sensitive to intracellular injection of KCl), and was blocked by the $GABA_A$ antagonist bicuculline. The late IPSP, in contrast, was longer in duration, displayed a smaller increase in membrane conductance (14 nS), reversed near presumed E_K (-94 mV) and was substantially reduced by local application of the $GABA_B$ antagonist phaclofen. Application of ACh or the muscarinic agonist MCh resulted in a hyperpolarizing, depolarizing sequence which showed all of the properties reported previously for guinea pig cerebral cortex. NE caused a reduction in the slow AHP and/or a slow depolarization. Glutamate caused rapid depolarizations which did not display prominent voltage dependence. These results indicate that actions of classical neurotransmitters in the human cerebral cortex are similar to those previously reported in lower mammals.

111.2

VOLTAGE-CLAMP STUDY OF RAT CHROMAFFIN CELLS (CCs) AND THEIR RESPONSE TO MUSCARINE. A. Neely*+ & C.J. Lingle. Dept. Anesthesiol., Wash. Univ. Sch.Med., St. Louis, MO 63110; +Dept. Biol. Sci, FSU, Tallahassee, FL 32306.

We are interested in the basic electrophysiological properties of rat adrenal CCs and their possible modulation by secretagogues. Muscarine appears to induce secretion from rat CCs by releasing Ca^{+2} from intracellular stores following phosphoinositide breakdown (Malhorta et al., J.Biol.Chem., 5:2123).

Whole-cell patch clamp recording of primary cultures of rat CCs reveals a prominent Na-current and one, sustained component of Ca-current. Outward currents are dominated by voltage-dependent, Ca-activated K-currents. Single-channel recording reveals at least 3 types of voltage-dependent K-channels. Muscarine suppresses spontaneous action potentials in these cells. 0.5-50 μM muscarine induces a slow outward current. This response persists in 5 mM $CoCl_2$. Voltage ramps during the response show that the major conductance increase is voltage independent and reverses around E_K. This is consistent with activation of a voltage-independent, Ca-dependent, outward K-current subsequent to the muscarine-induced increase in intracellular Ca. (supported by AHA & DK-37109)

111.3

PRETREATMENT WITH THE PARTIAL AGONIST DECAMETHONIUM (C_{10}) ENHANCES CARBACHOL-INDUCED DESENSITIZATION. L.M. Coniglio* and R.L. Parsons (SPON: W. Pendlebury). Department of Anatomy and Neurobiology, University of Vermont, Burlington, Vermont 05405.

Brief exposure to a conditioning dose of carbachol (10uM) accelerates the desensitization produced by subsequent exposure to higher doses of carbachol (162-756uM) (Fiekers et al., J. Physiol. 391: 109-124, 1987). The present study was done to test whether pretreatment with other nicotinic agonists, especially partial agonists, also accelerates carbachol-induced desensitization. Carbachol-induced desensitization was studied in snake twitch muscle fibers maintained in a isotonic potassium propionate solution and voltage-clamped to +50mV. Carbachol induced a transient outward current which peaked within a few seconds and then slowly decayed toward the baseline. The time course of current decay, which developed with two exponential components, was used to estimate the time course of desensitzation onset. A 30 second pretreatment with 10uM C_{10} accelerated 216uM carbachol-induced desensitization by decreasing the time constant of both the fast and slow components. C_{10} pretreatment also decreased the peak EPC_{carb} amplitude. Application of 10uM C_{10} alone produced no outward current; measurable outward currents occurring only with concentrations above 350uM. Supported by a grant from the MDA.

111.4

ADENOSINE INCREASES K^+ CONDUCTANCE OF RAT LOCUS COERULEUS NEURONS. W.J. Pan, S.S. Osmanovic and S.A. Shefner, Department of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60680.

We have previously shown that adenosine causes a dose-dependent inhibition of firing and hyperpolarization of locus coeruleus (LC) neurons which is blocked by theophylline. In the present study, the mechanism of adenosine effects on LC neurons was studied with intracellular recording in a submerged rat brain slice preparation. LC neurons showed the usual regular pattern of spontaneous firing at rates of 0.25-3.0 Hz. Bath application of adenosine (100 μM) reversibly decreased the firing rate in 80% of spontaneously active LC neurons (n=20). With current clamp recording, adenosine (100 μM) caused hyperpolarizations (2-7 mV) in 73% of the LC neurons tested (n=22). These hyperpolarizations were accompanied by increased input conductance (11±2%). The reversal potential for the adenosine-induced hyperpolarization was calculated from the intersection of voltage-current curves under control conditions and in the presence of adenosine. The mean adenosine reversal potential was -116 mV (n=13), very similar to the K^+ equilibrium potential as estimated from the reversal potential of the afterhyperpolarization which follows a train of action potentials. Adenosine caused a small outward current in 3 LC neurons tested under voltage-clamp conditions. These data indicate that adenosine-induced hyperpolarization of LC neurons is due to an increase in K^+ conductance. GRANT SUPPORT: US PHS AA 5846.

111.5

PROCTOLIN-SENSITIVE CA^{2+} CHANNELS IN ISOLATED CRAYFISH TONIC FLEXOR MUSCLES. M.E. Krouse*, C.A. Bishop and J.J. Wine, Department of Psychology, Stanford University, Stanford, CA 94305

The pentapeptide proctolin is a cotransmitter in 3 of 6 identified motoneurons and, although it has no observable effect on resting muscles, its release greatly potentiates depolarization-induced tension by acting at multiple sites in the excitation-contraction pathway (Bishop, *Neuro. Abst.*,1988). We have used the patch-clamp technique to investigate the possible effects of proctolin on membrane channels. Isolated muscle fibers were treated with collagenase IA for 20 min (2.2 mg/5ml saline) and were then placed in a bath containing 137 mM $BaCl_2$, 1 mM $CaCl_2$, 2.6 mM $MgCl_2$, and 10.1 mM Trizma (pH 7.0). The pipet solution contained 200 mM Na-Gluconate, 2.6 mM $CaCl_2$, 2.6 mM $MgCl_2$, and 10.1 mM Trizma (pH 7.0). Experiments were performed at 21°C on inside-out patches. In the absence of proctolin, 4 channel types were observed: two linear Cl^- channels (8 pS and 24 pS in 280 mM Cl^-) which were activated by hyperpolarization and then remained active, and 2 Ca^{2+} (137 mM Ba^{2+}) channels. Activity of the Cl^- channels was unchanged by the presence of proctolin. However, the Ca^{2+} channels, which showed outward rectification and had chord conductances of 20 and 80 pS at +50 mV, became more active during depolarizations when the muscles were bathed in $5x10^{-9}$ M proctolin; the effect was especially pronounced for the 80 pS channel and was due to a sharp decline in the mean closed time. This voltage-activated increase in channel opening rate is consistent with tension experiments where the cells must first be depolarized for proctolin to increase tension. Also consistent are the results at desensitizing concentrations of proctolin (10^{-8} M), where the 80 pS channel was detected in only 1 of 4 patches (compared to 4 out of 5 patches in $5x10^{-9}$ M proctolin) and its activity was confined to bursts separated by long closed periods of several seconds. (Supported by NIH and Muscular Dystrophy Assn.)

111.6

SUBSTANCE P-INDUCED INWARD CURRENT IN GUINEA-PIG SYMPATHETIC NEURONS. T. Miyazaki*and N. J. Dun (SPON:R.S.Schmidt). Loyola Univ. Med. Ctr., Maywood, IL 60153

Inferior mesenteric ganglion (IMG) neurons were voltage clamped using a single electrode voltage clamp method. At the holding potential of -40 mV, pressure application of substance P (SP) induced an inward current associated with increase (type I), decrease (type II) or no appreciable change (type III) of membrane conductance in different IMG neurons. In type I response, the SP-induced current became larger on membrane hyperpolarization, and the extrapolated reversal potential was about -25 mV. While SP did not appreciably affect the slow inward current induced by hyperpolarizing command pulse, it increased instantaneous transient current at the start and end of command pulse. In type II response, the SP-induced current was nullified at about -70 mV in a portion of the cells, whereas it became larger on hyperpolarization in a few cells. The slow inward current as well as the instantaneous currents were reduced by SP. In type III response, the SP-induced current was accompanied by little or no change of membrane conductance. In a portion of the IMG neurons the slow inward current reduced by SP was also sensitive to muscarine, Co and Cd, suggesting that it probably reflects a Ca-dependent K current. The results suggest that SP induced an inward current in IMG neurons either by increasing membrane conductance to Na and/or Ca, or by decreasing a Ca-dependent K current and leakage current. (Supported by NS18710)

111.7

A CALCIUM-DEPENDENT SLOW AFTERDEPOLARIZING POTENTIAL (s-ADP) RECORDED IN RAT DORSOLATERAL SEPTAL NEURONS IN VITRO. H. Hasuo*, K.D. Phelan, M.J. Twery, and J.P. Gallagher, Dept. of Pharmacol. & Toxicol., University of Texas Medical Branch, Galveston, Texas 77550.

Intracellular recording of action potentials (APs) in rat dorsolateral septal nucleus (DLSN) neurons revealed several types of spike afterpotentials: a fast afterhyperpolarization, a fast depolarizing afterpotential, a slow afterhyperpolarization, and a slow afterdepolarizing potential (s-ADP). In the present study, we investigated the ionic mechanism of the s-ADP. A typical s-ADP, could be evoked by a single AP, was associated with a decreased input resistance, and had a duration of several seconds. Often bursts of APs were triggered on top of the 2 to 10 mV in amplitude s-ADP when recorded at normal resting potential levels (-60 to -70 mV). The s-ADP was not affected by tetrodotoxin, but was markedly reduced by sodium replacement with choline. The s-ADP was abolished by cadmium (0.2 mM) or by a low calcium solution. Changing extracellular chloride did not affect the s-ADP. The s-ADP was voltage clamped following directly-evoked spikes and a plot of its current versus membrane potential was linear between -50 to -90 mV and had an extrapolated reversal potential of -30 mV.

We conclude that this s-ADP in rat DLSN neurons may be mediated by a calcium-activated non-specific cation channel. This potential may underlie some bursting patterns recorded from rat DLSN neurons. (Supported by DAMD-17-86-C-6032)

111.8

SYNAPTIC TRANSMISSION AND PASSIVE MEMBRANE PROPERTIES OF NEURONS IN RAT DORSOLATERAL SEPTAL NUCLEUS ARE AFFECTED BY SOMATOSTATIN IN VITRO. M. J. Twery and J. P. Gallagher, Dept. of Pharmacology and Toxicology, Univ. Texas Med. Br., Galveston, TX 77550.

Immunohistochemical studies indicate somatostatin (SS) may act as a neurochemical messenger in septal nuclei. The present study used intracellular recording techniques to investigate effects of SS-14 on neuronal membranes and orthodromically evoked postsynaptic potentials in a submerged rat brain slice preparation containing the dorsolateral septal nucleus (DLSN). Membrane hyperpolarization was the predominant response to superfused SS (0.001-1 μM) or SS (50-100 μM) applied by pressure ejection from a micropipette. Hyperpolarization (2-14 mV) inhibited spontaneous activity and was associated with an apparent increase in K^+ conductance. Neither bicuculline (10 μM), TTX (0.5 μM), nor low Ca^{++}/ high Mg^{++} media blocked SS-induced hyperpolarizations. Depolarizing responses (1-2mV) to SS were observed infrequently and were blocked by superfusion of a low Ca^{++}/high Mg^{++} medium. All membrane potential (Vm) changes were slow in onset (0.5-5 min) and recovery (2-10 min). SS also decreased the amplitude of GABA-mediated inhibitory postsynaptic potentials (IPSPs) evoked by electrical stimulation of the medial septum. The effect on IPSP amplitude occurred with a slower time course than the changes in Vm and were variable with repeated application of the peptide. Repeated or continuous application of SS resulted in "desensitization".

The results indicate that SS has potent actions at pre- and post-synaptic neuronal membranes in the DLSN. These findings suggest that SS could serve as an inhibitory transmitter and synaptic modulator in this nucleus. (Supported by NIMH Grant MH-39163).

111.9

NICOTINIC RECEPTOR ACTIVATION OF RAT DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS RECORDED IN VITRO. Linda A. Wong* and Joel P. Gallagher (SPON: O.S. Steinsland). Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

Our previous studies suggested that carbachol and pyridostigmine act on nicotinic receptors in rat DLSN (*Soc. Neurosci. Abstr.* 13:268). To characterize further the mechanisms of central nicotinic receptor activation and possible nicotinic-modulation of GABA inhibition in DLSN, we investigated the actions of two specific nicotinic agonists: nicotine and DMPP (dimethylphenylpiperazinium). When applied by superfusion (1-10 μM) or pressure ejection (10mM, 10-300 mS, 5-10 psi) both drugs acted in a concentration-dependent manner to hyperpolarize the resting membrane potential (4-14 mV; n=19). This hyperpolarization was long lasting (minutes); associated with an increase in K^+ conductance; persisted in TTX or low Ca^{2+}/high Mg^{2+} solutions; and was reversibly blocked by mecamylamine (50 μM; n=3) but not by α-bungarotoxin (0.5 μM; n=3). Single electrode voltage clamp studies revealed a drug-induced outward current (150-180 pA; n=2). Both drugs consistently depressed GABA-mediated synaptic responses, and evoked delayed effects which altered the normal rhythmic firing pattern of DLSN neurons.

We conclude that nicotinic receptor activation leads to a multiplicity of effects that may result from: (1) direct postsynaptic alteration of intrinsic neuronal properties; (2) presynaptic modulation of GABA release; and (3) possible second-messenger mediated effects on action potential profile. (Supported by DAMD-17-86-C-6032).

111.10

A TRANSIENT INWARD CALCIUM CURRENT IN CAT AND RAT THALAMIC CELLS. V.Crunelli, C.E.Pollard* and J.W.Hynd*. Pharmacol. Dept., St.George's Hosp. Med. Sch., London SW17 ORE, U.K.

Low threshold Ca^{++} spikes are central to the oscillatory activity displayed by thalamic relay cells. The current responsible for their generation has been studied with the single electrode voltage clamp technique in brain slices of cat and rat dorsal lateral geniculate nucleus bathed at 25°C in a medium containing 1mM Ca^{++}, 3mM Mg^{++}, 0.5μM TTX and 3mM CsCl. From a holding potential (V_h) of -100mV, depolarising voltage steps positive to -70mV activated a transient, inward current (I(t)), the size of which was very voltage sensitive between -65mV and -55mV. I(t) was completely inactivated positive to -70mV but the inactivation was removed as V_h approached -100mV. The removal of inactivation was time dependent with the maximal current only being achieved after a 1 sec hyperpolarisation to -100mV. I(t) decayed exponentially with a time constant of 65 - 75ms when fully activated at -50mV. Deactivation was studied by activating I(t) maximally and returning to a V_h of -100mV at the current peak. The tail currents generated decayed exponentially with a time constant of around 5ms. I(t) was relatively resistant to 0.5mM Cd^{++} but was abolished by 0.5mM Ni^{++} or by using 8mM Mg^{++} as a competitive antagonist. I(t) characteristics suggest the involvement of T-type Ca^{++} channels. Because of the narrow voltage activation range of I(t), even small changes in membrane potential will strongly influence the size of low threshold Ca^{++} spikes.

111.11

GABA$_B$ RECEPTOR MEDIATED SYNAPTIC TRANSMISSION IN THE CAT LATERAL GENICULATE NUCLEUS. I. Soltesz*, S. Lightowler*, N.Leresche*, J.W.Hynd* and V.Crunelli (SPON: L.Valzelli). Pharmac.Dept., St.George's Med. Sch., London SW17 ORE, UK.

In projection neurons of the cat dorsal LGN, recorded in a brain slice that did not contain the perigeniculate nucleus, the "classical" EPSP-GABA$_A$ IPSP sequence evoked by electrical stimulation of the optic tract was always followed by a long latency (30-45msec), long duration (200-300msec) IPSP. This IPSP was K^+ dependent and blocked by phaclofen (0.5-1mM), indicating that it represents a GABA$_B$ IPSP. Its amplitude decreased by about 25% at stimulation frequencies higher than 0.05Hz. Bicuculline (10-50μM) and picrotoxin (25μM) blocked the GABA$_A$ IPSP but invariably increased the amplitude of the GABA$_B$ IPSP. GABA$_B$ IPSPs could be recorded from cells located in lamina A, A$_1$ and C, and whose morphology, analyzed following intracellular injection of HRP, was very similar to that of X, Y and W cells. We suggest that the physiological role of the GABA$_B$ receptors in the LGN is to mediate a late, long lasting IPSP that could be involved in the subcortical processing of information in all three main visual channels. Thus in the rat ventral LGN, where no long lasting inhibition had been observed in vivo, optic tract stimulation evoked GABA$_A$ but not GABA$_B$ IPSPs. Finally, the possibility of an increase in GABA$_B$ receptor mediated synaptic transmission should be carefully considered when using bicuculline as a selective blocker of GABA$_A$ mediated inhibition.

111.12

ROLE OF NMDA RECEPTORS IN THE GENERATION OF RHYTHMIC THALAMIC ACTIVITY. N. Leresche*, M. Haby*, D. Jassik-Gerschenfeld*, I. Soltesz*+ and V. Crunelli+ (SPON: European Neuroscience Ass.). Dept. Neurosciences de la Vision, Institut des Neurosciences, Univ. Paris VI, France and +Dept. of Pharmacol., St. George's Hosp. Med. Sch., Cranmer Terrace, London SW17, Great Britain.

Intracellular recordings from projection cells in rat and cat lateral geniculate and ventrolateral posterior nuclei were performed in a slice preparation. In control medium containing 2mM Ca++, 2 mM Mg++, no spontaneous activity was observed. However, removal of Mg++ from the perfusion medium produced rhythmic depolarizations (15-20 mV amplitude, 210-230 ms duration, 0.5-2.5 Hz frequency). Each depolarization could reach threshold for firing and evoked a burst of 1-7 action potentials. This rhythmic activity was voltage dependent (observed only between -65 and -75 mV), and could be more consistently observed in the presence of 3-4 mM Ca++. TTX (1 μM) abolished the bursts of action potentials but had no effect on the underlying depolarizations. This activity was reversibly abolished by (+-)2-amino-5-phosphonovaleric acid (20-50 μM), ketamine (10 μM), Mg++ (50-200 μM) and 8-Br-cyclic-AMP (1 mM) and was unaffected by bicuculline (1-50 μM), strychnine (20 μM), phaclophen (0.5-1 mM) and atropine (10 μM).

Our results suggest that thalamic projection cell in vitro are capable of rhythmic membrane potential oscillations that are similar to those observed during thalamic synchronization and that require the activation of NMDA receptors.

111.13

TRANSDUCTION MECHANISM FOR MUSCARINE-INDUCED CURRENTS IN FROG SYMPATHETIC NEURONES. P.A. Smith, J.A. Zidichouski* and A.A. Selyanko*, Dept. Pharmacol., Univ. Alberta, Edmonton, Canada and Bogomoletz Inst. Physiol., Kiev, U.S.S.R.

Muscarinic outward currents in C-neurones (I_{MO}) and muscarinic inward currents (I_{MI}) in B-neurones were studied in Rana pipiens sympathetic ganglia using the whole cell patch-clamp technique. Inclusion of 100μM cyclic AMP or the protein kinase C inhibitors, H-7, (50μM) or gold sodium thiomalate (GST, 50μM) failed to antagonize either response although both were prolonged by the non-hydrolysable GTP analogue, GTP-γ-S (50μM). I_{MO} was antagonized rather than mimicked by the protein kinase C activator, phorbol-12-myristate-13-acetate (PMA, 2-5μM). Although PMA also produced an inward current as a result of M-current supression in both B- and C-cells and antagonised I_{MI}, this effect was not antagonized by H-7 or GST, suggesting that protein kinase C is not involved in the effects of PMA. Although the inclusion 1μM Ca^{2+} in the patch pipette failed to mimic I_{MO} or steady state I_{MI}, the latter current was supressed by 0.5mM IP_3. Thus, neither adenylate cyclase inhibition, nor the second messengers Ca^{2+} or diacylglycerol seem to mediate G-protein-coupled muscarinic responses in frog sympathetic neurones. However, a Ca^{2+}-independent role for IP_3 in the generation of I_{MI} cannot be excluded.

111.14

OUTWARD AND INWARD CURRENTS PRODUCED BY ADRENALINE AND MUSCARINE IN FROG SYMPATHETIC NEURONES. A.A. Selyanko*, J.A. Zidichouski* and P.A. Smith. (SPON: D.P.J. Boisvert). Dept. Pharmacol., Univ. Alberta, Edmonton, Canada and Bogomoletz Inst. Physiol., Kiev, U.S.S.R.

The effects of adrenaline and muscarine on neurones dissociated from Rana pipiens sympathetic ganglia were studied using the whole cell patch-clamp technique. The larger B-cells exhibited a fast, transient outward current (A-current) which was not present in the smaller C-cells. Drug responses were more readily observed when 100μM cyclic AMP ± 100μM GTP was included in the patch pipette. 10μM muscarine suppressed the M-current in all 16 B-cells tested and thereby produced an inward current (Adams et al., J. Physiol., 330, 537, 1982). A similar effect of 10 or 100μM adrenaline was seen in 5 of these 16 cells. In contrast, muscarine and adrenaline produced outward currents in 15 and 14 out of 22 C-cells respectively (cf. Dodd & Horn, J. Physiol., 334, 271, 1983). This current reversed at E_K and displayed marked inward rectification. Luteinizing hormone releasing hormone (10μM) suppressed the M-current in all B- and C-cells tested. Outward currents produced by muscarine and adrenaline were not additive and both agonists produced > 50% inhibition of the M-current. These data indicate that muscarine and adrenaline share the same potassium channels to generate either an inward current in B-cells or an outward current in C-cells. Supported by MRC and AHFMR.

111.15

GTPγs MIMICS THE EFFECTS OF CARBACHOL ON CAT SPINAL MOTONEURONS. L. Zhang and K. Krnjević. Departments of Anaesthesia Research and Physiology, McGill University, Montreal, Quebec H3G 1Y6, Canada.

We investigated the effects of carbachol and their intracellular mediation in cat spinal motoneurons in situ, by using intracellular recording and iontophoresis. Cats were fully anaesthetized with sodium pentobarbital. Intracellular iontophoresis was made by triple-barrelled electrodes. For extracellular iontophoresis, the injecting barrel was glued to recording one. Extracellular injection of carbachol caused a depolarization with increase in input resistance, reduced the afterhyperpolarization (AHP), and prolonged the spike duration. The effects are probably due to blockade of K conductance. To test if a G protein is involved, we used GTPγs, a non- hydrolyzable analog that causes prolonged activation of G protein. Intracellular injection of GTPγs induced a depolarization with rise of input resistance, depressed the AHP and IPSP, and slightly increased the spike duration. The effects of GTPγs are quite similar to those of carbachol, suggesting the possibility that G protein may be involved in coupling of ACh receptors to K conductance. Further experiments are necessary to examine whether blockade of G protein prevents the effects of muscarinic agents. Supported by Medical Research Council of Canada.

111.16

PROPERTIES OF THE POTASSIUM CONDUCTANCE DECREASED BY REAL AND PUTATIVE NEUROTRANSMITTERS IN SUBMUCOUS PLEXUS NEURONES. K-Z.Shen* and A.Surprenant. Vollum Institute, Oregon Hlth.Sci.Univ., Portland, OR. 97201.

Slow excitatory synaptic potentials (sEPSPs) are a dominant feature of submucous plexus neurones; the peptide and non-peptide projections to these neurones are now well established. We used single-electrode voltage-clamp methods to study the inward current associated with the slow EPSP and the responses produced by application of substance P, 5-HT, muscarine, VIP and forskolin. All agonists produced identical responses in a single cell, responses which were identical to the sEPSP in the same cell. These responses could be mimicked by intracellular injection of ATP-γ-S but not GTP-γ-S and were pertussis toxin insensitive. In 50% of cells this response consisted of a voltage-independent (between -40 to -130 mV) K conductance (gK) decrease. In the other cells, an inward current and decreased gK was observed between -40 to -75 mV; there was an inward current only without associated conductance change from -80 to -130 mV. Ion replacement studies showed that a concomitant cation conductance increase was not responsible for this behaviour. However, when noradrenaline or somatostatin, which appear to increase a separate gK at somatic sites only, were present, the response became a voltage-independent gK decrease.

111.17

GDP RELEASE FROM RAT SYNAPTIC MEMBRANES IS STIMULATED BY RECEPTORS LINKED TO BOTH ACTIVATION AND INHIBITION OF ADENYLATE CYCLASE. T.K. Narayanan and R.S. Aronstam. Department of Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA 30912.

The binding of agonists to many neuronal receptors facilitates their association with transducer G proteins, thereby promoting the release the GDP from the G protein. This release is the rate limiting step in the G protein transduction cycle. To measure this release we incubated rat cortical and striatal membranes with alpha-[^{32}P]GTP under conditions which favored GTP hydrolysis. Following extensive washing of the membranes at 4°, [^{32}P]GDP release was measured in an assay medium containing 25 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.5 mM DTT, 0.1 M NaCl, 5 mM $MgCl_2$ and 0.2 mM Gpp(NH)p in a volume of 200 ul. Released nucleotides were separated by thin layer chromatography on polyethyleneimine cellulose plates using 1.5 M LiCl. Both histamine and carbamylcholine caused a dose-dependent release of GDP which was blocked by receptor antagonists. Both transmitters (100 uM) increased release 1.5 to 2 fold. Release was linear for 7 min and plateaued after 10 min. Inclusion of either agonist during the labelling period increased the [^{32}P]GDP content of the membranes. Thus, GDP release is mediated by receptors which are coupled to adenylate cyclase in both stimulatory and inhibitory manners. (Supported by GM-37948, AA-07698 and the Georgia Heart Association.)

111.18

AUTOPHOSPHORYLATION OF Ca^{++}/CaM-DEPENDENT PROTEIN KINASE II IN RAT BRAIN POST-SYNAPTIC DENSITIES. D.P. Rich* and T.R. Soderling (SPON:T.Inagami). Department of Molecular Physiology and Biophysics and Howard Hughes Medical Institute, Vanderbilt University, Nashville, TN 37232.

CaM Kinase II comprises 30-50% of the protein in the post-synaptic density (PSD), thus placing it in a strategic locale for exerting effects on post-synaptic responses. Ca^{++}/CaM-dependent autophosphorylation of PSD CaM Kinase II caused generation of 50-70% Ca^{++}-independent activity within 1-2 minutes. Subsequent addition of excess EGTA to the autophosphorylation reaction resulted in loss of stimulation of CaM Kinase II by Ca^{++}/CaM. Autophosphorylation of PSD CaM Kinase II at 4°C incorporated phosphate into both 50 and 60 kDa subunits (50kDa:0.024 pmol ^{32}P/μg PSD protein, 73% P-Thr, 27% P-Ser; 60kDa:0.021 pmol ^{32}P/μg PSD protein, 60% P-Thr, 40% P-Ser). HPLC peptide mapping of CNBr ^{32}P-peptides showed Thr-286 to be a major site of autophosphorylation. Kinetic analysis of the autophosphorylated PSD enzyme using Syntide-2 as substrate gave a Km= 12μM and Vmax= 2.77μmol/min/mg compared to a Km= 17μM and Vmax= 5μmol/min/mg for the Ca^{++}-dependent, nonautophosphorylated PSD enzyme. A synthetic peptide corresponding to a putative inhibitory sequence near the CaM binding region of the 50kDa subunit gave inhibition (IC_{50}=10-12μM) of the Ca^{++}-independent form of the PSD kinase. All these results indicate that CaM Kinase II in the PSD is regulated very similarly to the purified cytosolic kinase (Hashimoto et al.(1987) JBC 262,8051;Schworer et al.(1988) FASEB J 2,A992;Colbran et al.(1988) FASEB J 2,A992).

ENDOCRINE CONTROL AND DEVELOPMENT I

112.1

PRENATAL STRESS ALTERS THE DORSOLATERAL NUCLEUS OF THE SPINAL CORD. W. Grisham, M. Kerchner* & I. L. Ward. Dept. Psych., Villanova Univ., Villanova, PA 19085

The dorsolateral nucleus (DLN) of the spinal cord is sexually dimorphic and innervates the ischiocavernosus muscles. These penile muscles are responsible for aspects of copulatory behavior in male rats. Many male rats stressed prenatally fail to mate, a phenomenon attributed to altered patterns of plasma testosterone during fetal ontogeny. We examined the DLN in prenatally stressed and control rats, screened for their ability to ejaculate or not ejaculate.

Regardless of behavior, DLN cells from stressed males had smaller somal ($\bar{x}$ = 876 μ^2, $p < .005$) and nuclear ($\bar{x}$= 167 μ^2, $p < .036$) areas than controls ($\bar{x}$ = 974 μ^2 and $\bar{x}$ = 185 μ^2, respectively). Cell number in copulators tended to be asymmetric, with more cells in the left ($\bar{x}$ = 329) than right ($\bar{x}$ = 315, p = .056) DLN. Since there are no differences in the weight of the IC/BC muscle complex of stressed and control rats, we conclude that the differences in DLN neuron dimensions are not related to peripheral target organ size. (Supported by 2R01 HD-04688 from NICHHD and 2K05 MH00049 from NIMH.)

112.2

SEIZURE-INDUCED DELAY OF PUBERTY IN FEMALE RATS: EFFECTS OF AGE AND OPIOID ANTAGONISTS. R. Carson* and M. Wilkinson. Physiol. & Biophys., Dalhousie U., Halifax, Canada B3H 4H7

We have shown that pre- and post-pubertal female rats are sensitive to seizures. For example, daily convulsions commencing at age 24 days delay puberty (Exp. Brain Res. 56 32, 1984). Here we examine the effect of seizures at various ages. In addition, because opioid peptides are implicated in regulating puberty onset and are activated by convulsions, we also investigate the effect of opioid antagonists in the seizure-induced delay of puberty.

A single daily electroshock (ECS) was given for 10 days to neonatal (days 2-10), infantile (days 15-24) and juvenile (days 21-31) rats. The treatment delayed vaginal opening (VO) in juvenile rats. Neonatal and infantile rats were unaffected. VO is also delayed by daily ECS for only 5 days in the late juvenile (days 27-31) period. The opioid receptor antagonists naloxone and nalmefene (2.5mg/kg 10 mins before and 20 mins after single daily ECS) were unable to block this effect of ECS on VO. To examine whether the effect of ECS is related to stress, we examined several stressors known to induce opioid-mediated alterations in gonadotropin secretion. Footshock, immobilization and ether stress administered in the juvenile period (days 27-31) did not affect timing of VO. In addition, rats anaesthetized with halothane, and then given ECS, still showed a delay of VO. These data demonstrate that rats in the late juvenile stage of development are most sensitive to convulsions. We also suggest that opioids are not critical to the mechanism by which the ECS disturbs puberty and that ECS elicits its effect seemingly independent of the convulsive stress.

Supported by the Canadian MRC and the Savoy Foundation.

112.3

PRENATALLY-STRESSED FEMALE RATS AND FERTILITY AND FECUNDITY: INVOLVEMENT OF POSTCOITAL PROLACTIN SURGES. C.H. Kinsley, P.E. Mann & R.S. Bridges, L.H.R.R.B., Harvard Medical School, Boston, MA 02115

Prenatally-stressed (P-S) female rats exhibit reduced fertility and fecundity. Such females also show dampened prolactin (Prl) secretion following stimulation by estradiol. Since coital-induced Prl is involved in both fertility and fecundity, we examined P-S female rats for altered Prl surges following coitus. Pregnant rats were exposed to a thrice-daily regimen (Days 14-21) of heat and restraint stress; Controls were unhandled. As adults the female offspring (P-S & Controls) were mated on the evening of proestrus and sex behavior was observed and recorded. 24 hours following mating all P-S and Control females were fitted with intra-atrial cannulae. Blood sampling began 48 hours after mating with samples being taken at 2-hour intervals beginning at 2200h and continuing until 0600h (lights on 0500h-1900h) for a total of 3 days. Plasma Prl was determined by RIA. Fewer P-S females became pregnant or carried a pregnancy to term. Of those P-S females who delivered, litter size was smaller than that of Controls. No differences were found in these early (post-coital Days 2, 3 & 4) nocturnal Prl surges. Thus, the deficits in fertility and fecundity in P-S female rats do not appear to be tied directly to alterations in coital-induced Prl secretion during early pregnancy. Other potential factors, however, may include reduced sensitivity to Prl and/or changes in gonadal steroid secretion/sensitivity.

112.4

INHIBITION OF HIPPOCAMPAL CELL PROLIFERATION BY STRESS IN DEVELOPING RATS. P.J. Gutierrez* and J.S. Meyer. Dept. of Psychology, Univ. of Massachusetts, Amherst, MA 01003.

Developing rats and mice display a relative stress-nonresponsive period (RSNP) during which adrenocortical responses to stress are greatly attenuated. Because high doses of glucocorticoids inhibit a variety of brain growth processes including cell proliferation, the RSNP has been thought to protect the developing brain from the deleterious effects of elevated steroid levels. However, there is little evidence that stress-induced increases in corticosterone (CS; the endogenous glucocorticoid) can cause retardation of brain development. In the present study, young rats were stressed by several s.c. injections of histamine over the course of 2 days. Mitotic activity was then assessed in hippocampus, a major CS target area, and cerebellum by measuring the activity of thymidine kinase (TK). Compared to untreated controls, stressed rats showed significant reductions in hippocampal TK both during (days 11 and 13 postnatal) and near the end (day 18) of the RSNP. No effect was found in the cerebellum. When 10-day-old animals were adrenalectomized and implanted with CS-containing minipumps to hold circulating CS concentrations constant, subsequent histamine treatment failed to inhibit hippocampal TK activity measured on day 13. These results support the hypothesized role of the RSNP by showing that even small elevations in CS can alter the timing of developmental events in the hippocampus.

Supported by BRSG RR07048 and a Healey Endowment Award.

112.5

AGE-RELATED EFFECTS OF HANDLING, DIAZEPAM EXPOSURE, AND GONADECTOMY ON CORTICOSTERONE RESPONSE TO STRESS IN THE RAT. R. Guillet* and C.K. Kellogg. (SPON: M. Mangiapane). Dept.of Psychology, Univ. of Rochester, Rochester, NY 14627.

The effects of handling and acute diazepam (DZ) exposure on the corticosterone (CS) response to a novel environment are dependent upon pubertal age and can be modified by prepubertal gonadectomy (GDX). Male Long Evans rats were left intact or gonadectomized at 21 days of age, then examined at 28, 42, or 70 days of age. Beginning one week prior to experimentation, one half of these rats were handled daily, one half left undisturbed. Within each group one half of the rats then received DZ (2.5 mg/kg, i.p.)and one half vehicle (DMSO) at t=0. They were moved to a novel environment at t=30 min, and were sacrificed by rapid decapitation at t=45 min. Trunk blood was collected and assayed for CS. Additional cohorts of intact rats were treated similarly except that they were not exposed to a novel environment. At all ages examined, the CS response to a novel environment was the same in the unmanipulated rat. At 28 days, the CS response was decreased by 25% by either DZ and/or handling in the intact state, but only by DZ in the GDX state. At 42 days, either DZ or handling (but not both together) decreased the response in the intact state; conversely, only DZ plus handling decreased the response in the GDX state. At 70 days, the CS response was not decreased by manipulations in the intact state and was increased in all cases by 75% in the GDX state. Injection alone (without novel environment) resulted in no increase over baseline CS at 45 min. These results suggest that: 1. the effects of handling and of DZ on CS response to stress are dependent upon pubertal age; 2. handling and DZ may affect this response via different mechanisms; 3. the effect of prepubertal GDX on the stress response is manifested differently depending on the age at testing; and 4. the gonadal axis may interact with the adrenal axis in the adult rat to influence stress-responsiveness. Thus, we hypothesize that the CS response to stress is mediated via multiple systems and that these systems are still evolving during puberty. Furthermore, manipulation of the gonadal axis in the prepubertal rat influences the evolution of these systems. Supported in part by Grant No. MH 31850.

112.6

PUBERTAL-RELATED INFLUENCES ORGANIZE NEURAL MECHANISMS UNDERLYING SOCIAL INTERACTION IN RATS AND THE EFFECTS OF DIAZEPAM THERE ON. R. Primus and C.K. Kellogg. Dept. of Psychology, Univ. of Rochester, Rochester, NY 14627.

The social interaction test of anxiety demonstrated that the organization of this behavioral response to a novel environment occurs during puberty. The impact of a novel environment and the influence of diazepam (DZ) and prepubertal gonadectomy (GDX) on social interaction was examined in 28, 35, and 60 day old male rats in order to demonstrate that changes during puberty organize the behavioral respone to a novel environment. In the social interaction test, 60 Long Evans rats from each age group received five days of pretreatment with either DZ (1.0 or 0.5 mg/kg/day) or vehicle (tween 80 & saline) and were then tested in one of two environmental conditions, familiar or unfamiliar. The amount of time spent in active social interaction was scored. At 35 and 60 days, vehicle treated rats exhibited the characteristic decreased interaction in the unfamiliar condition. This decrease was not evident in 28 day old rats. Social interaction was differentially affected by DZ depending on the rat's age. At 28 days, DZ treatment increased interaction in both conditions whereas at 60 days DZ increased interaction in the unfamiliar condition only, the typical effect of an anxiolytic compound. At 35 days, DZ had no effect on interaction in either condition. GDX at 19 days of age diminished the impact of the environment at each age (N=24/group). Overall, GDX decreased interaction in both conditions. Furthermore, at 35 and 60 days, there was no effect of the unfamiliar environment on interaction (the typical response of intact rats at 28 days). And finally, prepubertal GDX altered the effect of DZ on social interaction. In summary, social interaction and the ability of DZ treatment to affect interaction depends on pubertal-related influences. Thus, the change in the behavioral response to a novel environment that occurs during puberty reflects organization of the neural mechanisms underlying this response. Supported by Grant No. MH31850.

112.7

CORTICOSTEROIDS SUPPRESS EXPRESSION OF GAP-43. H.J. Federoff*, E. Grabczyk*, and M.C. Fishman. Howard Hughes Medical Institute and Medical and Neurology Services, Massachusetts General Hospital, Boston, MA

The hormonal milieu is one important determinant of neuronal structure. For example, corticosteroids can perturb neuronal development (Bohn, Neurosci. 5:2003-2012, 1980) and diminish sprouting (Scheff, et al. Exp. Neurol. 68:195-201, 1980). The GAP-43 gene is one of a set of genes regulated during neuronal growth and remodeling and hence a potential target for the corticosteroid effect. We are investigating the effects of nerve growth factor and glucocorticoids on the regulation of GAP-43 gene expression in the clonal PC12 cell line. We have found the following: 1) glucocorticoids diminish GAP-43 expression in a dose-dependent fashion; 2) nerve growth factor stimulates the accumulation of GAP-43 mRNA (Karns, et al., Science, 236: 597-600, 1987); 3) glucocorticoids suppress the NGF effect when they are added concurrently; 4) both the NGF and glucocorticoid effects are primary, that is they do not require de novo protein synthesis; 5) nuclear run-on experiments reveal that the glucocorticoid effect is predominantly transcriptionally regulated; 6) glucocorticoids similarly reduce GAP-43 mRNA in cultured neurons of the superior cervical ganglion. We are at present identifying the regions of the GAP-43 gene involved in this regulation.

112.8

Prenatal nicotine administration decreases testosterone levels in male neonatal rats. A.C.Segarra, A.Mauro*and F.L.Strand. Biology Department.New York University. Washington Square, New York, N.Y. 10003.

Our previous studies indicate that prenatal nicotine administration decreases sexual behavior and testosterone plasma levels in adult male rats (Segarra & Strand, in press). To extend these studies we investigated the effect of pre-(0.25 mg/kg 2X/day) or postnatal (0.25 mg/kg/day) administration of nicotine on various neonatal parameters. Anogenital distance and body weight of newborn rats was assessed. At 6 days of age, the animals were sacrificed, the testes weighed, trunk blood collected and testosterone assayed by RIA. Prenatal nicotine decreased plasma testosterone levels and anogenital distance in 6 day old pups when compared to controls. Postnatal nicotine treatment had no effect on either of these parameters. There were no differences in body nor testes weights following pre- or postnatal nicotine administration. These results extend our previous findings and suggest that the decreased sexual behavior in males treated prenatally with nicotine is correlated to decreased testosterone levels in male neonates. Nicotine appears to exert an organizational effect during the critical period of sexual differentiation of the brain; it's effect on testosterone seems to be confined to prenatal development. (Supported by CTR)

112.9

NICOTINE MAY REQUIRE ENDOGENOUS ACTH TO ACCELERATE NEUROMUSCULAR DEVELOPMENT IN RATS. K.J. Rose and F.L. Strand. Center for Neural Science.New York University. Washington Square, New York,N.Y. 10003.

A prenatal critical period exists for the neuromuscular acceleratory developmental effects of the noncorticotropic fragment of adrenocorticotropic hormone (ACTH 4-10), and for nicotine in the Sprague-Dawley rat, this period being gestational (G) days G3-G12 (Rose, K.J. and F.L. Strand. 1988. Synapse. [in press]; Rose K.J. et al. 1987. Soc. Neurosci. Abs. 13[3]:1521). Nicotine triggers the endogenous release of ACTH from the pituitary (Cam, G.R. et al., 1979. Arch. int. Pharmacodyn. 237:49-66). We show that two-week-old postnatal pups from pregnant dams that had received orally the ACTH-blocker betamethasone on G8-G9 [20 ug/ml; 17:00 h - 17:00 h] and nicotine [0.25 mg/kg/2x day] on G9 had significantly slower extensor digitorum longus (EDL) muscle contractile times compared to saline controls, pups treated with vector and betamethasone, or with nicotine alone. We conclude that the acceleratory effects of nicotine on neuromuscular development may require endogenous ACTH. (Supported by The Council for Tobacco Research.)

112.10

TIMECOURSE OF ADRENAL STEROID RESPONSE TO ETHER STRESS OR CRF IN FIFTEEN DAY OLD CHEMICALLY HYPOTHYROID RATS. L.A. Meserve and L.M. Juarez.* Dept. Biological Sciences, Bowling Green State Univ., Bowling Green, OH 43403-0212.

In earlier studies, thiouracil-induced hypothyroidism has caused delayed pituitary-adrenal stress response. The present study determined the influence of chemical hypothyroidism on corticosterone levels 15, 30, and 45 min after ether stress or injection of corticotropin-releasing factor (CRF) in 15 day old rats. Thiouracil (0.25%) was given in the maternal diet from day 1 of pregnancy, and pups were either exposed to ether fumes (1 min), or were injected i.p. with CRF (10 ng/g) or with injection vehicle. Young were decapitated before or 15, 30 or 45 min after stimulation, and serum thyroxine (T_4) and corticosterone levels, and hypothalamic CRF content, were determined by RIA. Thiouracil depressed body and adrenal weights while producing goitrous thyroid glands and reducing T_4 levels by 80%. Basal corticosterone levels were not modified by thiouracil, but response to ether stress was half of normal at each time point. Thiouracil depressed hypothalamic CRF content, but not response to CRF injection. Indeed, it appeared that hypothyroid animals were better able to distinguish mock and CRF injections than were controls. This study supports hypothalamic modification of pituitary-adrenal response in hypothyroidism. (Supported by the Faculty Research Committee, BGSU).

112.11

ONTOGENY OF PROOPIOMELANOCORTIN IN THE ANTERIOR AND INTERMEDIATE PITUITARY LOBES: mRNA QUANTIFICATION AND PEPTIDE CONTENT. D.M.Vazquez* and H. Akil (SPON: N. Alessi). Mental Research Institute, University of Michigan, Ann Arbor, MI 48109.

The proopiomelanocortin (POMC) producing cells present in the anterior lobe (corticotrophs) and the intermediate lobe (melanotrophs) of the pituitary are derived from a common primordium originating in the Rathke's pouch. However, in the adult rat, these cells exhibit tissue specific post-translational processing, differential regulation of peptide secretion and gene expression. Unlike the adult pituitary where the anterior and intermediate lobe POMC products are distinct, the developing pituitary lobes have similar POMC products. A definite adult pattern is not achieved until at least the twenty-first day of life. To our knowledge, patterns of POMC gene expression as reflected by mRNA levels have not been described in the developing animal, especially during the first two weeks of life when the adrenocortical response to stress is greatly reduced. For this reason, anterior and intermediate lobes of rats ranging between 1 and 21 days of age were extracted separately using the proteinase K extraction method (LET). The homogenized lobes were used to quantitate POMC mRNA by Northern gel analysis and peptide content by RIA (ACTH, βE, NAcβE and αMSH). Plasma samples were likewise assayed for peptide content using RIA. We will report on the POMC mRNA levels present in the anterior and intermediate lobe of rats 1 to 21 days old and its relation to pituitary peptide content and secretion during this time.

112.12

NEURONAL OXYTOCIN MRNA REGULATION BY GONADAL STEROIDS IN DEVELOPING AND ADULT RATS. F.D.Miller, R.J. Milner, and F.E. Bloom, Div. Preclinical Neurosci., Research Inst. Scripps Clinic, 10666 N. Torrey Pines Rd., La Jolla, CA 92037.

Gender dependent differences in neuronal structure and innervation patterns have been largely restricted to neurotransmitter systems arising from sexually dimorphic nuclei of the preoptic region.. Vasopressin immunoreactive neurons are more numerous in the male bed nucleus of the stria terminalis (van Leeuwen et al, J. Comp. Neurol. 233: 236,1985), but no comparable data exist for oxytocin (OXY). During studies of genes expressed in developing rat brain (Miller et al, 1988 J. Neurosci.7:2433,1987), we employed *in situ hybridization* (ISH) to determine the location, number, intensity, time course and hormonal sensitivity of brain OXY mRNA. Anti-sense single stranded RNA probes labeled with S^{35} were generated from a genomic clone of the rat oxytocin gene (Gift of H. Schmale, University of Hamburg). ISH gave sensitive, readily discriminable neuronal signals for mapping and quantitative comparisons. OXY mRNA was expressed in far more neurons in adult males than in females. In female rats, OXY mRNA was largely restricted to the supraoptic nucleus and perimeters of the paraventricular nucleus; sparser, but significant numbers of neurons in males were detected in several other nuclei between the anterior preoptic area and the zona incerta. In some regions, e.g., retrochiasmatic portion of the supraoptic nucleus, neurons were 1-2 fold richer in in male rats. Expression of OXY mRNA within positive sites of female rats can also be regulated. Although OXY mRNA expressing neuron numbers did not change in females post-natally, the intensity of the ISH increased approximately 2 fold per neuron and returned to prepubertal levels 3 weeks after ovariectomy. These data indicate that gonadal steroids regulate both number and magnitude of neurons expressing OXY and suggest that gender-dependent differences in OXY expression can exist within neuronal systems not otherwise considered sexually dimorphic. (Supported by Grant NS22347; FDM now at Dept. Anatomy and Cell Biology, Univ. Alberta).

ENDOCRINE CONTROL AND DEVELOPMENT II

113.1

NEUROGENESIS IN THE SEXUALLY DIMORPHIC AREA OF THE GERBIL HYPOTHALAMUS. C. Ulibarri and P. Yahr. Department of Psychobiology, Univ. of California, Irvine, CA 92717.

The sexually dimorphic area (SDA) of the Mongolian gerbil is a complex of hormone-accumulating cells on the border of the preoptic area and anterior hypothalamus. The lateral SDA (lSDA) is connected dorsally to the medial SDA (mSDA) by a bridge of darkly staining cells. This gives the male SDA a hooked shape, but the female SDA is more ovoid and diffuse. The most dimorphic portion of the SDA, the SDA pars compacta (SDApc), is present bilaterally in most males and absent in most females. The SDApc is present in both sexes at birth and is maintained in males by androgens. In females, the SDApc disappears within three weeks of birth. Females injected with testosterone neonatally retain their SDApcs as adults. In males, ^{3}H-thymidine autoradiography shows that neurogenesis occurs over a short time course in all parts of the SDA except the SDApc. Neurogenesis begins in the lSDA on embryonic day 13 (E13), (E1 = day of conception), and ends on E15. Cells in the bridge of the SDA are born on E19. Neurogenesis occurs in the mSDA on E18 and E19. The cells in the area under the hook are born on E15 and E16. In contrast, neurogenesis continues in the SDApc for five days (E17-E21). These patterns of neurogenesis are being compared to those found in normal and neonatally androgenized females.
This research was supported by NIMH Grant MH-26481.

113.2

SPECIFIC PROTEINS IN THE SEXUAL DIMORPHIC NUCLEUS (SDN) OF THE RAT DURING THE NEONATAL PERIOD. J.F. RODRIGUEZ-SIERRA, W.E. HEYDORN, R.P. HAMMER and D.M. JACOBOWITZ. Dept. Anatomy, Univ. Nebr. Med. Ctr., Omaha, NE 68105, Merrell-Dow Research Inst., Cincinnati, OH 45215; Dept. Anatomy and Reprod. Biol., Univ. Hawaii School Medicine, Honolulu, HI 96822; Lab. Clinical Sci., NIH-NIMH, Bethesda, MD 20892.

The development of the SDN in the medial preoptic area of the rat hypothalamus has been linked to several sexually dimorphic traits in this species. Opiate receptors appear very rapidly in the SDN of the female rat, from day 3 to day 6 of life, while male rats do not show this. We used the technique of 2-dimensional gel electrophoresis and spectrophotometry of the separated proteins to determine the amount of protein. We compared male and female rats at 1 and 6 days of age. Our results show that very few proteins were significantly greater on Day 1 of age than on Day 6 for either sex. The greatest effect was observed in the number of protein spots that showed a significant increase from Day 1 to Day 6 in the female rats (19 spots), while the males did not show this dramatic effect (only 2 spots). Our results suggest that during the development of the SDN, the males suppress the formation of a number of specific proteins in this area. (Sponsored by the NIH HD 13219 and HD19951 and funds from the Univ. Nebr. Med. Ctr.).

113.3

HORMONE-DEPENDENT SEXUAL DIFFERENTIATION IN DEVELOPING RAT HYPOTHALAMUS. S.A. Tobet and T.O. Fox. Prgm Neuroscience, Harvard Med. Sch. and Dept of Biochemistry, E.K. Shriver Ctr. for Mental Retard., 200 Trapelo Rd. Waltham, MA 02254.

Morphological sex differences in adults can result from differential gonadal steroid exposure during critical perinatal periods. We are developing molecular markers to study mechanisms of sexual differentiation of brain structure and function. One hypothesis is that certain transient molecular events during development are hormone dependent, leading to sexual dimorphisms. Antibody AB-2 (Tobet et al., Endo.120[Suppl]:235,1987) detected immunoreactive cellular elements, including radial glia, transiently during the perinatal period. Peak reactivity in radial glia was on embryonic day 19 (E19) in males and on postnatal day 1 (P1) in females. On P1, AB-2 immunoreactivity in radial glia was 2-fold greater in females than in males. Activity detected in males was localized more on one side of the brain than the other (2- to 4-fold, depending on the region). Exposure of females to testosterone propionate on E18 resulted in lower levels of AB-2 immunoreactivity in radial glia on P1 relative to control females. The pattern was bilaterally asymmetric, approaching that of males. Whether these differences are in levels of antigen or in detection of antigen, reflecting another brain difference that is hormonally regulated, is being investigated. As a marker AB-2 demonstrates that the sequence of events during development can be influenced by gonadal steroids. (HD07251-SAT, HD20327-TOF, HD04147-MR)

113.4

RELATIONSHIP BETWEEN MALE SEXUAL BEHAVIORS AND DORSAL MEDIAL SPINAL NUCLEUS IN ANDROGEN-INSENSITIVE *Tfm* MICE. K.L. Olsen, C.K. Wagner and L.G. Clemens. Dept. Psychiatry, SUNY-Stony Brook, NY 11794; Neurosci. Prog.& Dept. Zoology, Michigan State Univ., East Lansing, MI 48824.

In testicular feminized (*tfm*) rats, the spinal nucleus of the bulbocavernous (SNB) is markedly reduced (Breedlove & Arnold,'80;81) although they can mount (Olsen,'79). The *Tfm* mutation is characterized by defects in the androgen but not estrogen receptor systems suggesting that masculinization of the SBN morphology and behavior are independent and mediated by different hormones. In contrast, *Tfm* mice display little or no male sexual behavior and the present data reveal that the mutants and their female siblings have less volume and fewer cells in the dorsal medial (DM) spinal nucleus than wild-type males. *Tfm* mice can be induced to display the ejaculatory pattern by various neonatal and/or adult hormone treatments. This study examines the morphology of the DM spinal nucleus in these mice.

At birth, mice were exposed to either testosterone propionate (TP), dihydrotestosterone (DHT), R1881, or estradiol benzoate (EB). When adult, these mice were gonadx, injected daily with TP or DHT+EB and given six weekly 1 hr tests for male sexual behavior. Other adult gonadx *Tfm* mice were given EB (10 μg) for the three days prior to testing.

Androgen-insensitive mice exposed neonatally to DHT (250 ug/2 days) but not to TP (500 μg) or to EB (10 μg) exhibit the ejaculatory pattern. *Tfm* mice given EB in adulthood also eventually display this response. Thus, the potential of the *Tfm* mice to show ejaculatory behavior can be dissociated from neonatal exposure to exogenous androgen. The effect of these treatments on morphology are being analyzed. These data will provide information about the relationship between male sexual behaviors and the sexual dimorphic DM spinal nucleus.

113.5

BRAIN AROMATASE ACTIVITY OF FETAL AND ADULT GUINEA PIGS. P.B. CONNOLLY*, C.E. ROSELLI, J.A. RESKO* (Spon: H. Spies). Physiology Dept, Oregon Health Sci U, Portland, OR 97201, Oregon Reg Primate Res Ctr, Beaverton OR 97006

Conversion of androgens to estrogens by aromatase activity (AA) may be important for organizing nervous tissues that mediate sexual behavior or gonadotropin release. Although testosterone (T) is a definite effector of androgen action in the guinea pig (GP), the role of in situ conversion to estradiol in sexual differentiation of the brain is less clear. We undertook a study to characterize and localize microsomal AA in brains of fetal, neonatal and adult male GP. Hypothalamic tissues from 50-day fetuses and adult males contained AA with an apparent Km of 19.6 and 16.6 nM, respectively. Fetal and neonatal brain AA showed significant ($p<0.01$) between tissue differences (amygdala [AMG]>preoptic area [POA]> basal hypothalamus≥septum>cortex); no sex difference was apparent. AA was highest at 40-days gestation, declined ($p<0.01$) with gestational age (50 and 60 days) and was lowest at 5-days post-partum. Adult male brains showed a similar distribution of AA to fetus with greatest activity in AMG and POA. AA in adults was greater ($p<0.01$) than in neonates. Castration (2 weeks) had no effect on AA in adult brains. These data demonstrate the existence of AA in fetal, neonatal and adult GP brain and suggest that AA in the brain of this species may not be androgen dependent. Supported by HD-07133 and HD-16022.

113.6

SEX AND AGE-RELATED DIFFERENCES IN NUCLEAR ESTROGEN BINDING CAPACITY AND PROGESTIN RECEPTOR INDUCTION IN THE RAT BRAIN T.J. Brown, N.J. MacLusky, E.E. Jones and F. Naftolin. Dept. of Ob/Gyn, Yale Univ. Medical School, New Haven, CT 06510

Previous studies have suggested parallels between the mechanisms underlying sexual differentiation and age-related loss of reproductive cyclicity in the female rat. Both appear to involve the actions of estrogen on the brain; and are associated with a reduction in hypothalamic estrogen sensitivity. The present studies were performed to directly compare the effects of sex and aging on cell nuclear estrogen receptor (ERn) capacity and cytosol progestin receptor (PRc) induction in the rat brain and pituitary gland. Young (2.5 mo) and middle-aged (8-10 mo) male and female, and old (19 mo) female rats were gonadectomized and adrenalectomized two weeks before use. ERn capacity and estrogen induction of PRc were measured in the pituitary (PIT), periventricular preoptic area (PVPOA), medial preoptic area (mPOA), bed nucleus of the stria terminalis, arcuate-median eminence region, ventromedial nucleus (VMN) and corticomedial amygdala. RESULTS: ERn levels and PRc induction were lower in the PVPOA, mPOA and VMN of the male as compared to the female. In the female, although the level of PRc induction in the PIT and the six different brain regions was unaffected by age, ERn capacity in the mPOA, VMN and PIT was significantly lower at 19mo than at either 2.5 or 8-10mo. ERn capacity in the PVPOA was identical in young and old animals. CONCLUSIONS: These results demonstrate that the effects of sexual differentiation and aging on the hypothalamus involve similar, but not identical, region-specific reductions in ERn binding capacity. The consequences of these reduced ERn levels in terms of the PRc response to an estrogen challenge are, however, different in male as compared to old female rats. (Supported by NIH grants HD13587, AG05004 and NS07807)

113.7

FETAL ETHANOL EFFECTS ON AROMATASE ACTIVITY IN THE HYPOTHALAMIC-PREOPTIC AREA IN THE MALE NEONATAL BRAIN. P.K. Rudeen, J.A. Creighton*, W. Kelce* and V.K. Ganjam*. Depts. of Anatomy and Vet. Biomed. Sci., Univ. of Missouri School of Medicine and College of Vet. Medicine, Columbia, MO 65212.

The fetal ethanol induced demasculinization of the sexually dimorphic nucleus of the preoptic area in male rats may be due to the inhibition of fetal testosterone synthesis by a 50% reduction of 17α-hydroxylase activity. This study was conducted to determine the effects of fetal ethanol exposure on aromatase activity in the anterior hypothalamic-preoptic area of neonatal male rats. Estrogen formation in individual brains was assayed in vitro using the stereospecific production of ^3H_2O from (1β-3H)- androstenedione as a measurement of aromatase activity. Aromatase activity was significantly higher ($p< 0.05$) in the fetal ethanol exposed animals compared to enzyme activity in animals whose mothers were given the chow diet (92.2 ± 7.5 and 61.1 ± 6.4 pmoles/hr/mg protein, respectively). Animals exposed to the pair fed diet had intermediate enzyme activity. The increase in aromatase activity in FEE animals may be due to a compensatory increase in enzyme activity due to reduction in the availability of testosterone from fetal testes. (Supported by Grants AA05893 and AA00107).

113.8

DEVELOPMENTAL CHANGES AND SEX DIFFERENCES IN THE ACTIVITY OF TESTOSTERONE- METABOLIZING ENZYMES IN THE BRAIN OF THE ZEBRA FINCH. A. Vockel*, E. Pröve* and J. Balthazart, Dept. Ethology, Univ. Bielefeld, FRG and Lab. Gen. and Comp. Biochemistry, Univ.Liège, Belgium.

Many effects of testosterone (T) in the zebra finch (*Taenopygia guttata*) can be mimicked by T metabolites, mainly oestradiol and 5α-dihydrotestosterone. We have therefore characterized T-metabolizing enzymes and studied their neuroanatomical distribution by means of the Palkovits punch technique combined with radioenzyme assay in the brain of adult and young male and female zebra finches. The kinetic parameters (Km, Vmax) of the aromatase, the 5α- and 5β-reductase were first determined in the hypothalamus of birds of both sexes at different ages between 5 and 100 days post-hatch. We observed a large decrease of the Vmax and Km of the 5β-reductase and a substantial decrease of the Km of the aromatase with increasing age. No significant sex difference in these parameters was detected. The activity of these enzymes was then studied by a one point assay in 4 nuclei of the song system (*X, RA, ICo, HVc*), 2 nuclei of the visual system (*ectostriatum*, *n. rotundus*) and in limbic and hypothalamic areas. Very noticeable was the presence of a very high not sexually dimorphic aromatase activity in the hippocampal region and in the *nucleus taeniae* and the absence of this enzyme in *ICo*. We found a higher aromatase activity in male than female preoptic area-anterior hypothalamus and a higher 5α- but lower 5β-reductase activity in *RA* of males compared to females. Many age-related metabolic differences were also detected but these do not have a clear interpretation since the Km of these enzymes also changes with age. Extremely low levels of 5β-reductase activity were found in the nuclei of the visual system in adult birds while this enzymatic activity was very high in young birds. The function of this change with age remains obscure. Correlations are thus observed between the neuroanatomical distribution of T-metabolizing enzymes and of androgen and estrogen receptors with the important exception of *ICo* which has no aromatase but contains high concentrations of estrogen receptors. T-metabolizing enzymes are however also present in areas which are not known as steroid targets.

113.9

ESTROGEN STIMULATES THE INCORPORATION OF NEW NEURONS INTO AN AVIAN SONG NUCLEUS DURING ADOLESCENCE. E.J. Nordeen and K.W. Nordeen. Psychology, U. Rochester, Rochester, NY, 14627.

The avian song nucleus hyperstriatum ventralis pars caudalis (HVc) contains more neurons in adult males (who sing) than in females. In part, this sex difference develops because HVc neurons born during song learning are added in greater numbers in young males than in females. We report here that estradiol (E2) masculinizes HVc by stimulating the addition of these newly-generated neurons.

Two days after hatching, female zebra finches received silastic implants of E2. Beginning 20 days after hatching, these females and normal males and females were injected with ^{3}[H]-thymidine daily for 20 days. On day 25, the E2-treated females were implanted with either empty or dihydrotestosterone (DHT)-filled silastic tubes. Birds were killed at 64 days of age and coronal sections (10um) were processed for autoradiography.

In females, early E2 treatment increased the addition of HVc neurons born after 20 days of age. Exposure to DHT during the juvenile period did not augment this increase. The percentage of labeled HVc neurons in males (19.9%), E2-treated females (23.4%) and E2+DHT-treated females (19.0%) was greater than in normal females (11.9%). These data suggest that sex differences in HVc neuron number arise because in males, early E2 exposure promotes the production or survival of HVc neurons born during the period of song learning.

113.10

EFFECT OF HVC LESIONS ON ESTRADIOL-INDUCED MASCULINIZATION OF ZEBRA FINCH SONG SYSTEM. K. Herrmann* and A.P. Arnold (SPON. S.C. Lee) Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The song system of male zebra finches is much larger than in females. Early treatment with estradiol (E2) can masculinize female song areas by increasing both the number and size of neurons. The mechanism by which this masculinization occurs is unknown. One hypothesis is that E2 acts transsynaptically by first masculinizing one nucleus which then masculinizes the development of other song regions. We tested this idea by lesioning HVc (caudal nucleus of the ventral hyperstriatum) unilaterally in 20 day old female zebra finches which were implanted with a 200 ug E2 pellet. At day 60 the birds were sacrificed and the volume of RA (robust nucleus of the archistriatum), area X and MAN (magnocellular nucleus of the anterior neostriatum) were analyzed for interhemispheric differences.

Most pronounced left/right differences after unilateral HVc lesions were seen in area X which was virtually absent or very small on the side of the lesion. RA volume was reduced by about 30% ipsilateral to the lesioned HVc, whereas MAN seemed to be masculine in morphology and symmetric and therefore not affected by the lesion. These data suggest that the E2-induced masculinization of area X, and to a lesser degree of RA, is dependent on the presence of HVc, whereas the masculinization of MAN does not seem to require an intact HVc.

Supported by a grant from the DFG (He1539/1-1) and NIH grant NS 19645.

113.11

ULTRASTRUCTURAL CORRELATES OF SONG DEVELOPMENT IN THE ZEBRA FINCH. R.P. Clower and T.J. DeVoogd. Dept. of Psychology, Cornell University, Ithaca, NY 14853.

The production of birdsong is controlled by a well defined system of brain nuclei, the core elements of which include HVc, RA, and nXII. In the male zebra finch, all three of these areas are clearly recognizable at 10 days of age, but the projection from HVc to RA does not appear to become functional until about day 30 (Konishi and Akutagawa, 1985). Kirn and DeVoogd (1985, 1986, and 1987) have shown that neurogenesis and cell death combine during development to produce the nucleus volumes and cell densities typical of adults.

While the sensitive period for song learning may last from 20 or 30 to as late as 85 (Price, 1979) days of age, the most dramatic behavioral changes, and probably most syllable aquisition, take place before day 60. It also appears to be during these first two months that the gross structure of the descending song system is established. We have found that substantial ultrastructural modification is also taking place during this period (Clower and DeVoogd, 1987). In RA of male zebra finches, synapse density increases across 5, 10, and 20 days, and by day 30 has reached the level seen in adults. In HVc, increases in synapse density seem to result in a state of overproliferation around day 30; the density in adults is about 31% lower.

To complement the above developmental data, we are now examining the emergence of synapses in female and isolate male zebra finches. If females do not sing in part because the anatomy of the system will not support song, this may be reflected in fine structure. The trend in RA toward higher synapse density with age seems to hold for females. At days 10, 20, 30 and 100+, values for synapses/100μ^2 are 4.1, 11.5, 16.5, and 19.8 respectively. In adult male zebra finches, the volume of RA is 4.8 times that in females (Nottebohm and Arnold, 1976). The density at 100+ days is somewhat higher than that found in males (13.7 synapses/100μ^2), probably due to what amounts to a compressed condition of the female nucleus relative to the male.

113.12

MORE EVIDENCE CONCERNING HYPERMASCULINIZING EFFECTS OF ANTIESTROGENS IN THE ZEBRA FINCH SONG SYSTEM. G. A. Mathews* & A. P. Arnold. Dept. of Psychology and BRI, UCLA, Los Angeles, CA 90024.

Tamoxifen, an antiestrogen, hypermasculinizes the song system of male zebra finches, *Poephila guttata* (Mathews et al., 1987). This result does not support the hypothesis that estrogen normally masculinizes the male's song system after hatching. Here we report similar hypermasculinizing effects with two other antiestrogens, and at two different ages. Zebra finch chicks of both sexes were subcutaneously injected daily with CI628 (25ug/3ul propylene glycol [PG]), LY117018 (50ug/3ul PG), or 3ul PG (controls) for the first 20 or 25 days post-hatch. The males in the 20-day groups were castrated at Day 20 and all animals in these groups were sacrificed at Day 60. The animals in the 25-day groups were sacrificed on Day 25.

Neuronal soma areas in MAN (magnocellular nucleus of the anterior neostriatum) were increased in males, but not females, at both ages by CI628 (p = .004) and by LY117018 (p < .001). At Day 60, but not at Day 25, areas of neuronal somas in HVc (caudal nucleus of the ventral hyperstriatum) were larger in treated males and females than in controls of each sex (p < .001). Neither antiestrogen affected neuronal soma areas in RA (robust nucleus of the archistriatum). Area X was the only song region whose volume was affected by the two antiestrogens. Females injected with both drugs possessed Area X, unlike controls. Area X in LY117018 males was smaller than in controls at Day 25 (p = .01) but larger than in controls at Day 60 (p = .004). Although the effects reported here are weaker and less consistent across song regions than those found with tamoxifen (Mathews et al., 1987), our results suggest that tamoxifen is not unique among antiestrogens in its hypermasculinizing effects. Moreover, like tamoxifen, the present antiestrogens failed to demasculinize young males. Supported by NIH Grant NS19645.

113.13

CASTRATION AND ANTI-STEROID TREATMENT IMPAIR VOCAL LEARNING IN MALE ZEBRA FINCHES. S.W. Bottjer & S.J. Hewer. Department of Biology, USC, Los Angeles, CA 90089-0371.

Both song behavior and its neural substrate are hormone-sensitive: castrated adult male zebra finches need replacement of gonadal steroids in order to restore normal song behavior, and sex steroids are necessary to establish neural song-control circuits during early development. This pattern of results suggests that hormones may be necessary for normal song development, but direct evidence that steroids are necessary for normal neural and behavioral development during song learning is lacking.

We addressed this question by attempting to eliminate gonadal steroids in juvenile male zebra finches between the time of initial song production and adulthood. Males were anesthetized and castrated at 20 days; they received Silastic implants of either an anti-estrogen (tamoxifen) and/or an anti-androgen (flutamide). Control birds were treated similarly but were sham-operated and received empty implants. At day 40, all birds were housed in individual cages and their song behavior was recorded until day 90.

Preliminary results indicate that the stereotypy of the overall song pattern as well as the production of individual syllables was disrupted in experimental birds. All birds were assigned a number between 1 and 5 indicating song quality (1 = very poor; 5 = very good). The $\bar{X} \pm$ SD for control (n = 7) and experimental (n = 6) birds, respectively, was 4.0 $\pm$ 1.4 (median = 5.0) and 1.7 $\pm$ 1.2 (median = 1.0). These results indicate that gonadal steroids are necessary during the time that male zebra finches are learning to produce the efferent commands that correspond to their normal song pattern.

113.14

CHRONIC TESTOSTERONE ADMINISTRATION IMPAIRS VOCAL LEARNING IN MALE ZEBRA FINCHES. S. Korsia & S.W. Bottjer. Department of Biology, USC, Los Angeles, CA 90089-0371.

Juvenile male zebra finches learn their song during a specific period of development. Once their vocal pattern becomes stereotyped, it is preserved unchanged throughout adult life. In adulthood, gonadal hormones are necessary for normal song production, but song learning occurs even in the absence of gonadal steroids.

Prompted by evidence that testosterone (T) modulates neural and behavioral plasticity, we investigated the effects of chronic administration of T on song learning and behavior in male zebra finches. Continued exposure to T might impair brain plasticity and consequently decrease learning ability.

We treated male zebra finches from the day of birth until adulthood with T. Subcutaneous implants were made so as to maintain a rate of release of roughly 10mgT/kg/day during the first 20 days of life. At day 20, the birds received Silastic implants packed with 10mm of crystalline T. At day 60, the birds were housed in individual cages and their song behavior was recorded until day 90, when stereotyped song has crystallized.

Preliminary results indicate that the number of different syllables produced during each song bout was decreased, resulting in a very limited repertoire. Furthermore most of the birds had stereotyped songs, with a tendency to repeat a single syllable in a pattern lacking the phrasing characteristic of normal song. Thus the results indicate that chronic administration of T disrupts the normal process of song learning and produces significant abnormalities in song structure relative to normal male songs.

113.15

NEURONAL TYPES IN CANARY HVc, A TELENCEPHALIC SONG CONTROL NUCLEUS: A QUANTITATIVE GOLGI STUDY. B. E. Nixdorf, S. Davis* and T. J. DeVoogd. Cornell University, Department of Psychology, Ithaca, N.Y.14853

The song control system of the canary brain is a fascinating system in which to study the effects of steroids on brain development and function, adult neurogenesis and neuronal plasticity, recovery of function, lateralization and sexual dimorphism - issues of general interest to neurobiology. The brain pathways for song control are well described (Nottebohm et al. 1976). Hyperstriatum ventralis, pars caudalis (HVc) receives direct or indirect input from Field L, a major telencephalic auditory station and projects indirectly to the syrinx, the avian vocal organ. Lesion and electrophysiological studies (Margoliash,1983; McCasland,1986) support a central role of HVc in integrating auditory and motor aspects of song. HVc in canaries is much larger in males than in females, a difference parallel to sex differences in singing behavior. The sex difference in HVc size seems closely related to the developmental and adult hormonal status of the animal. Each of these discoveries concerning HVc make it more clear that many exciting sorts of integration occur here and that there are many questions which presently cannot be answered.

To form a structural foundation for experimental manipulation and study of HVc, we have identified neuronal types in HVc in rapid Golgi-stained material, tracing them with the aid of a computer system which allows three dimensional reconstruction and analysis . Sholl analyses, branch morphology and dendritic spine density at various dendritic sites have been used for classification. We have analyzed a total of 103 neurons from adult male and female canaries. We find seven different cell classes in HVc, at least two of which appear to be sexually dimorphic. Dendritic fields range in size from 900 to more than 3800 µm and consist of from 30 to 80 branch segments. Spine density ranges from near zero on smooth stellate-like cells to 2.3 spines / µm on the class most densely packed with spines. Work is in progress to determine to which of these classes adult neurogenesis contributes.

113.16

ANDROGEN-INDUCED SEXUAL DIMORPHISM: PHENOTYPIC ALTERATION OF AN INTRASPINAL SUBSTANCE P (SP) PATHWAY. B.W. Newton and R.W. Hamill. Dept. of Neurology, Monroe Community Hospital / University of Rochester, Rochester, NY 14603

The sexually dimorphic male (M) cremaster nucleus (CN), in the rat L1,2 ventral gray spinal cord, receives a massive intraspinal SP innervation which diverges over CN motoneurons. These M SP patterns are nearly absent in females (F). To test whether the expression of the SP CN-pathway is androgen dependent, F rats were injected with testosterone propionate (TP; 1mg s c.) on postnatal days (P)0,2,4; and on P20,40,60 the SP patterns were examined in littermate M, F, and F-TP rats with the PAP technique. Anti-SP (IncStar lot # 8642009) was diluted 1:8000.

P20 M had a dense SP CN-pathway and moderate CN SP. These patterns were nearly absent in F. F-TP rats had a moderate SP CN-pathway but sparse CN SP. P40 M had a dense SP CN-pathway and dense CN SP. F had an adult SP pattern: sparse SP in the CN-pathway and CN. F-TP rats had a nearly M-like CN-pathway and moderate CN SP. **On P60 both M and F-TP had a M adult-like pattern:** a very dense SP CN-pathway and dense CN SP. F had no change from P40.

Thus, early postnatal TP causes the expression of the M intraspinal SP phenotype in adult F rats. This suggests that androgens either directly or indirectly influence F SP intraspinal neurons to produce SP at levels which are immunohistochemically similar to M. Supported by NS 22103 and Monroe Community Hosp. / U of R research funds.

113.17

IMMUNOHISTOCHEMICAL PATTERNS OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN RAT LUMBAR SEXUALLY DIMORPHIC NUCLEI. R.W. Hamill and B.W. Newton. Depts. of Neurology, and Neurobiology and Anatomy, Monroe Community Hospital / University of Rochester, Rochester, NY 14603

The male (M) rat lumbar spinal cord contains three known sexually dimorphic (s.d.) nuclei (spinal n. of the bulbocavernosus (SNB), dorsolateral n. (DLN) and cremaster n. (CN)) which contain a greater number of larger motoneurons than females (F). CN innervation is sexually dimorphic for 5HT, and substance P (M>F). Other peptides, such as CGRP, may also be s.d. Adult M and F rats (12) were perfused (Zamboni's) for the PAP technique and anti-CGRP (1:10,000) was applied. No colchicine was used.

In all cases M>F for SNB-, DLN- and CN-CGRP fibers. M CN CGRP fibers >> than all other M & F s.d. nuclei. Only occasional CGRP fibers were observed in M & F SNB and DLN, but CGRP fibers in all s.d. nuclei were > than surrounding ventral horn. However, CGRP fibers in entire VGH << than in laminae I-IV. Most VGH motoneurons contain CGRP except for the s.d. nuclei. M & F SNB and DLN contain scattered CGRP motoneurons whereas the M & F CN motoneurons do not apparently contain CGRP in non-colchicinized rats.

Thus s.d. nuclei are innervated by more CGRP fibers than the surrounding VGH but contain far fewer CGRP-containing motoneurons. CGRP innervation of s.d. nuclei may carry nociceptive information. Supported by NS 22103 and MCH/U of R research funds.

113.18

NERVE GROWTH FACTOR (NGF) PLAYS A PHYSIOLOGICAL ROLE IN FEMALE SEXUAL DEVELOPMENT. **H. Lara*, J.K. McDonald, S.R. Ojeda. Div Neuroscience, OR Reg Pri Res Ctr, Beaverton, OR 97006, and Dept Anat (JKM), Emory Univ, Atlanta, GA.**

Little is known regarding the role of growth factors in the development of neuroendocrine reproductive function. High titer polyclonal antibodies against NGF were produced in rabbits and injected into neonatal rats (12.5 μl/g BW, sc, postnatal day 1-3). While body growth and age at vaginal opening were normal, the age at first ovulation was significantly delayed, and the subsequent estrous cyclicity severely disrupted. Reproductive performance was diminished, as determined by the inability of 50% of the treated rats to sustain pregnancy. The ovaries from antiNGF-treated rats showed an absence of noradrenergic nerves and a marked reduction in neuropeptide Y and calcitonin-gene related peptide nerve fibers. The ovarian estradiol response to hCG was blunted. At a central level, median eminence LHRH nerve terminals from antiNGF-treated rats responded significantly less than controls to stimulation with prostaglandin E_2, a mediator of norepinephrine-induced LHRH release. The results indicate that: a) NGF, through its trophic effect on peripheral sympathetic neurons, plays a role in the acquisition of female reproductive capacity, and b) NGF may be involved in facilitating the development of the reproductive hypothalamus. (Grants HD24870 & RR00163 from the NIH, & The Rockefeller Fnd)

NEUROPEPTIDES AND BEHAVIOR I

114.1

POSSIBLE MU-1 OPIOID RECEPTOR INVOLVEMENT IN MATERNAL BEHAVIOR IN RATS. P.E. Mann, G.W. Pasternak, & R.S. Bridges. LHRRB, Harvard Medical School, Boston, MA 02115, Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Ctr., New York, NY 10021

Maternal behavior (MB) is under an inhibitory influence of opiates. Systemic and central administration of morphine (MOR) disrupts MB in steroid-primed, pup-induced virgin and lactating rats. Naloxonazine (NAZ), a selective and irreversible antagonist of mu-1 opioid binding sites, blocks certain pharmacological effects of opiates. The aim of the present study was to determine if MOR disruption of MB involves the mu-1 binding site. Virgin, Sprague-Dawley rats were mated and on day 3 postpartum jugular catheters were implanted in all rats. On day 5 the rats received one of the following treatments: MOR alone (10 mg/kg, n=10, s.c.); MOR (10 mg/kg) 24 h after NAZ pretreatment (10 mg/kg, n=10, i.v.); MOR (10 mg/kg) 1 min after naloxone pretreatment (NAL, 1 mg/kg, n=10, s.c.); or MOR (10 mg/kg) 24 h after NAL pretreatment (10 mg/kg, n=8, i.v.). MOR alone completely disrupted MB (0% responded) which was blocked by NAL administered 1 min before (100%). NAZ treatment partially blocked MOR disruption of MB (36% responded) which was significantly different from MOR alone. NAZ blocked MOR's effect on pup retrieval to an even greater degree (70% responded vs. 10% in MOR alone). NAL administered 24 h before MOR had no effect. These results suggest involvement of the mu-1 opioid receptor in the regulation of maternal behavior.

114.2

CORRELATION BETWEEN PARENTAL BEHAVIOR AND INFUNDIBULAR VIP-LIKE IMMUNOREACTIVITY IN THE DOVE. R. Cloues* and R. Silver (SPON: S. Stone), Psychology Department, Barnard College of Columbia University, NY, NY 10027.

It is well established that there is an association between prolactin and parental behavior in birds. Recent evidence suggests that the neuropeptide VIP releases prolactin in hens (MacNamee et al, GCE, 1986). We used immunohistochemical techniques to assess the possibility that VIP plays a role in naturally occuring changes in plasma prolactin during breeding in ring doves (Streptopelia roseogrisea). First, we determined the distribution of VIP-like immunoreactive cells throughout the brain. These cells are found in the septum, hyperstriatum, hypothalamus, and infundibular region. Next, we compared VIP profiles in breeding and non-breeding birds. There were differences between parental and non-parental birds in the ventral portion of the infundibular region. More specifically, there is an increase in VIP cell size and staining density in this region in breeding birds compared to simultaneously processed tissue from control animals. In both sexes, these changes in immunoreactivity are detectable at incubation day 5, anticipating the increase in plasma prolactin. Supported by NIMH grant 29380.

114.3

MATING ALTERS OXYTOCINERGIC TOPOGRAPHY AND CONTENT IN MALE MICE

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The impact of mating on the distribution of hypothalamic oxytocin neurons and on oxytocin content was studied immunocytochemically and with radioimmunoassays of microdissected hypothalamic nuclei. 15 male unmated white mice were exposed to receptive females overnight. Mating was confirmed by the presence of sperm in the vaginal smear. The males were killed immediately thereafter. Another group of males was exposed to receptive females permanently for two months, unmated males served as controls. In singly mated animals oxytocin immunostained neurons appeared in the paraventricular- and supraoptic nuclei and were scattered in the ventral and dorsal preoptic region. In unmated and repeatedly mated males additional intensely immunostained oxytocin neurons were found in the medial and dorsal preoptic region, in the perifornical region, in the zona incerta and in the ansa lenticularis. In radioimmunoassays of the magnocellular nuclei, smaller amounts of oxytocin were measured in singly mated males as compared with controls or repeatedly mated males. The observed dynamics of the male oxytocinergic brain topography are perhaps due to steroid-dependent changes of the secretory activity of oxtocinergic systems. It is likely that oxytocin is involved in physiological and behavioral changes known to occur in males during sexual activity.
(Supported by DFG Grant Ji 10/1-1)

114.4

IN VIVO MEASUREMENT OF BIOGENIC AMINES FROM THE PARAVENTRICULAR NUCLEUS (PVN) USING PUSH-PULL PERFUSION: RELATION TO OXYTOXIN (OXY) INDUCED YAWNING. N.J. Laping and V.D. Ramirez. Dept. of Physiology, University of Illinois, Urbana, IL 61801.

Young adult male rats implanted with a push-pull cannula directed at the PVN were perfused with modified KRP buffer. Samples were collected every two minutes for one hour at a flow rate of 18μl/min while yawns were simultaneously recorded. After a ten minute basal collection period, the normal KRP was replaced with KRP containing 50ng/μl OXY for a ten minute duration. Samples were analyzed for dopamine (DA), DOPAC, and 5-HIAA using HPLC-EC. Detectable levels of DOPAC were recorded in all rats with basal levels of 5-100pg/min. Most animals also showed detectable levels of 5-HIAA with basal levels of 150-300pg/min. Samples from one animal also revealed detectable levels of DA with a release pattern mimicking that of DOPAC and basal values of 0-12pg/min. After the OXY treatment yawning was observed in 4 out of 5 rats. Levels of DOPAC during yawning behavior were compared with pre-OXY DOPAC values. This analysis revealed that yawning is associated with momentary decreases in DOPAC release from the PVN (-7.6 ± 4.0pg/min) vs. randomly selected DOPAC changes (10.5 ± 6.1pg/min), suggesting an inhibitory control of DA on yawning behavior.

114.5

ARCUATE NUCLEUS LESIONS MODIFY YAWNING BEHAVIOUR IN THE RAT. E. Doger* and B. Holmgren* (SPON: M.R. Budelli) Dept. de Ciencias Fisiológicas ICUAP, Univ. Aut. de Puebla.MEXICO

Yawning is a discrete behavioural item, that occurs spontaneously at variable frequencies, and is under a complex set of neurotransmitter influences: cholinergic excitatory, dopaminergic (DA) excitatory/inhibitory and other. Yawning can also be elicited by peptidergic hormones: ACTH, MSH, prolactin, oxytocin. Different experimental techniques have led to the proposition that many brain structures (septum, rombencephalon, striatonigral system, hippocampus, paraventricular hypothalamic nucleus, cerebral cortex) and the hypophysis are somehow involved in yawning. In the search for neuroanatomical sites of interactions between neurotransmitters and hormones in the control of yawning, we have made radiofrequency lesions of the arcuate hypothalamic nucleus (AHN) in male Sprague Dawley Rats. The AHN has an important number of DA and pro-opiomelanocortin neurons, and is known to exert an inhibitory control over the adenohypophysis. Bilateral lesions of AHN (n=17) produce a sixteen fold increase in average yawning frequency (normalized values), 24 h. after surgery, effect which declines progressively along 8 days. Unilateral lesions (n=11) induce a lower effect and sham lesioned rats show no change in yawning. Our results thus suggest that the AHN exerts a tonic inhibitory influence on yawning, and that AHN and the adenohypophysis might represent a DA-peptidergic link in the control of this behaviour.

114.6

ANTERIOR HYPOTHALAMIC VASOPRESSIN BLOCKADE INHIBITS INTRASPECIFIC AGGRESSION IN MALE HAMSTERS. M. Potegal and C. Ferris. N.Y. State Psychiatric Inst. and U. Mass. Medical Center, Worcester MA

Vasopressin-sensitive neurons in the anterior hypothalmus have previously been shown to affect flank marking, a behavior which occurs with high probability in hamster agonistic encounters. The contribution of these neurons to intraspecific aggression itself was evaluated in two experiments involving intrahypothalamic injections of the V_1-receptor antagonist $d(CH_2)_5Tyr(Me)AVP$. In the first experiment, in which two adult hamsters were paired in a neutral cage with both, one or neither animal having been microinjected with the antagonist, the drug significantly reduced aggression. The second experiment determined if the drug suppressed aggression directly or just affected the animals' aggression eliciting qualities. We found that the antagonist produced a significant, dose-related suppression of resident's attacks on a small intruder hamster. Because these intruders neither initiated aggression nor counter-attacked V_1 receptor blockade in the anterior hypothalamus appears to suppress aggression directly. Furthermore, the drug did not affect social contact nor sexual behavior toward an estrous female. Linkage between the vasopressin control of flank marking and aggression will be explored.

Supported by NIH Grant NS-23557 to C. Ferris

114.7

FLANK MARKING FOLLOWING LESION OF VASOPRESSINERGIC NUCLEI IN GOLDEN HAMSTERS. R.W. Irvin*, J.F. Axelson and C.F. Ferris. Dept Physiology, Univ Mass Medical Ctr. Worcester MA 01655

Flank marking in hamsters is controlled by vasopressin (VP)-sensitive neurons in the anterior hypothalamus (AH). The VP innervation to the AH involved in the control of flank marking is unknown. The present study examines flank marking prior to and following kainic acid lesions of the supraoptic nucleus (SON) (n=9), suprachiasmatic nucleus (SCN) (n=5), paraventricular nucleus (PVN) (n=5), bed nucleus of the stria terminalis (BNST) (n=6) and the nucleus circularis (NC) (n=10) of the AH. Hamsters were sacrificed and the lesion sites examined following ICC with anti-AVP using PAP with DAB. Lesioning VP nuclei outside the AH had no appreciable effect on flank marking despite the loss of VP immunoreactivity in the area of the lesion. The only lesion sites that consistently abolished flank marking were localized the area of the NC. However, since the NC is found in the same region of the hypothalamus that also controls flank marking it is not possible to lesion these cells without damaging the surrounding AH. While these results indicate that the VP neurons of the SON, PVN, SCN, and BNST do not appear to play an essential role in the control of flank marking the role of the NC is still unclear.

(This work was supported by NIH Grant NS-23557 to C.F.F.).

114.8

POSTERIOR PITUITARY HORMONES AND SEPARATION DISTRESS IN CHICKS. Jaak Panksepp, Department of Psychology, Bowling Green State University, Bowling Green, OH 43403.

Avian and mammalian posterior pituitary peptides Arg-vasotocin (AVT) and oxytocin (OXY) were found to be remarkably effective in reducing separation induced distress vocalizations (DVs) in chicks while neither the constituent fragments, tocinoic acid and Pro-Leu-Gly, nor Arg-Vasopressin (AVP) exert such an effect. The effects appeared to be behaviorally specific.

Domestic chicks between 2-14 days of age were injected into the 4th ventricle region with the above peptides just prior to separation. At doses above 10 picomoles of either AVT or OXY, highly reliable reductions in DVs were observed, the effect lasting about 2-3 hrs at 0.1 - 1.0 nmole doses. The animals exhibited no apparent sedation. Following these doses of AVT and OXY, three other discrete behaviors were markedly potentiated --1) lateral head shaking movements, 2) yawning, and 3) wing flapping. The wing flapping produced by AVT and OXY required social-facilitation for full expression, while the head shaking and yawning did not. Head shaking and yawing were potentiated by conjoint treatment with CRF (1 ug) while OXY-induced wing-flapping was reduced by CRF. Peripheral administration of these peptides yielded none of the above effects. The OXY and AVT effects did not show apparent tolerance during 7 successive test sessions. None of the above effects were observed by comparable doses of the ring (tocinoic acid) or linear segments (Pro-Leu-Gly) of OXY. AVP (1 ug) yielded modest increases in DVs with no other clear behavioral effects.

These results suggest that a normal brain function of OXY and AVT peptide systems is the reduction of separation distress, raising the possibility that social bonding mechanisms and related emotions may be mediated by such neurochemistries. An additional impression of the present investigator was that OXY and AVT treated animals appeared to be more less "fearful" or more "courageous" than controls.

114.9

a-MSH EFFECTS ON SEPARATION INDUCED DISTRESS VOCALIZATIONS IN CHICKS. B. Abbott* and J. Panksepp (Spon, F.G. DeEskinazi), Dept of Psychology, Bowling Green State University, Bowling Green, OH 43403

Past work has demonstrated that brain opioids very specifically reduce activity in separation distress vocalization (DV) circuits of the brain. Since a-MSH is co-localized in the same neurons as B-endorphin, it was of interest to determine whether that peptide would also modulate separation distress induced by isolating young animals from social stimuli. In 2-12 day old domestic chicks, centrally administered a-MSH (into the 4th ventricle region), but not peripheral a-MSH yielded powerful biphasic effects on DVs. Following an initial modest elevation of DVs (which lasted about 15 minutes, vocalizations were markedly reduced for an hour and a half. The elevation of vocalizations was observed at doses of .04, 0.2, 1.0 and 5.0 µg, but not at .008 µg, and was most clearly seen when base line levels of DVs were reduced by either testing animals in *pairs* or *ndividually* in mirrored test chambers. The two effects may be controlled by different mechanisms since low doses (.04 µg) were more effective in elevating DVs while high doses (1.0 µg) were more effective in reducing DVs. The effects of a-MSH (1 µg) did not exhibit tolerance over four repeated days of testing. Naloxone and morphine interactions studies indicated these effects were independent of *mu*-opioid receptor systems. Birds pre-treated with naloxone (1 mg/kg, i.p.) exhibited no change in the ability of 1 µg of a-MSH to reduce DVs. Likewise, the quieting effects of morphine (5 mg/kg i.p.) were not reduced by central a-MSH.

All doses of a-MSH which reduced DVs produced a strong squatting tendency resembling freezing responses normally seen during fear. Fear is known to reduce DVs, and a-MSH induced reductions in DVs could be explained by such a mechanism of action. Since fear increases measures of tonic-immobility (TI), we evaluated the effects of 1 µg of a-MSH on duration of TI in 3 week old birds. Duration of TI was doubled by this dose of a-MSH suggesting that central a-MSH participates in the generation of behavioral inhibition which normally accompanies fear. Whether a-MSH promotes just the behavior or the central affective state of fear needs to be evaluated in future research.

114.10

THE PROFILE OF NEUROBEHAVIORAL EFFECTS PRODUCED BY NEUROPEPTIDE Y. A. Drumheller[1], R. Rivest[1], J.N. Michaud*[1], S. St-Pierre*[2] and F.B. Jolicoeur[1]. [1]Dept. Psychiatry, Fac. of Med., Univ. of Sherbrooke, Québec J1H 5N4 and [2]INRS, Blvd. Hymus, Point Claire, Québec H9R1G6.

Neuropeptide Y (NPY) is a recently discovered member of the pancreatic polypeptide family and has been shown to be widely distributed in mammalian brain (Allen et al., Science 221:877, 1983). Studies concerned with delineating the possible neurobehavioral effects of this peptide have concentrated mainly on the effect of NPY on food and water intake in rodents. However, in view of the pervasive distribution of the peptide in the CNS we thought it worthwhile to design experiments aimed at constructing a more complete profile of the neurobehavioral effects of NPY following intracerebroventricular injections. More specifically, the effects of several doses (2.5-20µg) of the peptide injected into the lateral ventricle on spontaneous motor activity, muscular tone, body temperature, nociception and food intake, as well as its potential for producing catalepsy, were examined. Results indicate that starting at 5.0 µg, motor activity of animals was significantly decreased in a dose related fashion. At 10 µg, NPY induced significant catalepsy, and increased both muscular tone and food intake. Interestingly, body temperature was significantly increased by the smallest dose administered, but significantly decreased with 10 and 20 µg of NPY. In general, all neurobehavioral effects of NPY were already present at 15 min and persisted upto 60 min following administration. On the other hand, no dose tested significantly affected nociception in the animals as measured by the hot plate test. Together, our results indicate that the profile of NPY's neurobehavioral actions is more complex than previously demonstrated and suggests that the peptide might be implicated functionally in a variety of neurophysiological processes.

Supported by Medical Research Council of Canada PG 38

114.11

CHANGES IN THE CONCENTRATION OF BRAIN NEUROPEPTIDE Y (NPY)-LIKE IMMUNOREACTIVITY FOLLOWING OLFACTORY BULBECTOMY (OB) AND EFFECTS OF ANTIDEPRESSANTS IN RATS. Y.Kataoka* (1), M.Ushio* (2), S.Koizumi* (2), M.Niwa (1) and S.Ueki* (2) (Spon:M.Kabuto). (1) Dept. of Pharmacol.Nagasaki Univ.Sch.Med.Nagasaki 852, (2) Dept. of Pharmacol.Fac. Pharmaceu.Sci.Kyushu Univ.Fukuoka 812, JAPAN

OB-induced muricide (mouse-killing behavior) has been known to be a useful laboratory model for evaluating the property of antidepressants. Our previous study suggested that NPY might be involved in the regulation of muricide induced by OB in relation to that of noradrenaline (NA) in amygdala (AME) (Pharmacol.Biochem.Behav.,28,101,1987). The present study was, therefore, designed to investigate changes in NPY-like immunoreactivity (NPY-LI) and NA concentrations following OB and effects of chronic administration of desipramine (DMI) and mianserine (MIA). NPY-LI concentrations in hypothalamus (ventromedial:VMH, lateral:LH) and AME (basolateral:ABL, central:ACE) increased significantly by 25% and 50-70%, respectively, 28 days after OB. Chronic treatment (7 days) with DMI inhibited the elevation of NPY-LI in accordance with the suppression of muricide. The effect of chronic administration of MIA was similar to that of DMI, though the effect was not pronounced in potency.

Our findings provide further evidence for the involvement of NPY in the pathophysiology of depression and the neurochemical mechanism of action of antidepressants.

114.12

EFFECTS OF ALPHA-HELICAL CRF (9-41) ON CRF-AND FEAR-POTENTIATED ACOUSTIC STARTLE RESPONSE (ASR) IN RATS. K.T. Britton (1), N.R. Swerdlow (1) and G.F. Koob (2). (1) Dept. Psychiatry, UCSD Sch. of Med., San Diego, CA 92193; (2) Preclin. Neurosci., RISC, La Jolla, CA 92037.

The acoustic startle response (ASR) is a reflex contraction of the skeletal musculature following exposure to an intense acoustic stimulus. ASR amplitude is increased by conditioned fear, i.e. when the acoustic stimulus follows a cue previously associated with shock. We have reported that ASR amplitude is also increased by intraventricular (ICV) infusion of CRF, a neuropeptide implicated as a substrate in several fear- or stress-enhanced behaviors. We studied the relationship between CRF- and fear-potentiated ASR using alpha-helical CRF (9-41), a peptide known to antagonize the effects of CRF and fear on other behavioral and endocrine indices.

In the first experiment, pretreatment with CRF (9-41) (0-25 µg ICV) was found to reverse CRF-potentiation of ASR amplitude. CRF (9-41) alone did not significantly alter ASR baseline. In a second study, animals were trained in a conditioned-fear paradigm. CRF (9-41) significantly reversed the effects of conditioned fear on ASR amplitude. Doses of CRF (9-41) that reversed CRF- and fear-potentiated ASR were similar. In a third study, CRF (9-41) failed to antagonize the ASR-potentiating effects of strychnine (0.75 mg/kg, i.p.). These results suggest overlapping neural substrates for CRF- and fear-potentiated acoustic startle and suggest that endogenous CRF systems in the brain may be involved in behavioral responses associated with fear and stress.

114.13

THE IMPORTANCE OF CENTRAL AND PERIPHERAL NORADRENERGIC SYSTEMS IN THE ENHANCED CONDITIONED FEAR PRODUCED BY CORTICOTROPIN RELEASING FACTOR. B. J. Cole* and G. F. Koob. Scripps Clinic and Research Foundation. La Jolla. CA 92037.

Both central administration of corticotropin releasing factor (CRF) and activation of the norepinephrine (NE) containing nucleus locus coeruleus (LC) induce behaviors normally exhibited in threatening situations. Furthermore, CRF has been shown to activate NE neurones in the LC. The purpose of the present series of experiments was to investigate the role of NE in the enhanced fear (as measured in the conditioned emotional response paradigm) and the behavioral activation (as measured by locomotor activity in photocell cages) produced by CRF, using the beta-adrenoceptor antagonist propranolol. d,l- Propranolol itself was found not to affect performance of a conditioned emotional response (CER), but it did antagonize the enhanced conditioned fear produced by CRF. This antagonism was specific to l, propranolol, suggesting that it is receptor mediated. In contrast, propranolol was shown to potentiate the locomotor hyperactivity induced by CRF. These results suggest that activation of beta-adrenoceptors may be an important mechanism in the 'anxiogenic' effects of CRF. In addition, they suggest that the neurochemical mechanisms that underlie the 'anxiogenic' and the 'activating' behavioral effects of CRF are neuropharmacologically distinct. Since propranolol acts both in the central and peripheral nervous system, and CRF has also been shown to activate the autonomic nervous system, we are also investigating the relative importance of central and peripheral NE systems in mediating the behavioral effects of CRF using ST91. This drug is a non-lipophilic, selective alpha-2 agonist, that `does not cross the blood-brain barrier.

114.14

CORTICOTROPIN-RELEASING FACTOR PRODUCES ANXIOGENIC & BEHAVIORAL ACTIVATING EFFECTS FOLLOWING MICROINFUSION INTO THE LOCUS COERULEUS. P.D. Butler, J.M. Weiss, J.C. Stout*, C.D. Kilts, L.L. Cook*, and C.B. Nemeroff. Depts. Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.

Intracerebroventricular (ICV) injection of CRF produces anxiogenic effects and increases locomotor activity in rats. The norepinephrine-rich locus coeruleus (LC) may be of importance in producing these CRF effects; CRF terminals innervate the LC, CRF increases the firing rate of LC neurons, and CRF concentrations are increased in the LC following acute and chronic stress. To examine behavioral activation, struggling and floating were measured in Long-Evans hooded rats for 15 min using the modified Porsolt swim test. Forty-five min following ICV injection, CRF produced a dose-dependent decrease in floating, with the lowest effective dose being 500 ng. However, bilateral infusion of CRF directly into the LC produced significant decreases in floating at doses as low as 10 ng, consistent with the hypothesis that the LC may mediate CRF-induced increases in activity. To examine anxiogenic activity, rats were placed in an open field containing a small darkened compartment for 15 min. Forty-five min following infusion into the LC, CRF significantly increased the time rats spent in the compartment and decreased the amount of time spent exploring the outside of the compartment or venturing into the inner squares of the open field, all indices of anxiogenic behavior. Thus the LC also appears to play a role in the anxiogenic properties of CRF. Preliminary results indicate that bilateral infusion of CRF into the LC produces significant increases in the concentration of the NE metabolite DHPG in several forebrain projection areas of the LC (i.e. the posterior hypothalamus and the amygdala). These data, taken together, suggest that CRF may produce its locomotor activating and anxiogenic effects, in part, by increasing activity of LC NE neurons. (NIMH MH-42088, MH-40406 and MH-42637.)

114.15

NORADRENERGIC ACTIVATION OF CRF SECRETION MEDIATES STRESS-INDUCED CHANGES IN EXPLORATORY BEHAVIOR. Craig W. Berridge and Adrian J. Dunn, Department of Neuroscience, University of Florida, Gainesville, FL 32610

Exploratory behavior as measured by the time an animal spends investigating objects in a complex novel environment has been previously shown to be decreased by various stressors (Arnsten et al., 1985; Berridge & Dunn, 1986). Stress activates the release of both norepinephrine (NE) and corticotropin releasing factor (CRF) from neurons in the brain and both have been implicated in regulating behavioral responding. Thus, either or both NE and CRF may be responsible for the stress-induced decrease in exploratory behavior.

This effect of stress can be mimicked in mice by intracerebroventricular (ICV) CRF (5-100 ng) or by promoters of noradrenergic activity; a noradrenergic α-agonist, phenylephrine (50-100 ng injected ICV), and the α_2-antagonist, idazoxan (1.0 mg/kg). Further, the effect of restraint-stress can be antagonized by an α_1-antagonist (prazosin, 100-200µg/kg), an α_2-agonist (clonidine, 25 µ/kg), a noradrenergic neurotoxin (DSP-4) which destroys norepinephrine-containing neurons, and a CRF-antagonist (alpha-helical CRF_{9-41}, ICV 10-50 µg). These data implicate both CRF and NE in the stress-induced change in behavior observed in this paradigm

The behavioral response to ICV CRF is not affected by any of the above mentioned noradrenergic antagonists (DSP-4, prazosin, clonidine). However, the response to the noradrenergic α-agonist, phenylephrine (50-100 ng; ICV) can be reversed by alpha-helical CRF_{9-41} (20 µg). Therefore, these results suggest that behavioral responding during stress, involves an activation of noradrenergic systems which in turn stimulates the secretion of CRF.

This research was supported by NIMH (25486).

114.16

CENTRALLY ADMINISTERED GROWTH HORMONE-RELEASING FACTOR AFFECTS FEEDING DIFFERENTIALLY ACROSS PHOTOPERIODS. D. Feifel and F.J. Vaccarino. Departments of Psychology and Psychiatry, University of Toronto, Canada.

We have previously reported that intracerebroventricular (ICV) administration of GRF in doses ranging from 0.4 to 40 picomoles produces a short-term facilitation of eating in the rat. Since all previous investigations were restricted to light photoperiods the present set of studies sought to investigate possible light/dark differences in GRF's feeding effect. Twenty rats were administered 0 (vehicle), 0.4, 4 and 40 picomole rhGRF ICV in the dark or light phase of a 12h:12h light:dark photoschedule. Feeding for the 90 minutes following injections in the light was dose-dependently facilitated similar to our previous results. Conversly, in the dark, a significant dose-dependent inhibition of eating was produced. Both facilitatory and inhibitory effects of GRF were limited to short-term feeding since no effect was seen on 24 hour consumption. Furthermore, since an inactive GRF analogue had no significant effect on eating and GRF itself did not significantly alter locomotor activity in either photoperiod, the differential effects of rhGRF on feeding seem not to be the result of non-specific neurochemical or behavioural influences. Together with other evidence from our lab, this study suggests that GRF may play some role in the endogenous regulation of circadian eating.

114.17

ACIDIC FIBROBLAST GROWTH FACTOR (aFGF) ACTING AS FEEDING SUPPRESSANT. Y. Oomura, K. Hanai*, N. Shimizu*, Y. Kai* and H. Kimura* Dept. of Physiol., Fac. of Med., Kyushu Univ., Fukuoka 812, Japan.

Regulation of feeding behavior and control of body weight involve neuronal and humoral factors. We investigated the effects of aFGF on food intake in the rat.

A phasic increase in aFGF activity was detected in the cerebrospinal fluid (CSF) after feeding. Intraperitoneal glucose injection also produced the increase with dose-dependent manner. CSF samples before and after feeding were applied on a heparin-sepharose column and washed with 1.0 M NaCl. aFGF was eluted in 1.0 M NaCl fraction. From the mitogenic activity change (using BALB/c 3T3 cells) recovered in this fraction before and after feeding, aFGF increased 10 fold after feeding. Intracerebroventricular injection of aFGF (8-20 pmol) suppressed food intake. Electrophoretically applied aFGF specifically suppressed the glucose-sensitive neurons activity in the lateral hypothalamic area (LHA). Neurons in the ventromedial hypothalamus (VMH) were unaffected by aFGF. Immunohistochemical study by aFGF antibody revealed aFGF containing neurons in the LHA, zona inserta, amygdala, hippocampus, globus pallidus, reticular thalamic nucleus, cortex etc. and ependimal cells, but not in the VMH. The glucose injection released aFGF from the neurons. In situ hybridization study indicated aFGF production in the neurons. The results suggest that aFGF participates in the central regulation of feeding.

114.18

DOPAMINE BLOCKADE RELEASES DE-ENCEPHALIZED JUMPING TRIGGERED BY POSTURAL INSTABILITY. T.K.Morrissey*, S.M. Pellis, V.C. Pellis and P. Teitelbaum, Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Rats made cataleptic by systemic haloperidol (0.5-10 mg/kg) jump explosively (up to 86 cm). This seems paradoxical, since the akinesia and inability to initiate movement in catalepsy are often emphasized. However, such catalepsy leaves intact a set of allied reflexes which defend stable static equilibrium (eg. by standing, clinging, bracing and righting). We show that such jumping is not paradoxical but is an allied reflex because it only occurs when postural instability causes the animal's hindlegs to slide forward out from under its body, e.g., by tilting the rat's head down on a board.

The jump under haloperidol is a de-encephalized form, intermediate between decerebrate and normal. Decerebrate cats leap whenever weight is shifted onto the hindlegs and the neck dorsiflexed, even if stable. Such decerebrate jumps are undirected. In contrast, intact rats, given haloperidol, only jump when a key releasing stimulus (instability) is added, but like decerebrate cats, they do not guide their jumps visually and thus leap into walls and over the table edge. In undrugged, intact rats, such jumping is fully adaptive, occurring only in response to postural instability, and only if it can be visually guided to land in a 'safe' location. By these modifications, the primitive jump becomes encaphalized.

114.19

EVALUATION OF THE CENTRAL NERVOUS SYSTEM (CNS) DEPRESSANT EFFECTS OF MURAMYL DIPEPTIDE. K.A. Serpa, W.H. Moos, and L.T. Meltzer. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105

Benzodiazepines (BDZ) are currently the drugs of choice for the treatment of insomnia. Unfortunately, clinically important adverse effects are associated with BDZ use (e.g., CNS depression and drug interactions). Thus, the development of new agents is warranted.

An endogenous muramyl peptide has been identified as the active component of urinary sleep factor S. The synthetic muramyl peptide, N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP), induces sleep in rats, cats, rabbits, and monkeys. In the present studies, possible CNS depressant effects of MDP were evaluated.

In rabbits, a somnogenic dose of flurazepam induced ataxia (CNS depression) while an equisomnogenic dose of MDP did not induce ataxia.

In rats, triazolam produced a greater potentiation of ethanol-induced effects than did equisomnogenic doses of MDP. Both triazolam and MDP increased the percent of animals losing their righting reflex, the increase being greater after triazolam. Only triazolam increased the duration of the ethanol-induced loss of righting reflex.

Taken together, these data indicate that MDP produces less general CNS depression than do benzodiazepines.

114.20

Psychological induction of brain c-fos mRNA. P.F. Morgan, T. Nakajima[1] and M. Linnoila*. LCS, NIAAA and [1]BPB, NIMH, NIH, Bethesda, MD 20892, USA.

Transient induction of the proto-oncogene c-fos mRNA in mouse brain tissue has been reported following chemically- (Morgan, J.I. et al, Sci., 237:192,1987) and electrically- (Nakajima, T. et al, Neurosci. Lett., In press) evoked seizures. Expression of c-fos mRNA was studied in male Hartley guinea pigs 15 minutes after experimental procedures. Total RNA was extracted from whole brain and separated by agarose gel electrophoresis. The RNA was transferred to nitrocellulose membranes and hybridized with a nick-translated ^{32}p-c-fos DNA probe under high stringency conditions. Autoradiograms reveal hybridization to a single band of 2.2 kb. Male Hartley guinea pigs exhibit a delayed righting reflex, which can be of several minutes duration. We report that brain c-fos mRNA can be induced by a 1-minute inversion of such animals ($p<0.01$, compared to control, ANOVA/Scheffe). Brain c-fos is also induced by gently hand-immobilized such animals for a 1-minute period ($p<0.05$, compared to control, ANOVA/Scheffe). These results reveal that c-fos mRNA can be induced by non-invasive manipulations and may be a sensitive marker of psychological activity.

NEUROPEPTIDES AND BEHAVIOR II

115.1

CHOLECYSTOKININ: A MEMORY MODULATOR? E.E.Quinton and D.A.Quinton*. Dept. of Psychology, Univ. of Louisville, Louisville Ky. 40292.

The octapeptide fragment of cholecystokinin, 26-33(CCK-8), is widely distributed throughout the mesolimbic system. It has been claimed that the peptide functions as a memory modulator, but experimental results have been inconsistent when passive avoidance(PA) has been used as the learning paradigm. This study uses a PA procedure that has been extensively utilized to study memory. Groups of male C57BL/6j mice were injected subcu. with 200ug/kg CCK-8, saline, or 5mg/kg proglumid (PROG, a CCK-8 antagonist) immediately after training and 30 min before the test trial 72 hrs later, in a counter balanced design. The test performance of the three groups that received CCK-8 immediately after training was superior to all other groups except the PROG-PROG group, which was also superior to all remaining groups.These data suggest that CCK-8 administered immediately after training can enhance test performance on a PA task, but has no effect on test performance when administered shortly before the test trial. PROG needs to be further investigated.

115.2

EFFECT OF CENTRALLY ADMINISTERED CCK RESEMBLES THAT OF NEUROLEPTIC TREATMENT ON APOMORPHINE INDUCED YAWNING IN RATS. P. Curzon, E. Danaher*, A. Nadzan* and D. Britton. Neuroscience Research Division, Pharmaceutical Discovery, Dept. 47H, Abbott Laboratories, Abbott Park, IL 60064.

Apomorphine (APO) at very low doses (.032-1.78 mg/kg, s.c.) rapidly elicits a yawning response in rats with an inverted "U" shaped dose-response curve. The peak number of yawns occurs at between 0.056 and 0.078 mg/kg. ICV injection of 10 and 100 pmol of either CCK-8-S or BOC-CCK-4 prior to APO significantly reduced the number of yawns at either 0.056 or 0.078 mg/kg.

We have confirmed earlier reports (Protais et al., 1985; Argiolas and Melis, 1987) that the yawning response is antagonized by both typical and atypical neuroleptics. Haloperidol (.1 and .2 mg/kg), clozapine and sulpiride (each at 1.0 and 2.0 mg/kg) were tested against several doses of APO. All three drugs not only reduced the number of yawns but also shifted the dose of APO that produced the peak number of yawns to the right.

When CCK-8-S (10 or 100 pmol) is injected i.c.v. 15 min. prior to varying doses of APO, yawning is reduced by both doses of CCK-8-S and is significantly suppressed at 0.078 mg/kg of APO. These results suggest some common factors between CCK and neuroleptic pharmacology.

115.3

A "PERIPHERAL TYPE" CCK RECEPTOR ANTAGONIST (A-65186) BLOCKS BEHAVIORAL EFFECTS OF ICV AND IP ADMINISTERED CCK IN MICE. D.R. Britton, L. Yahiro*, M.J. Cullen*, J. Kerwin*, H. Kopecka* and A. Nadzan*, Neuroscience Research Div., Pharmaceutical Discovery, D-47H, Abbott Laboratories, Abbott Park, IL 60064.

Cholecystokinin octapeptide (CCK-8) administered centrally (icv) suppresses several types of behavioral activity in mice including exploratory locomotion. Systemic (ip) administration of CCK-8 also suppresses activity. At doses equimolar to those active for CCK-8, neither desulfated CCK (CCK-8-DS), nor the protected tetrapeptide fragment, BOC-CCK-4, is behaviorally active. Boc-CCK-4 failed to antagonize the effects of centrally administered CCK. A potent and selective antagonist to the peripheral type (Type A) CCK receptor, A-65186, when given systemically, blocked the effects of systemically administered CCK but failed to block the effects of icv administered CCK. Central administration of A-65186 blocked the effects of icv administered CCK. These results demonstrate that administration of exogenous CCK to mice can suppress exploratory locomotion by acting either centrally or peripherally and that in either case the behavioral effects are mediated via a "peripheral" type (Type A) CCK receptor.

115.4

LORDOSIS RESPONSE TO SITE-SPECIFIC CHOLECYSTOKININ (CCK-8) IN THE MALE. GJ Bloch*, WA Dornan, AM Babcock*, RA Gorski, and PE Micevych, Dep't of Anatomy and Lab. of Neuroendocrinology, Brain Res. Inst., UCLA School of Medicine, Los Angeles, Ca. 90024.

In females, CCK-8 inhibits lordosis when injected into the ventromedial hypothalamus (VMH;Babcock et al, Physiol Behav:42,'88) and facilitates lordosis when injected into the medial preoptic area (MPO;Priest et al, Neurosci Abst:14,'88)). Intracerebroventricular CCK-8 has a dramatic facilitatory effect on lordosis in estrogen-primed males. We tested the lordosis response to site-specific injections of CCK-8 in males. Male rats were castrated as adults, then given injections of CCK-8 into the VMH or MPO 2 weeks later and their receptivity was assessed by determining a lordosis quotient (LQ). Animals with MPO cannulae were primed with 5 ug estradiol benzoate (EB) per dayX 3, while animals with VMH cannulae were given 5 ug EB/day X3 before each of 3 testing sessions, then 30 ug EB/day X3 before an additional 2 testing sessions. MPO injection of as little as 5 ng CCK-8 had a dramatic facilitatory effect (LQ=87.5±7.3 with CCK-8 vs 39.8±11.4 with vehicle, $p<0.01$). In contrast, VMH injection of 5 ng to 100 ng CCK-8 had no effect on the LQ when animals were either low- or high-receptive. These results demonstrate that: 1) CCK-8 injected into the MPO facilitates lordosis behavior in the estrogen-primed male, and 2) CCK-8 injected into the VMH inhibits lordosis in the female but not in the male. Supported by NS 21220 (PEM) and HD 01182 (RAG).

115.5

FACILITATION OF LORDOSIS BY INJECTION OF CCK-8 INTO THE MEDIAL PREOPTIC AREA IN THE FEMALE RAT. C.A. PRIEST*, W.A. DORNAN, G. BLOCH*, P.E. MICEVYCH, Department of Anatomy and Lab. of Neuroendocrinology, Brain Res. Inst., UCLA School of Medicine, Los Angeles, CA 90024.

Accumulating evidence supports a role for cholecystokinin (CCK) in the neural mediation of estrogen-induced sexual receptivity (lordosis) in the female rat. One neural substrate that has been reported to participate in the CCK mediation of lordosis is the ventromedial nucleus of the hypothalamus (VMH). Direct injections of CCK-8 into the VMH were found to inhibit lordosis in female rats. Recently, the VMH and the medial preoptic area (MPOA) have been shown to functionally interact in the activation of lordosis. Since the MPOA has extensive CCk terminal fields, we were interested in examining the effects on lordosis following CCK-8 injections into the MPOA. Adult Long-Evans ovariectomized female rats were implanted with unilateral guide cannulae aimed at the MPOA. One week following surgery, each female was injected with 5ug estradiol benzoate at 10 day intervals 53 hours before a mating test, for a total of 5 tests. Ten minutes prior to each test females were injected with artificial CSF or CCK (5, 50, 100 ng). The effects of these injections on lordosis were assessed by recording the number of lordotic responses following 10 mounts with pelvic thrusting by a sexually vigorous male. Injections of CCK-8 directly into the MPOA produced a facilitation of lordosis in ovariectomized estrogen treated females. In a previous study in our lab, peripherally injected CCK-8 facilitated lordosis when receptivity was low, and attenuated lordosis when receptivity was high. In contrast, in the VMH study injections of CCK-8 inhibited lordosis when receptivity was high and had virtually no effect on lordosis when receptivity was low. Taken together, these results strongly implicate CCK as a functional link in the reciprocal control of lordosis by the VMH and MPOA. (Supported by NS21220 PEM).

115.6

SUBSTANCE P IMMUNOREACTIVE NEURONS IN THE VENTROMEDIAL NUCLEUS PROJECT TO THE DORSAL MIDBRAIN CENTRAL GRAY. W.A. DORNAN, T.R. AKESSON, P.E. MICEVYCH, Department of Anatomy and Lab. of Neuroendocrinology, Brain Res. Inst., UCLA School of Medicine, Los Angeles, CA 90024.

Numerous studies have revealed the importance of the ventromedial nucleus (VMN) and the midbrain central gray (MCG) for the expression of sexual receptivity (lordosis) in the female rat. Cells within the VMN have been shown to project to the MCG and as a result, this pathway has been implicated in the regulation of lordosis. Recently, Dornan, Malsbury, and Penney (Neuroendocrinol., 45: 498, 1987) have demonstrated that substance P (SP) facilitated lordosis following injections into the dorsal MCG. SP-containing cells as well as SP receptors have been localized in the VMN and MCG respectively. Collectively, this suggested to us that perhaps SP neurons originating within the VMN terminate in the dorsal MCG and form part of the neural circuitry involved in the regulation of lordosis. Fluoro-gold was iontophoretically injected into the dorsal MCG of adult female Long-Evans rats. After a 14 days survival period all animals received ventricular injections of colchicine (100 ug) and were sacrificed 2 days following colchicine treatment. Only brains that had intense iontophoretic injection sites restricted to the dorsal MCG were subsequently processed for SP immunofluorescence (FITC). Alternate fluorescent illumination revealed cell bodies in the ventral lateral portion of the VMN for both FG and FITC (double-labelled). Double labelled cells were more abundant in the caudal aspect of the VMN. In addition, some doubled-labelled cells were also found in the central and dorsomedial subdivisions of the VMN. These results provide morphological evidence for a SP-specific projection from the VMN to the dorsal MCG, and strongly implicate this pathway as an important part of the neural circuitry modulating lordosis in the rat. Supported by NIH grants NS 21220 (PEM) and HD 22869 (TRA).

115.7

VAGOTOMY BLOCKS THE PERIPHERAL EFFECTS OF CCK-8 ON LORDOSIS BEHAVIOR IN THE RAT. AM Babcock*, JC Barton*, GJ Bloch*, PE Micevych (Spon:Adinolfi,AM). Dept of Psychology, University of S. Alabama, Mobile AL 36688 and Lab. of Neuroendocrinology, Brain Res. Inst., UCLA School of Medicine, Los Angeles, CA 90024.

We have previously reported that i.p. injection of CCK-8 alters lordosis behavior in estrogen-primed female rats (Physiol Behav:39,217,'87). Since the effects of i.p. CCK-8 on feeding and exploratory behaviors are blocked by vagotomy, we investigated the effects of CCK-8 on lordosis behavior in vagotomized rats. Adult female rats were ovariectomized and underwent vagotomy (VAG;n=12) or a sham (SHAM;n=12) procedure. One week following surgery, rats were injected with 10 ug estradiol benzoate and tested 53 hrs later. CCK-8 (3 ug/kg) or saline vehicle (SAL) was injected i.p. 10 minutes before behavioral testing. To evaluate increased levels of receptivity, rats were retested every 8 days for a total of 4 behavioral tests. Lordosis quotients (LQ) were evaluated using an independent t-test. Injection of CCK-8 significantly stimulated LQ in SHAM animals on behavioral tests 2 (CCK,60±15 vs SAL,26±9;$p<.05$) and 4 (CCK,70±12 vs SAL,25±11;$p<.05$). CCK-8 failed to alter the LQ of VAG animals for any of the 4 behavioral tests. These findings suggest that the stimulatory effects of i.p. CCK-8 on lordosis are, in part, mediated through afferent vagal fibers. Supported by USARC 3-61332 (AMB).

115.8

ANALGESIA INDUCED BY RESTRAINT STRESS IS ATTENUATED BY CCK AND ENCHANCED BY THE CCK ANTAGONISTS MK-329, L-365,031 AND CR 1409. **C.T. Dourish, M.L. Clark* and S.D. Iversen* Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, CM20 2QR, U.K.**

The neuropeptide cholecystokinin (CCK) attentuates opiate-mediated analgesia induced by morphine injection or front paw shock (Faris et al, 1983). The selective CCK antagonist MK-329 enhances morphine analgesia and prevents morphine tolerance (Dourish et al, 1988). This study examined the effects of CCK and three CCK antagonists on analgesia induced by acute restraint stress. Analgesia was assessed using the radiant heat tail-flick method, as previously described (Dourish et al, 1988). Basal tail-flick latencies were determined 20 min prior to drug; test latencies 25 min post-restraint. Drug treatments were given prior to restraint as follows: CCK (10 min), naloxone (15 min), CCK antagonists (30 min). Data were expressed as % of maximum possible effect (% MPE). Restraint for a 15 min period produced analgesia, ranging from 25-40% MPE. The analgesia was dose-dependently reduced by naloxone (0.4-2.5 mg/kg sc) indicating that it was opioid mediated. Similarly, CCK (4-16 ug/kg ip) dose-dependently blocked the response. In contrast, the CCK antagonists MK-329 (0.05, 0.1 mg/kg sc), L-365,031 (0.5-2.0 mg/kg sc) and CR 1409 (0.3-1.0 mg/kg sc) significantly enhanced restraint-induced analgesia to 70-80% MPE. Thus, CCK antagonists enhance analgesia produced by endogenous or exogenous opioids, suggesting that they may be of value in the treatment of pain.

Faris P.L. et al (1983) Science, 219: 310.
Dourish C.T. et al (1988) Eur. J. Pharmacol., 147: 469-472.

115.9

CHOLECYSTOKININ-TETRAPEPTIDE INDUCES PANIC ATTACKS IDENTICAL TO SPONTANEOUS PANIC ATTACKS IN PATIENTS SUFFERING FROM PANIC DISORDER. J. Bradwejn, G. Meterissian* and D.Koszycki*. St. Mary's Hosp., McGill Univ., Montreal, Quebec, Canada, H3T 1M5.

Cholecystokinin (CCK) is found in high concentrations in the limbic system of mammalian CNS. When applied on limbic neurons, CCK produces an excitation. Benzodiazepine receptor agonists selectively antagonize this excitation. Therefore, it has been suggested that CCK might play a role in anxiety and/or panic. We undertook this study to compare the effects of CCK-tetrapeptide (CCK-4, 30-33) with spontaneous panic attacks of patients suffering from panic disorder.

Eleven patients (6 males, 5 females; aged 20 to 51 years) were studied. Using a randomized, double-blind design, they received i.v. CCK-4 (50μg) and i.v. saline separately, on two different days.

CCK-4 induced a panic attack in all patients whereas saline had no effect. The attacks began 20 $\pm$ 3 seconds after the end of the injection and lasted 20.7 $\pm$ 7.3 minutes. The attacks were reported to be identical in symptoms to spontaneous panic attacks. The appearance of symptoms of CCK-4-induced attacks was identical to the one experienced during spontaneous panic attacks.

The reproduction of symptoms of spontaneous panic attacks by CCK-4 suggests that CCK might play a role in the neurobiology of panic disorder.

115.10

ANXIETY AND PANIC ATTACKS INDUCED BY THE TETRAPEPTIDE CHOLECYSTOKININ IN HEALTHY VOLUNTEERS. C. de Montigny. Dept. of Psychiatry, McGill University, Montreal, Canada.

From the observation that benzodiazepines antagonize cholecystokinin(CCK)-induced activation of CNS neurons in the rat, it was postulated that CCK might be involved in the pathogenesis of anxiety disorders. To put this hypothesis to the test, the tetrapeptide form of CCK (30-33) (CCK-4) was injected intravenously to 10 healthy volunteers (8 males; 2 females).

Seven of the ten subjects experienced a panic attack, lasting for 1 to 4 min, with doses of CCK-4 ranging from 20 to 100 µg. In the three others, doses of 80 to 100 µg induced a marked anxiety, but no panic. The CCK-4 injection produced pronounced gastrointestinal symptoms in all subjects.

Pretreatment of subjects, in whom CCK-4 had induced a panic attack, with lorazepam, but not with meprobamate or naloxone, prevented the panicogenic effect of CCK-4.

Intravenous injections of the sulphated octapeptide (26-33) (CCK-8S) induced intense gastrointestinal symptoms which precluded the administration of more than 40 µg. At this dose, CCK-8S did not induce any panic or anxiety.

It is concluded that CCK-4 can induce panic attacks or anxiety in healthy volunteers. Definite evidence remains to be provided that CCK-4 produces its panicogenic/anxiogenic effect through direct activation of CNS neurons of the limbic system.

115.11

CHOLECYSTOKININ POTENTIATES DOPAMINE-INDUCED HYPOLOCOMOTION IN THE VENTRAL TEGMENTAL AREA OF THE RAT. J.N. Crawley. NSB, NIMH, Bethesda, MD 20892.

Cholecystokinin (CCK) coexists with dopamine (DA) in ventral tegmental neurons projecting to the nucleus accumbens in the rat. To investigate the possible behavioral functions of CCK in the ventral tegmental area (VTA), CCK was microinjected alone or in combination with DA via indwelling cannulae at stereotaxic coordinates 5.8 mm caudal and $\pm$ 0.5 mm lateral to bregma, and 6.9 mm ventral to the surface of the skull. CCK (100 pg to 100 ng), had no effect alone on locomotion, but CCK (1 ng to 400 ng) significantly potentiated the hypolocomotion induced by DA (5 ug). Topographical analysis showed that CCK potentiated DA-induced hypolocomotion only in the medial and caudal VTA. Pharmacological analysis showed that unsulfated CCK-8 and CCK-4 also potentiated DA-induced hypolocomotion in the nanogram dose range, and that peripheral-type CCK receptor antagonists were less potent blockers of CCK potentiation of DA-induced hypolocomotion in the VTA, than of CCK potentiation of DA-induced hyperlocomotion in the nucleus accumbens (Crawley et al., JPET, 1986). These data suggest that CCK acts as a facilitatory modulator of DA in both the cell body and the postsynaptic regions of the mesolimbic pathway. The actions of CCK at the VTA site resemble the pharmacology of the central-type CCK receptor, while the actions of CCK at the nucleus accumbens site resemble the pharmacology of the peripheral-type CCK receptor.

115.12

MICROINJECTIONS OF CCK INTO THE NUCLEUS ACCUMBENS ATTENUTE THE LOCOMOTOR ACTIVATING EFFECTS OF SYSTEMIC HEROIN. F. J. Vaccarino, J. Mogil* and J. Rankin*. Departments of Psychology and Psychiatry, University of Toronto, Toronto, Ont. M5S 1A1.

Activation of opiate receptors in the nucleus accumbens (NA) is important for the locomotor activating properties of systemically administered heroin (Amalric and Koob, 1985, Vaccarino and Corrigal, 1988). Findings showing that cholecystokinin (CCK) terminals are present in the NA and that central CCK has neuromodulatory effects raise the possibility the NA CCK may influence the expression of heroin-induced locomotor activity. In order to examine this possibility, the present study investigated the effects of intra-NA CCK microinjections on locomotor activity induced by systemic heroin.

Rats with cannulae implants aimed bilaterally at the NA were tested for their locomotor response (over 120 minutes) to *systemic heroin (0.3 mg/kg, s.c.) followed by intra-NA* microinjections of sulfated CCK octapeptide in doses of 0.0 (saline vehicle), 0.5, 1.0, 2.0 and 4.0 µg. Each rat received all drug treatments in random order. Intra-NA CCK was administered in a volume of 0.25 µl per side over a 1 minute period using a microdrive pump. A minimum of 2 days separated drug tests.

The results demonstrated that CCK had opiate antogonist-like effects on heroin-induced locomotor activity. Intra-NA CCK dose-dependently attenuated the initial hypolocomotion and the subsequent hyperlocomotion produced by systemic heroin treatment. These results suggest that NA CCK plays a modulatory role in heroin-induced behavioural activation. Opiate antagonist-like effects of CCK have also been reported in morphine-induced analgesia studies, suggesting that CCK/opiate antagonism is present in other behavioural systems.

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115.13

CYSTEAMINE-INDUCED DEPLETION OF CENTRAL SOMATOSTATIN AND PASSIVE AVOIDANCE CONDITIONING IN RATS.

G. R. Sessions, M. D. Matthews*, and G. F. Koob. Walter Reed Army Institute of Research, Washington, DC 20307, Drury College, Springfield, MO 65802 and Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Intracerebroventricular (ICV) injections of somatostatin in laboratory rats have been shown to inhibit the extinction of active avoidance responding *(Vescie, et al., Acta Phys. Hung., 62:205, 1983)*, and to block ECS-induced amnesia *(Vescie, et al., Peptides, 4:293, 1983)*. Animals with depletions of somatostatin induced by ICV injections of cysteamine acquire an active avoidance task and inhibit punished avoidance responding in a normal manner *(Sessions, et al., Neurosci. Abs., 11:1113, 1985)*, but have been reported to be deficient in learning a one-trial passive avoidance task *(Bakhit & Swerdlow, Bn. Res., 365:159, 1986)*. Two experiments further investigated the capability of somatostatin-depleted animals to learn and retain a passive avoidance response. Groups of rats received ICV injections of cysteamine (250 or 350 µg in 2 µl saline) or saline for three (Exp. 1) or four (Exp. 2) consecutive days. Forty-eight hr following the last injection, they received one shock-punishment trial in a step-through passive avoidance task. Twenty-four hr later the animals were tested for retention of the passive avoidance response. In both experiments, results showed no differences in retention of the passive avoidance response between cysteamine-injected and saline-injected controls. These results lend no support for findings suggesting a passive avoidance deficit in animals with depletions of cerebral somatostatin, and, together with similar negative findings in a previous active avoidance study, suggest that somatostatin depleted animals do not suffer memory deficits detectable in avoidance paradigms.

115.14

STRUCTURE ACTIVITY STUDY OF NEUROTENSIN IN AN ANIMAL MODEL OF PARKINSON'S DISEASE. R. Rivest[1], S.St-Pierre*[2] and F.B. Jolicoeur[1]. [1]Dept. Psychiatry, Fac. of Med., Univ. of Sherbrooke, Quebec J1H 5N4 and [2]INRS, Blvd. Hymus, Pointe-Claire, Quebec H9R 1G6.

Intracerebroventricular (ICV) administration of neurotensin (NT) has been shown recently to attenuate specificParkinson-like symptoms induced by injection of 6-OHDA into the medial forebrain bundle (MFB) of rats (R. Rivest, F.B. Jolicoeur, Soc. Neurosci. Abst. (12) no. 41.22, 1986). In the present study, the effects of two NT fragments, NT_{8-13} and NT_{1-10}, and two NT analogues, [D-Tyr^{11}]-NT and [Ala^{11}]-NT, on muscular rigidity and tremors induced by 6-OHDA treatment were investigated. Male hooded rats received bilateral injections of 26 µg 6-OHDA in the MFB (A-0.8; L-2.0; V-8.0 in relation to bregma), and were implanted with an indwelling left ventricular cannula. The 6-OHDA treatment resulted in several neurobehavioral anomalies such as hypoactivity, catalepsy, recurrent bouts of hind leg tremors and muscular rigidity as measured by evaluation of tail rigidity and a prolonged grasping time. Peptides were administered ICV in doses ranging from 0.45 to 120 µg/10 µl and presence and intensity of the above symptoms were recorded over a 2 hr period following injections. Muscular rigidity and tremors were not altered by either NT_{1-10} or [Ala^{11}]-NT even at the highest dose, while 60 µg of NT_{8-13} decreased grasping time 15 min after injection. However, the NT analogue [D-Tyr^{11}]-NT proved to be markedly more potent in its anti-Parkinson-like effects. Compared to NT, which significantly decreased grasping time, tail rigidity and tremors at 30.0, 7.5 and 30.0 µg respectively, the analogue produced similar effects at 1.8, 0.9 and 0.9 µg respectively. Together these results indicate that our previous observations of anti-Parkinson's actions of NT are not solely due to unspecific effects of peptide administration, are largely dependent on the carboxy terminal of NT, and that the tyrosine residue in position 11 plays a critical role in these actions of NT.

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115.15

EFFECTS OF HALOPERIDOL ON BOMBESIN-INDUCED HYPOTHERMIA AND APHAGIA IN FOOD DEPRIVED RATS. L. Perez,*K. Tyler,*A.M. Babcock,*(SPON: C.W. Brown). Dept. of Psychology, University of South Alabama, Mobile AL 36688.

Intranigral injections of bombesin (BBS) produce hypothermia and aphagia in food deprived rats. Since the substantia nigra (SN) contains dopaminergic elements which are implicated in feeding and thermoregulation, we evaluated the effects of haloperidol (HAL) on BBS-induced aphagia and hypothermia.

Food deprived (18 hrs) rats, previously implanted with cannulae aimed at the SN, received the following treatments in random order: HAL (.5 mg/kg; I.P.) + BBS (100 ng/.5 ul), HAL vehicle (.3% tartaric acid) + BBS, HAL + BBS vehicle (saline), HAL vehicle + BBS vehicle. HAL was given 60 minutes prior to BBS. Food intake was measured for 30 minutes following BBS or saline injection. Rectal temperature was measured before and after presentation of the test diet. Data were evaluated using ANOVA and Newman Keuls post hoc procedures ($p<.05$).

Intranigral injections of BBS significantly decreased food intake and core body temperature. Treatments of HAL alone significantly reduced food intake, but did not alter body temperature. Administrations of HAL attenuated BBS-induced hypothermia and aphagia.

These findings suggest that BBS-induced hypothermia, and perhaps aphagia, are in part mediated through an interaction with dopamine elements within the SN.

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115.16

CONOTOXINS FROM A SNAIL-HUNTING CONE VENOM: BIOCHEMICAL CHARACTERIZATION AND CLONING. D.R. Hillyard*, S. Woodward*, C. Ramilo*, G.P. Corpuz*, B.M. Olivera and L.J. Cruz. Howard Hughes Medical Institute and Dept. of Biology, University of Utah, Salt Lake City, UT 84112, and Marine Sci. Inst., Univ. Philippines, Metro-Manila, Philippines.

We purified and biochemically characterized 3 major toxins from the venom of a mollusc-hunting snail, Conus textile, and are presently cloning the genes for these toxins. The 3 toxins, all 27 amino acids long, have quite different physiological effects on vertebrates and do not have related sequences. One of these toxins, the "King-Kong" peptide causes a convulsive-like state when injected into molluscs, and an abnormal "dominant" postural gait when injected into crustaceans. The other two peptides are Y-carboxyglutamate (Gla) containing, but not homologous to each other. One of these causes a mouse to phenocopy the "spastic" phenotype; the other peptide causes rapid running. The discovery of two Gla-containing major toxins in the venom of a snail-hunting cone suggests that neuroactive Gla-containing peptides will be widely found within Conus venoms.

cDNA clones have been obtained from Conus textile venom; the "king-Kong" peptide gene is presently being sequenced. A comparison of sequences of various toxin genes should reveal processing signals, at both the N terminus and C terminus, as well as sequences that are potentially important in proper peptide folding.

115.17

FMRFamide perfusion of the isolated Aplysia gill prevents habituation of siphon evoked gill withdraval. E. Colebrook, D. Cawthorpe, A. Higgins and K. Lukowiak. Dept. Med. Physiol. Faculty of Med. University of Calgary, Alberta, Canada.

The evoked gill withdrawal response (GWR) of the Aplysia gill which has been isolated from the abdominal ganglion has been described to exhibit forms of learning such as habituation and sensitization which may be modulated by peptides.

The results reported here are similar to those previously reported about the effects of FMRFamide upon the gill evoked GWR (Cawthorpe et al. 1988 Reg. Pep. in press). In these experiments the effects of FMRFamide perfusion upon the siphon evoked GWR was examined. FMRFamide perfusion of the gill via the afferent vein was found to increase the amplitude of the siphon evoked GWR with threshold effects around 3 nM. Habituation of the GWR to repeated siphon stimulation was prevented by perfusion of 10 nM FMRFamide through the gill.

These findings further support the role of FMRFamide as an endogenous modulator of gill adaptive behaviors.

115.18

DEVELOPMENTAL RESPONSIVENESS OF THE HEART AND FEEDING SYSTEM OF THE TERRESTRIAL SLUG, LIMAX MAXIMUS, TO SCP_B. I.G. Welsford and D.J. Prior. Dept. Biological Sciences, Northern Arizona University, Flagstaff, AZ. 86011-5640.

SCP_B had no effect on heart activity (rate or apparent force of heart contractions) or feeding activity in stage IV (7-10 days after laying) embryos of the terrestrial slug Limax maximus (0/5) but caused dose-dependent increases in heart activity in 100% (n=5) of stage V (12-15 days after laying) embryos with an approximate threshold concentration of 10^{-9} M. In contrast, SCP_B had no effect on feeding activity in stage V embryos (0/5). SCP_B caused dose-dependent increases in heart activity and initiated patterned feeding in 100% (n=5) of stage VI (22-25 days after laying) embryos with an approximate threshold concentration of 10^{-9} M. SCP_B had the same effects on 100% (n=4) of hatchling Limax 4 $\pm$ 2.3 hr. post-hatch in age and 80% (4/5) of hatchling Limax 70 $\pm$ 1.8 hr. post-hatch in age. For both groups, the approximate threshold SCP_B concentration was 10^{-9} M. Although SCP_B caused dose-dependent increases in heart activity in 100% (n=5) of Limax 1 mo. post-hatch in age, it initiated only the appetitive phase of feeding behavior in these animals(4/5). The threshold concentration of SCP_B for the increase in heart activity was 10^{-9} M while that for the effect on feeding was 10^{-7} M. These results suggest that peripheral targets in Limax are responsive to SCP_B earlier in development than central targets and central responsiveness to SCP_B is altered after 70 hr. post-hatching.

115.19

IMPAIRED BEHAVIORAL HABITUATION IN VASOPRESSIN DEFICIENT BRATTLEBORO RATS IS INDEPENDENT OF GENETIC BACKGROUND. **A. Cerbone and A.G. Sadile, Inst. Human Physiol. & Biomed. Phys., Univ. Naples, 1st Med.Sch., 80138, Naples, Italy.**

Aim of this study was to investigate the role of the genetic background and that of perinatal vasopressin (AVP) in determining the behavior of adult vasopressin deficient Brattleboro rats. To this end the genetic pool of Brattleboro rats, homozygous for diabetes insipidus (DI/DI: Inst. Anatomy, Univ. Oxford) was shuffled with that of normal, Long-Evans rats (+/+). The F2 hybrids, homozygous (DI/DI), heterozygous (DI/+) and normal (+/+), shared the genetic pool, differying for the vasopressin gene only. During perinatal development all rats were raised by normal mothers in presence of circulating plasma vasopressin. This group was compared with DI/DI rats born from DI/DI mothers, with no perinatal vasopressin. At the age of 80-90 days, all rats were tested in a Làt's maze for three 10min-exposure at 24 hr interval. Corner crossings and rearings were monitored per 30sec-blocks. DI/DI rats from the perinatal AVP group showed higher body and brain weight than those from the no vasopressin group. DI/DI rats were more active, the within exposure activity decline was similar across groups. The between exposures activity decrement (long-term habituation) was impaired in all DI/DI rats, including original Oxford-rats. The lack of response inhibition to novelty is a constant feature of DI/DI rats and does not result from a specific constellation of genes from high inbreeding.

Supported by CNR, Gruppo Scienze del Comportamento.

116.1

MIF ACTS AS A CS FOR CONDITIONED MORPHINE TOLERANCE. C. R. McLaughlin, A. H. Lichtman, M. S. Fanselow* and C. P. Cramer. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Pro-Leu-Gly-NH2 (melanotropin-release inhibiting factor, MIF) has been reported to inhibit the formation of morphine tolerance in rats (review in Bhargava, 1986). Because tolerance to morphine-induced antinociception can be an associative process that follows the laws of Pavlovian conditioning (Baker & Tiffany, 1985; Eikelboom & Stewart, 1982; Paletta & Wagner, 1986; Siegel, 1975), we tested the effect of MIF on the acquisition of tolerance and its efficacy as a cue predictive of morphine administration. Adult male rats were administered either MIF (4 mg/kg, ip) or water two hr prior to daily morphine administration (10 mg/kg, ip) for 10 days. MIF did not attenuate the acquisition of tolerance to morphine-induced antinociception, as measured by the latency to hindpaw lick in a hotplate test of analgesia. Half of the animals in each group were then reassigned to the other pretreatment group. Animals tested 72 hr later with a different pretreatment displayed longer pawlick latencies than those given the same pretreatment, indicating a loss of tolerance.

We suggest that MIF can interfere with the expression of tolerance by acting as a CS that reliably predicts the antinociceptive properties of morphine.

116.2

OPIOID MODULATION OF PAVLOVIAN LEARNING FOLLOWING LESIONS OF THE AMYGDALOID CENTRAL NUCLEUS. L.L. Hernández and P.C. Gantt. Dorn Veterans' Hospital and University of South Carolina, Columbia, SC 29201.

Albino rabbits prepared with bilateral lesions of the amygdaloid central nucleus (ACN), and sham-lesioned controls, were treated intravenously with either d-ala2-met-enkephalinamide (DALA; 10 μg/kg), naloxone-HCl (0.5 mg/kg) or saline vehicle (0.2 ml/kg), and were subjected to a variety of behavioral and physiological tests. Both DALA and naloxone treatments decreased locomotor activity but had opposite effects on Pavlovian conditioned bardycardiac responses to tones paired with paraorbital shocks. ACN lesions had no effects on locomotor activity or bradycardiac orienting reflexes to novel tones, but did reduce the magnitude of Pavlovian conditioned bradycardiac responses and altered responses to unsignalled shocks. Interactions between the effects of the opioid treatments and ACN lesions support earlier findings suggesting that certain effects of systemic opioid and antagonist treatments on Pavlovian conditioning and related behavior involve the ACN.

Supported by VA Institutional Research funds and by USPHS Grant #R23-AA06817.

116.3

DYNORPHIN A: SELF-ADMINISTRATION IN CA3 HIPPOCAMPAL FIELD IN THE RAT. K.E. Stevens, G. Shiotsu*, J.D. Belluzzi and L. Stein. Dept. Pharmacol., Univ. Calif., Irvine, CA 92717

The presence of dynorphin terminals and kappa opioid receptors in the CA3 area of the rat hippocampus has been demonstrated by immunohistochemical and autoradiographic methods. Last year we reported *in vitro* operant conditioning of individual CA3 pyramidal cells in brain slices with locally applied dynorphin A (DYN) or U50,488H (U50) as reinforcement (Stein and Belluzzi, 1987). Here, we report evidence of DYN and U50 reinforcement at the behavioral level by demonstrating self-administration of these compounds in the hippocampal CA3 area in the intact animal. Rats were implanted with a unilateral guide cannula aimed at the CA3 pyramidal cell layer. Following recovery, they were connected to the EMIT self-administration system and allowed to self-administer DYN (0.01 - 33 pmol/inj.), U50 (0.1 - 33 pmol/inj.) or Ringer's vehicle by means of a nose-poke response which delivered 100 nl during a 5-sec infusion interval. Both kappa agonists were self-administered in a dose-dependant manner. Inverted-U dose-response curves were obtained for both agonists with peak response rates at 0.1 and 1 pmol/inj. for DYN and U50, respectively. Even the lowest dose of DYN used (0.01 pmol/inj.) supported significant self-administration behavior when compared to vehicle ($p < 0.05$); this dose is only about 3-fold greater than the reported endogenous concentration of DYN in the CA3 field. These behavioral results corroborate the *in vitro* single-cell operant conditioning data and suggest that dynorphin may be a natural "reinforcement neurotransmitter" in the CA3 area of the hippocampus.

116.4

DYNORPHIN A [1-17] PLACE PREFERENCE IN RATS: OPIOID-SPECIFIC AND DOPAMINERGIC. Edgar T. Iwamoto. Dept. of Pharmacol., Univ. of Kentucky, Lexington, KY 40536.

Sprague-Dawley male rats were administered 1.2 to 3.5 nmol of dynorphin A [1-17] (DYNA) or buffer into the lateral ventricle and then placed in one of two rooms of a place-conditioning apparatus for 20 min (Iwamoto, Alc Drug Res 6:327, 1986). The next day, the rats were not treated but simply placed in the other distinctive room for 20 min. On Day 3, rats were given free-access to explore the entire apparatus for 15 min, the number of sec spent in the treatment-paired (T) or the non-treatment-paired (NT) rooms was recorded, and a preference ratio (PR) was calculated as [T-NT]/[T+NT] for each rat. 2.3 nmol of DYNA in 1 or 2 ul of buffer induced a significant preference response as indicated by positive PRs greater than control: DYNA PR = 0.47 ± .07 vs buffer control PR = 0.18 ± .05 (N = 8 each). 3.5 nmol of DYNA also induced significant place preference; 1.2 nmol was without effect. 27.5 nmol, but not 5.5, of naloxone coadministered with 2.3 nmol of DYNA antagonized the reward response. Dynorphin A [2-17], a probable metabolite of DYNA that does not bind opiate receptors, did not condition place preferences at doses of 2.3 or 3.5 nmol. Twenty min pretreatment with 0.02 mg/kg SC of R(+)SCH23390 or 0.04 mg/kg of spiperone completely antagonized 2.3 nmol DYNA-induced reward indicating roles for D_1 and D_2 systems. The data indicate that DYNA may mediate or modulate neuronal substrates of CNS "reward". (KTRB and UKMCRF)

116.5

OPIATES MICROINJECTED INTO THE VENTRAL PALLIDUM/ SUBSTANTIA INNOMINATA (VP/SI) PRODUCE LOCOMOTOR RESPONSES THAT INVOLVE DOPAMINERGIC SYSTEMS. T.C. Napier, D. An*, M.C. Austin, and P.W. Kalivas. Dept. Pharmacol., Loyola Univ. of Chicago, Stritch Sch. Med., Maywood, Il 60153; Dept. VCAP, Washington State Univ., Pullman, WA 99164.

The VP/SI contains enkephalin peptides and projects to the mesencephalic locomotor region. Using rats implanted with guide cannulaes over the VP/SI, we reported at the 1987 Neuroscience meeting that opiate peptides microinjected into the VP/SI alter various motor functions in a naloxone-reversible fashion. One of these was an opiate-induced contralateral rotation. For the present studies, we determined if this circling involves brain dopamine (DA) systems. Cannulae-implanted rats were pretreated i.p. with 0.1 mg/kg SCH23390 or 0.5 mg/kg haloperidol (D1 and D2 DA receptor antagonists, respectively) 30 min before unilaterally-microinjecting the VP/SI with the ED_{50} for leu-enkephalin (3.3 nmoles) or DAGO (33 pmoles). SCH23390 blocked the contralateral rotations of both enkephalin- and DAGO-treated rats, while haloperidol was effective only against DAGO-induced circling.

These data suggest that motor functions of the VP/SI involve opiate and DA systems. Since DAGO, the *mu* receptor specific peptide, exhibited a smaller ED_{50}, and since only haloperidol blocked this response, it is likely that a DA-D2-receptor/opiate-*mu*-receptor interaction is an important VP/SI influence on motor behavior.

116.6

OPIOIDS MICROINJECTED INTO CENTRAL DOPAMINERGIC PATHWAYS: EFFECTS ON COPULATION IN MALE RATS. L. Band and E. Hull. SUNY at Buffalo, NY 14260.

Dopaminergic neurons are involved in morphine-induced reward and are thought to play a role in opiate enhancement of natural reward. The effects on sexual behavior of opiate receptor stimulation within A10 and A14 terminal areas were examined in the following experiments. Morphine (.01-6 nmol) and dynorphin (1-13; .01-3 pmol) were microinjected into the medial preoptic area (MPOA) and the nucleus accumbens (NA) of male rats. Morphine (10-100 pmol) and dynorphin (10-100 fmol) injected into the MPOA reduced both the latency to ejaculate and the number of intromissions triggering ejaculation; 6 nmol morphine produced a failure to resume copulating following the second ejaculation. Morphine (1-10 nmol) injected into the NA shortened the latency to the first intromission and lengthened the second postejaculatory interval. Morphine (1 nmol) injected into the NA increased the frequency of ejaculation in poor copulators. Naloxone (3 mg/kg IP) reversed morphine's effects on intromission latency and ejaculation frequency. Opioids and dopamine agonists injected into the MPOA and the NA produce change in the same copulatory factors; these findings are consistent with opiate modulation of a dopaminergic substrate.

116.7

REINFORCING PROPERTIES OF SUBSTANCE P, ITS FRAGMENTS AND MORPHINE DEMONSTRATED BY A NEW METHOD FOR PLACE CONDITIONING
M.S. Holzhäuer-Oitzl*, R. Hasenöhrl* and J.P. Huston (SPON: European Brain and Behavior Society). Inst. Physiological Psychology, Univ. Düsseldorf, D-4000 Düsseldorf, F.R.G.

Centrally applied substance P (SP) has reinforcing and memory promoting properties depending on the site of injection. Since peripherally injected SP facilitated the performance in various learning tasks in rats and mice, we hypothesized SP to have reinforcing properties as well.

A circular open field was used to assess the reinforcing effects of SP and its N- and C-terminal fragments. Morphine was used to validate the new method. Experimental sessions (15min) were run on 5 consecutive days. On Day 1 animals (male Wistar rats) were allowed to explore the apparatus. On Days 2,3 and 4 rats received an injection (i.p., 0.5ml/kg) of SP (5,50,250 µg/kg), SP1-7 (33,170,334 µg/kg), SP6-11 (5,27, 134 µg/kg), morphine (10mg/kg) and vehicle. After injection the rats were placed into the open field, which was divided by plexiglass barriers into 4 quadrants of equal size. On Day 5 rats explored the open field. Only rats treated with morphine, 50 µg SP and the eqimolar dose of SP6-11 (27 µg), but not SP1-7 (33 µg), significantly increased their time spent in the previously drug-paired quadrant, thus, reflecting a positive reinforcing effect. These results show that SP has positive reinforcing properties additional to its memory promoting effects, whereby the active components for reinforcement may lie within the C-terminal sequence of the SP-molecule.

116.8

STUDIES ON THE EFFECTS OF MICROINJECTIONS OF VARIOUS OPIATE PEPTIDES INTO THE MIDBRAIN RAPHE NUCLEI OR THE VENTRAL TEGMENTAL AREA.
M.A. Klitenick and D. Wirtshafter, Dept. Psychology, Univ. of IL at Chicago, Chicago, IL 60680.

Numerous studies have reported dramatic behavioral effects following various manipulations of the midbrain raphe nuclei. We have previously demonstrated that intra-MR microinjections of morphine elicit a dose-dependent increase in locomotor activity, comparable in magnitude to that produced by peripheral administration of amphetamine. In an attempt to further clarify the role of opiates within the MR, several selective opiate receptor agonists were injected into the MR and locomotor activity within a photocell cage was measured for a two hour period. We report here that intra-MR injections of DAGO (0, 13.67, 27.34, 54.68,109.4, 218.7 & 437.5ng) or DPDPE (0, 0.625, 2.5, 5, 10 & 20ug), selective agonists of the mu and delta opiate receptors, respectively, elicited dose-dependent increases in locomotor. Studies in which microinjections of DAGO, DPDPE or dynorphin A into the dorsal raphe nucleus (DR), rostral or caudal ventral tegmental area or the midline pontine tegmentum caudal to the MR, are currently in progress in order to better assess the most active site for opiate-induced hyperactivity. In other studies, preliminary results suggest that intra-MR injections of DAGO, DPDPE, dynorphin (1-8) or dynorphin (1-13) elicit pronounced dose-dependent increases in food and water intake and food spillage in non-deprived rats.

(Supported by NIH grant NS21350 to DW)

116.9

CYCLO-LEUCYL-GLYCYL ALTERS THE EFFECTS OF CHRONIC APOMORPHINE TREATMENT.
L.P. Gonzalez, O. Dimson*, J.F. Czachura*, and R.F. Ritzmann. Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.

Our previous research has indicated that administration of the peptide cyclo-leucyl-glycyl (CLG) can reduce the development of dopamine receptor supersensitivity during chronic haloperidol administration. In the present study, we examined the effects of CLG in male Sprague-Dawley rats treated chronically with the dopamine agonist apomorphine (APO). Response measures included APO-induced motility as measured in an automated movement sensor, APO-induced hypothermia, and haloperidol-induced catalepsy. Following a test of initial drug sensitivity rats were divided into two groups, receiving daily injections of 1 cc/kg saline or 10 mg/kg APO HCl (i.p.) for 25 days. These were divided into 4 subgroups which received 0, 2, 8, or 16 mg/kg CLG (s.c.) at 3-day intervals during the 25-day chronic exposure period. Acute APO effects were again determined at the end of the chronic exposure period and for the next 2 months thereafter. Chronic APO-treated rats showed significantly more APO-induced motility, reduced hypothermia, and increased haloperidol-induced catalepsy, for at least 45 days after the termination of chronic treatment. CLG was found to have significant dose-related effects on the motility and catalepsy responses after chronic APO, but had no effect on APO-induced hypothermia. Supported in part by the State of Oklahoma Health Research Program, Contract 1676.

116.10

MORPHINE EFFECTS ON POSTPARTUM BEHAVIOR, TEMPERATURE AND ANALGESIA IN LACTATING MICE.
M.J. Haney*, K.A. Miczek, (SPON: J. Ellingboe) Dept. Psychology, Tufts Univ., Medford, MA 02155.

Endogenous opioid systems may play a role in the behavioral and physiological characteristics of lactating animals. In order to assess the contribution of opioid receptors to aggressive behavior, pup care, pain response and body temperature, we challenged mice with morphine at different phases of the lactation period. For comparison, morphine's effects on temperature and tailflick were also determined in virgin females.

Lactating mice were assigned to 1 of the 3 weeks of lactation and to 1 dose of morphine (0,1,3,6,10 mg/kg i.p.). After morphine administration, temperature and tailflick were assessed. Behavior toward 3 pups was observed for 5 min, followed by an aggression test with an intruder.

Morphine increased the latency to retrieve pups at doses that do not decrease motoric activity. Compared to virgins, lactating mice are less sensitive to the analgesic actions of morphine but similarly sensitive to its hypothermic properties. This differential sensitivity to morphine induced analgesia suggests that lactating animals undergo functionally relevant changes in opioid regulation of pain. Furthermore, morphine's specific inhibition of retrieval supports the hypothesis that decreased opioid peptide activity is important for the expression of certain postpartum behaviors.

116.11

ELECTROPHYSIOLOGICAL EFFECTS OF OPIATES ON NUCLEUS ACCUMBENS (NAS): DOPAMINE (DA) AND OTHER AFFERENT INFLUENCES.
R.L. Hakan and S.J. Henriksen. Preclin. Neurosci., RISC, La Jolla, CA 92037

The NAS is an important structure for opiate reward (Psychopharm.,86:37-42,1985). However, the precise role of ventral tegmental area (VTA)-NAS afferents in this process is unresolved. Single-unit recordings from the NAS of halothane anesthetized rats reveal that microinfusions of morphine into the VTA primarily inhibit spontaneously active NAS units. These inhibitory effects are reversed by alpha-flupenthixol (s.c), suggesting dopamine (DA) mediation. VTA opiate infusions also inhibit fimbria-driven, silent cells recorded in the NAS. This inhibition is reversed by naloxone (s.c.), but not by alpha-flupenthixol, implying a non-DA mechanism. Iontophoretic morphine applied within the NAS inhibits spontaneous activity but not fimbria-driven activity. However, VTA-induced inhibition of fimbria-driven activity is reversed by systemic opiates, indicating that opiates can also exert effects through other, as yet unidentified NAS afferent systems. Thus, systemic opiate effects on NAS neurophysiology must be complex, involving both local actions and indirect actions mediated by VTA DA-ergic, VTA non DA-ergic and other afferent systems.

116.12

PREDATOR-INDUCED ANALGESIA IN DEER MICE: POPULATION DIFFERENCES AND BENZODIZAEPINE AND OPIOID INVOLVEMENT.
M. Kavaliers, Div. Oral Biology, Univ. Western Ontario, London, Ontario, Canada N6A 5B7.

Although there is abundant evidence for predator recognition by prey, little is known about the neurochemical mechanisms associated with these aversive responses. In the present study determinations were made of the effects of exposure to a predator, the short-tail weasel, on the nociceptive responses of deer mice that had different histories of exposure to this predator. Peromyscus maniculatus artemisiae were from the mainland and P. m. angustus and P. m. triangularis were from small predator (weasel) free islands. In the mainland derived population, a short (30 sec), ecologically appropriate exposure to the scent of the weasel elicited a relatively brief analgesic response that was blocked by the benzodiazepine antagonist, Ro15-1788 and insensitive to the opiate antagonist naloxone. A 5 min exposure to the weasel elicited an analgesic response that was sensitive to both naloxone and Ro15-1788, while a 15 min exposure induced just the opioid mediated analgesia. In contrast, exposure of the island derived populations of deer mice to the weasel elicited only the naloxone-reversible opioid mediated analgesic responses. These findings indicate the existence of genetic variations (population differences) for benzodiazepine and opioid mediation of fright behaviors and aversive responses in the presence of a predator.

116.13

NALOXONE, CHOLECYSTOKININ, AND BOMBESIN INTERACT TO INHIBIT ETHANOL INTAKE. P.J. Kulkosky, H.D. Moore*, R.L. Cannon*, and N. Chiu.* Dept. of Psychology, Univ. of Southern Colorado, Pueblo, CO 81001

Naloxone (NAL), an opiate receptor blocker, and cholecystokinin (CCK) and bombesin (BBS), brain-gut neuropeptides, have each been shown to reduce alcohol consumption after peripheral injection (Myers & Critcher, 1982; Kulkosky, 1985). To characterize the interaction of these agents in the control of alcohol intake, NAL, CCK and BBS were injected i.p. in rats, in single doses and combinations of doses, prior to ethanol consumption induced by water deprivation. After an 8-day adaptation procedure, rats were randomly assigned to receive injections of 0.0, 0.1, 1, or 10 mg/kg NAL, followed by 0, 1, 2, or 4 µg/kg CCK or BBS, prior to 30 min access to 5% w/v ethanol. NAL, CCK and BBS inhibited ethanol intake when injected in single doses. CCK and BBS and NAL and BBS combined to inhibit ethanol intake in a dose-additive manner, except at 4.0 µg/kg BBS + 1 mg/kg NAL, where intake was inhibited supra-dose-additively. NAL and CCK typically inhibited intake infra-dose-additively. These data suggest that NAL, CCK, and BBS inhibit alcohol intake by multiple, interacting mechanisms of action. (Supported by NIH Grant No. RR-08197).

116.14

VASOPRESSIN FAILS TO MODULATE VOLUNTARY ETHANOL INTAKE IN RATS. A. Ross*, E. Perlanski* and L.A. Grupp. Dept. of Pharmacology, Univ. of Toronto. Toronto, Canada M5S 1A8.

Recent research from our laboratory has indicated that manipulations which increase activity in the Renin-Angiotensin System (RAS), attenuate voluntary consumption of ethanol (EtOH) in rats, suggesting that the RAS may participate in the regulation and termination of EtOH intake. Since Angiotensin II (AII) is known to stimulate the release of Vasopressin (VP), the possibility exists that AII attenuates EtOH intake indirectly through stimulation of VP release. In order to investigate this, rats were offered a choice between 6% (w/v) EtOH and water, for 1 hr each day, using a limited access procedure. When EtOH intake had stabilized, each group received AVP or DGAVP (a VP fragment with minimal pressor or anti-diuretic activity) at a dose of 2, 10, 20 or 200 µg/kg injected subcutaneously, immediately prior to EtOH access. Control animals received injections of vehicle only. While consumption of EtOH was not significantly altered by AVP or DGAVP at any of the doses tested, there appeared to be a transient decrease in water intake at the higher doses of both peptides. These findings indicate that VP does not mediate the ability of enhanced RAS activity to attenuate EtOH intake. Furthermore, it appears that VP does not play a critical role in the control of established EtOH drinking behavior. Supported by the Alcoholic Beverage Medical Research Foundation.

116.15

REDUCTION IN VOLUNTARY ETHANOL CONSUMPTION BY ACE INHIBITION IS NOT MEDIATED BY PERIPHERAL ANGIOTENSIN II RECEPTORS. T.B. Lingham*, E. Perlanski* and L.A. Grupp. (SPON: G.A. King). Dept. of Pharmacol., Univ. of Toronto, Toronto, Ont., Canada M5S 1A8.

Angiotensin converting enzyme (ACE) inhibitors, are potent antihypertensive drugs, that also reduce voluntary ethanol consumption in rats (Spinosa et al. 1988). To further characterize this phenomenon, four groups of male Wistar rats received intraperitoneal injections of the ACE inhibitor, Abutapril: CGS-16617 (5, 10 or 20 mg/kg) or saline, and were offered a choice between ethanol (6% w/v) and water using the limited access procedure. Whereas water consumption increased immediately, ethanol intake was reduced only during the second week of drug administration. This finding suggests that ethanol and water intake show different thresholds of responsiveness to the ACE inhibitor.

In the second phase of the experiment animals which received Abutapril (10 mg/kg) or saline, were also given subcutaneous [Sar1,Thr8]-Angiotensin II (500 µg/kg), an Angiotensin II receptor antagonist that does not cross the blood-brain barrier. The antagonist failed to reverse the effect of Abutapril on ethanol consumption, suggesting that if ACE inhibitors reduce voluntary ethanol intake by modulating the renin-angiotensin system, then it must be through the central and not the peripheral renin-angiotensin system. Supported by Ciba-Geigy Canada Ltd.

ION CHANNELS: CELL FUNCTION I

117.1

CALCIUM EFFECTS ON IMPULSE AFTERPOTENTIALS AND SUPERNORMALITY IN EARTHWORM MEDIAN GIANT FIBER. Katherine Lowe Perkins* and Stephen A. George. Neuroscience Prog., Amherst College, Amherst, MA 01002.

The second of two closely spaced impulses travels faster than the first impulse in earthworm giant fibers. This supernormality (SN) requires the presence of Ca^{++} in the bathing medium, and is increased in elevated $[Ca^{++}]$.

Intracellular recordings in median giant fibers revealed depolarizing afterpotentials (DAPs) lasting up to 1 second. In 60% of fibers, the afterpotential was purely depolarizing; in 40%, a hyperpolarization lasting approx. 50 msec was followed by a late DAP. DAPs were enhanced in high $[Ca^{++}]$, and an early DAP could be induced by elevated $[Ca^{++}]$ in fibers lacking one in normal $[Ca^{++}]$. The amount of SN varied with the amplitude of the DAP at all times after the 1st spike, and under conditions in which the DAP amplitude was altered by changing $[Ca^{++}]$.

Different mechanisms are responsible for the early and late phases of the DAP. (1) The amplitude of the early DAP varied monotonically with $[Ca^{++}]$ in the bathing solution up to 18 mM. However, the amplitude of the DAP measured at 500 msec peaked at approx. 7 mM $[Ca^{++}]$, and declined at higher $[Ca^{++}]$. (2) Co^{++} increased the early DAP, but reduced the amplitude of the late DAP.

The DAP and supernormality may be associated with the production of burst discharges in this fiber.

117.2

IONIC CONDUCTANCES IN CRUSTACEAN STOMATOGASTRIC NEURONS IN PRIMARY CULTURE. W.D. Krenz* and P. Fischer*. Zoology Institute, Univ. of Basel, CH-4051 Basel, Switzerland

Individual unidentified neurons from the stomatogastric ganglion of adult crayfish, Pacifastacus leniusculus, were isolated by suction after incubation of ganglia in collagenase/dispase. Cells were plated on poly-L-lysine-coated glass coverslips in supplemented L-15 medium. Under these conditions about 40 to 50% of the explanted neurons survive and grow processes.

After 4 days in culture resting membrane potentials are between -40 and -65mV. Under current clamp most explanted cells show strong outward rectification and only local responses are generated by depolarization. Few cells became fully excitable. Plateau-like potentials are generated by depolarizing current in some neurons.

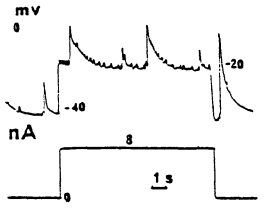
Voltage clamp measurements reveal a fast transient and a slow delayed outward current as well as fast and slow inward currents. These results suggest that primary culture of stomatogastric neurons may enable the detailed analysis of the membrane conductances mediating e.g. conditional bursting in stomatogastric neurons. We are now attempting to isolate identified neurons from the stomatogastric ganglion.

(Supported by the Swiss NSF and the Roche Res. F.)
(SPON: ENA)

117.3

IDENTIFICATION OF VOLTAGE-DEPENDENT ION CHANNELS IN THE CARDIAC FOLLOWER CELL MEMBRANE OF LIMULUS. A.J. Meuse and D.G. Gibson, III. Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609.

Intracellular recording was used to determine the response of Limulus cardiac follower cells to injected current. Seventy percent of the follower cells impaled responded to depolarizing current by increasing the frequency and number of secondary (attenuated) spikes. These additional spikes enhance neuromuscular transmission. The spikes continue as long as depolarization of approximately 50% of membrane resting potential is maintained. The duration of each secondary spike is usually less than 25 msec; spikes appear to be produced by voltage-dependent fast sodium channels, as determined by ion substitution and TTX experiments.



117.4

LOCALIZATION OF CURRENTS IN STOMATOGASTRIC NEURONS. K. Graubard, and D.K. Hartline, Dept. of Zoology, Univ. of Washington, Seattle, WA 98195; and Békésy Lab., Univ. of Hawaii, Honolulu, HI 96822.

A two-electrode somatic voltage clamp was used on pyloric neurons left intact within the Panulirus stomatogastric ganglion, retaining their chemical and electrical synapses with other cells. Spikes in the distant axon are seen in the soma as 2-8 nA brief inward currents which are blocked by TTX. A slow inward current underlying bursts is also abolished by TTX. A TTX-insensitive inward current is revealed in the presence of TEA or TEA + 4AP and is probably caused by distant calcium spikes. A rapidly inactivating, TEA-insensitive, 4AP-sensitive, outward current resembles the "A" current of molluscan neurons. It is inactivated near rest (-50 mV). Hyperpolarization removes inactivation over a range of ca.-65 to -100 mV. The current activates rapidly above -45 mV and then inactivates over a period of 50-80 msec. A-current depresses the firing rate of sodium and calcium spikes in both voltage clamped and unclamped neurons and reduces the size of the sodium spike current. We infer that A-current is distributed on or near the soma and in a non-space clamped region near the spike generating zones. A calcium-dependent outward current, J-current, activates rapidly at clamp steps above -25mV and then decays with a compound time course. This current inactivates at depolarizing holding voltages. The calcium-dependent outward current has no clear effect on cell firing. Increasing depolarization results in an increase in both J-current and in firing rate, and reduces the amplitude of the sodium spike current. Maintaining a depolarization causes inactivation of J-current, spike frequency adaptation, and a further decrease in the amplitude of the sodium spike current. Blockage of J-current with Cd causes little change in spike firing pattern. One explanation of these observations is that J-current is localized to the soma and nearby regions.

Supported by NIH grants NS15697 (K.G.) and NS15314 (D.K.H.).

117.5

WHOLE-CELL VOLTAGE-CLAMP RECORDINGS FROM MURINE RETINAL NEURONS IN CULTURE. M.W. Salter, I. Mody, M.C. Bartlett* & J.F. MacDonald. Playfair Neurosci. Unit, The Toronto Hosp. & Dept. of Physiol., Univ. of Toronto, Toronto, Ont.

Retinal cells were taken from embryonic mice (day 18) and grown in dissociated culture. Recordings were made using conventional whole-cell and single electrode switching voltage-clamp techniques. Electrodes contained (in mM) either 120 KCl and 35 KOH or 120 CsCl and 35 CsOH. In addition, the following were added (in mM): 10 HEPES, 11 EGTA, 4 $MgCl_2$, 2 K_2ATP and 2 $CaCl_2$. The extracellular medium was Hank's balanced salt solution containing HEPES (25 mM) and TTX (1 μM).

With electrodes containing KCl, outward voltage- and time-dependent currents sensitive to TEA were observed. In the presence of TEA (25 mM) and with CsCl electrodes, voltage-dependent inward currents carried by Ca^{2+} and blocked by Cd^{2+} (100 μM) could be recorded. Pressure application of kainic acid (200 μM) evoked currents which were inward at negative holding potentials, reversing between 0 and +5 mV.

The present results demonstrate that murine retinal cells in culture express voltage- and neurotransmitter-gated ionic currents. Hence, this culture preparation is suitable for electrophysiological investigation of mammalian retinal neurons.

Supported by the MRC of Canada. MWS is an Eastburn Fellow and IM is an MRC Fellow.

117.6

MEMBRANE PROPERTIES OF EDINGER WESTPHAL NEURONS: A SYSTEM FOR COMPARING SOMATA WITH SYNAPTIC TERMINALS. J.T.Fujii. Dept. of Physiology, University of Colorado School of Medicine, Denver, CO 80262.

A subpopulation of Edinger Westphal (EW) neurons form large calyciform synaptic terminals that have been studied using intracellular recording techniques (Martin & Pilar, 1963, 1964; Dryer & Chiappinelli, 1985). To compare the membrane properties of these terminals with their parent cell bodies, a slice preparation of the chick midbrain was developed, containing the EW nucleus.

EW neurons exhibited two types of responses to prolonged pulses of depolarizing current (500 ms, 0.4-2.0 nA). Some cells fired repetitively, usually for as long as the pulse was applied. Firing frequency increased as the depolarizing current was increased, ranging from 6 to 100 Hz, and at higher levels often exhibited accommodation, *i.e.*, increase of interspike interval and decrease of action potential amplitude at the end of a train. In the second group, cells meeting the same criteria as the first group (similar or higher resting potentials, input resistances, and time constants) fired only a few action potentials regardless of the size of the depolarizing pulse.

Replacement of calcium with magnesium increased firing frequency of repetitively firing cells and reduced the amplitude of the after-hyperpolarizations (AHP) following trains of action potentials. This suggests that a calcium-activated potassium current is involved in limiting the firing frequency of the cells and is responsible for at least part of the AHP.

The repetitive firing observed in some EW cell bodies resembles that reported for calyciform terminals. Supported by NIH NS09660.

117.7

VOLTAGE DEPENDENT IONIC CURRENTS IN SINGLE CEREBELLAR CELLS GROWN IN THREE-DIMENSIONAL CULTURE. B. Fermini*, P.W. Coates, J. Netzeband*, J.C. Strahlendorf and H.K. Strahlendorf. Depts. Physiology, Cell Biology & Anatomy and Neurology, Texas Tech University Health Sciences Center, Lubbock,TX 79430.

Neurons isolated from cerebelli of late gestation rat fetuses quickly express distinct neuronal features yet grow as *single* cells in three-dimensional culture (Coates and Dunn, this volume), which may prove useful to elucidate intrinsic electrical properties of such neurons. We used this culture system to determine electrophysiological properties of isolated cerebellar neurons from 20 day old rat fetuses grown in culture for 24 to 48 hrs. Whole-cell voltage clamp experiments were performed at 25° C. Several sizable voltage dependent conductances were observed. Depolarizing steps from a holding potential (HP) of -90 mV elicited two outward currents showing different time course, amplitude and sensitivity to the potassium channel blockers tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP). Addition of 4-AP (5 mM) to the bathing medium abolished within 5 min a current showing properties similar to I_A, a transient outward current described in other neurons. TEA (20 mM) greatly reduced the amplitude of an outward current showing delayed rectification properties similar to I_K. Combined application of TEA and 4-AP unmasked two inward conductances activated by depolarization. These currents, separated by using different HP (-90 or -50 mV) were selectively inhibited by tetrodotoxin (30 μM) or cobalt chloride (5 mM) and were identified as I_{Na} and I_{Ca}, respectively. Results demonstrate that single cerebellar neurons devoid of cell-cell communication rapidly exhibit a number of ionic conductances previously observed in more mature interconnected cells and tissue slices. Supported by NS19296 (JCS & HKS) and NS20802 (PWC). BF holds a fellowship from the Canadian Heart Foundation.

117.8

SLOW INWARD AND SLOW OUTWARD CURRENTS INDUCED BY HYPERPOLARIZING PRE-PULSES IN CAT PARASYMPATHETIC NEURONS. E. Kumamoto* and P. Shinnick-Gallagher. (SPON: P.M. Callahan). Dept. of Pharmacology, Univ. of Texas Medical Br., Galveston, TX 77550

We have used the single electrode voltage clamp to examine membrane currents recorded as a result of hyperpolarizing pre-pulses in cat bladder parasympathetic neurons. Hyperpolarizing pre-pulses (10 ms - 1 s) to -90 to -130 mV from holding potentials of -30 to -60 mV elicited slowly decaying inward and/or outward currents. A short duration pulse (20-40 ms) induced only a slow inward current (SIC; 0.13 nA and 8 s); with a longer pre-pulse (1 s) a slow outward current was superimposed on the SIC (SOC; 0.18 nA and 32 s). SIC and SOC became larger at depolarized holding potentials, with large hyperpolarizing pre-pulses, and with long pulse durations, but their relative amplitudes were independent of each other. SIC and SOC were associated with an increased membrane conductance, the SOC had an extrapolated reversal potential around -89 mV at a holding potential of -60 mV. SIC not SOC was depressed in low Na (26 mM); SOC not SIC was reduced in high K (10-20 mM), depressed in low Ca (0.5 mM), and potentiated in high Ca (5 mM). TEA (10 mM) depressed the SOC, but not SIC. 4-AP (1 mM) blocked a transient outward current (I_A), but potentiated SOC. TTX (0.5 μM) and Cs (3 mM) did not affect either current, whereas Ba (1 mM) and Cd (0.5 mM) inhibited both currents. These results suggest that the SIC is a slow Na current and the SOC, a calcium-dependent K current. Supported by NS16228.

117.9

A TRANSIENT LOW-THRESHOLD Ca^{2+} CURRENT UNDERLIES AN AFTERDEPOLARIZATION IN DORSAL ROOT GANGLION NEURONS ACUTELY DISSOCIATED FROM ADULT RATS. G. White*, D.M. Lovinger*, F.F. Weight, Section on Electrophysiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852

In some central and peripheral neurons, action potentials are followed by an afterdepolarizing potential (ADP). Using whole-cell patch-clamp recording, the ADP and the currents underlying the ADP were studied in single dorsal root ganglion cells within 10 hours of being dissociated from whole ganglia by enzymatic and mechanical means. At membrane potentials of -60 mV a small ADP was present which increased in amplitude to between 50 and 65 mV upon hyperpolarization from -60 to -80 mV (n=8). Repetitive cell firing was observed within a narrow window of ADP amplitude. The ADP was only observed in cells which also exhibited a transient low-threshold Ca^{2+} current ($Ca_{(t)}$, n>30). $Ca_{(t)}$ was studied in part by voltage-clamping the cell to -80 mV and applying voltage jumps to -50 mV, a potential at which the active currents are composed almost entirely of $Ca_{(t)}$. The amplitudes of the ADP and $Ca_{(t)}$ were relatively unaffected by equimolar substitution of Ba^{2+} for Ca^{2+}; whereas the addition of 500 uM Cd^{2+} or replacement of external Ca^{2+} with Mg^{2+} resulted in a 90% decrease in the amplitude of the ADP and $Ca_{(t)}$ (n=6). The amplitude of the ADP was comparable between cells in which chloride gradients differed by 40 fold (n=5). We conclude that a transient low-threshold Ca^{2+} current underlies the ADP in adult rat DRG cells. Modulation of this ADP could serve to alter voltage sensitive activities of the cell (Lovinger and Weight, this meeting).

117.10

WHOLE CELL PATCH CLAMP RECORDINGS FROM ACUTELY ISOLATED NEURONES OF THE DORSAL RAPHE NUCLEUS OF THE ADULT RAT N.J. Penington* and J.S. Kelly. Dept. of Pharmacology, Univ. of Edinburgh Med. Sch., Edinburgh EH8 9JZ.

Whole cell recordings were made from individual neurones of the dorsal raphe nucleus of the rat, isolated by the method of Kay and Wong (1986), using a switching voltage clamp amplifier. The pipette contained in mM K^+ Gluconate 122, Hepes 10, $CaCl_2$ 1, $MgCl_2$ 2, EGTA-KOH 11, pH 7.3 with KOH. The mean membrane potential was -56 mV. The passage of depolarizing current pulses caused repetitive firing of fast TTX (0.5 µM) sensitive spikes 80-100 mV in amplitude. The spikes were followed by small A.H.P.'s. Some cells fired spontaneously at rest and each spike was preceded by a slow depolarizing potential. In TTX this prepotential resembled a low threshold Ca^{++} spike; a high threshold Ca^{++} spike could also be evoked. In voltage clamp steps from -60 mV to -20 mV caused an inward/outward current sequence in which the outward current increased with time and was followed by an outward tail current. A graded transient outward current resembling "I_A" was also recorded. Large inward non-inactivating Ca^{++} currents were also seen on steps from -60 mV to -20 and above. Under certain conditions GABA, 100 µM, caused an increase in conductance and an inward current at -60 mV which reversed close to -33 mV (E_{Cl}).

Supported by the Wellcome Trust.

117.11

ELECTROPHYSIOLOGY OF THALAMIC-PROJECTING CHOLINERGIC BRAINSTEM NEURONS AND THEIR INHIBITION BY ACH. C.S. Leonard and R.Llinás, Dept. Physiol. & Biophys., NYU Med. Ctr. 550 First Ave. New York, NY 10016

Intracellular recordings were obtained from pedunculopontine (PPT) and laterodorsal tegmental (LDT) neurons in guinea pig brainstem slices. Three types of neurons were found. One type had "low threshold" Ca^{++} spikes (LTS). Another had an "A" K^+ conductance (A type) and the third type had both an A conductance and LTS (A+LTS type). Experiments were performed to resolve which types were thalamic-projecting and/or cholinergic. Rhodamine beads were injected into the dorsal-lateral thalamus 3-30 days prior to the experiments. Retrogradely labeled neurons were impaled, characterized electrophysiologically and stained with lucifer yellow. Nine doubly labeled neurons were all of the A or A+LTS type. Their mean diameter (20µm) and shape index (width/length = 0.7) agree well with those of PPT cholinergic neurons in rat (Rye et al., '87, JCN 259:483-528). The cholinergic nature of A and A+LTS neurons was confirmed for five neurons by combined lucifer yellow staining and NADPH-diaphorase histochemistry which selectively marks cholinergic neurons in PPT and LDT. In A and A+LTS neurons, Ach (3-30µM) produced dose dependent hyperpolarizations up to 20 mV with up to 60% reductions in input resistance in 10µM eserine. These effects were also evoked by carbachol and bethanechol and were blocked by atropine (0.5-10µM). In 2µM TTX or in Ca^{++} free/2mM Co^{++} medium, the effects were undiminished indicating a direct action of Ach on the impaled neurons. These actions of Ach likely arise from activation of a K^+ current since the Ach hyperpolarization is suppressed by hyperpolarization to E_K and it is not reversed by using 3M KCl electrodes. Preliminary evidence suggests this Ach inhibition is mediated by M_2 receptors since 1µM pirenzepine only partly blocks carbachol (30µM) hyperpolarizations while 10µM pirenzepine completely blocks them. We conclude that Ach inhibits thalamic-projecting cholinergic neurons of the PPT and LDT by activating a K^+ conductance via an M_2 muscarinic receptor. These results support the idea that M_2 receptors can play an autoregulatory role in Ach release by feedback inhibition. Support by NS07848 & NS13742.

117.12

OSCILLATORY MEMBRANE CURRENTS INDUCED IN RAT GONADOTROPES BY TREATMENT WITH GONADOTROPIN-RELEASING HORMONE. T.L. Croxton*, N. Ben-Jonathan, and W.McD. Armstrong*. Dept. of Physiology and Biophysics, Indiana Univ. Sch. of Med., Indianapolis, IN 46223

Cell-attached patch clamp studies were carried out with rat anterior pituitary gonadotropes identified in primary cultures by reverse hemolytic plaque assays. Under basal conditions, spontaneous current spikes were recorded. These could be eliminated by treatment with D600 and were apparently due to Ca^{2+} action potentials. Brief (15-30 sec) treatment with high K^+ produced a concurrent outward shift in current, consistent with membrane depolarization, and a delayed (3 min) period of inward current. Brief treatment with GnRH (2-100 nM) induced prominent oscillations in membrane current (10-30 per min) that persisted for several minutes. These consisted initially of clusters of action potentials but evolved over 5-10 min into a series of large amplitude inward current pulses. Similar treatments with TRH (100 nM) had no effect. In the presence of D600, GnRH stimulation did not induce inward currents but did produce oscillatory activation of small conductance K^+-channels. These results indicate that cellular mechanisms involved in GnRH responses have an intrinsic rhythmicity and that both K^+-channels and Ca^{2+}-channels are modulated by intracellular mediators. Our data suggest that GnRH stimulation leads to pulsatile increases in Ca^{2+} in gonadotropes. Supported by USPHS grants DK36575 and DK07554.

117.13

MUSCARINIC-DEPENDENT AFTERDISCHARGE IN RAT PREFRONTAL CORTEX. R. Andrade. Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

To determine the mechanisms involved in the actions of acetylcholine in the prefrontal cortex, I have conducted intracellular recordings using in vitro brain slices from the prelimbic and dorsal anterior cingulate regions.

Layer V neurons typically exhibit membrane potentials in the range of -70 to -80 mV and respond to a brief depolarizing current pulse (300-600 ms) with a rapidly accommodating burst of spikes. Carbachol (1 µM-100 µM) elicits a dose-dependent depolarization which often fails to reach threshold even after prolonged bath administration (> 5 min). However, when the carbachol stimulation is paired with a brief depolarizing pulse, the resulting spike burst initiates a long-lasting afterdischarge which greatly outlasts (several seconds to minutes) the depolarizing pulse. This period of sustained spiking is made possible by the appearance of a long-lasting depolarizing afterpotential (DAP). This DAP can be elicited by low-threshold calcium spikes in the presence of TTX but is blocked by Ca^{2+} removal of Mn^{2+} administration suggesting that it is triggered by Ca^{2+} influx. Since the depolarization and the DAP are blocked by atropine, these actions appear to be mediated via muscarinic receptors. The ability of muscarinic receptors to induce the appearance of a long-lasting DAP confers unusual associative properties to the cholinergic input to this region. (Supported by the Pharmaceutical Manufacturers Association Foundation.)

117.14

COMPARISON OF EXCITABLE MEMBRANE PROPERTIES IN IDENTIFIED NEURONS FROM MAMMALIAN VISUAL CORTEX. K. Giffin*, J.P. Doyle* and J.M. Nerbonne, Dept. of Pharmacology, Washington Univ. Med. Sch., St. Louis, MO. 63110.

In order to test the hypothesis that cortical cell "types" can be distinguished electrophysiologically, we are comparing excitable membrane properties in identified (projection and intrinsic) neurons derived from rat (Long Evans) primary visual cortex (area 17). Callosal-projecting cells are identified in vitro following in vivo retrograde labeling with rhodamine beads. Injections are made into the left hemisphere of area 17 at postnatal day 5, and ~2-15 days postinjection, cells from the contralateral area 17 are dissociated and placed in culture. Bead-labeled cells are identified prior to recordings under fluorescence illumination. The monoclonal antibody VC1.1 (generously provided by Dr. J.R. Naegele, Rockefeller) is being used to identify a population of intrinsic GABAergic neurons (Naegele, J.R. et al., J. Neurosci. 8:79-89); ~10-15% of dissociated rat area 17 cells are VC1.1-positive. For physiology, two methods are used to identify VC1.1-positive neurons: (1) prelabeling and visualization prior to recording, or (2) identification following recordings (from randomly selected cells) with Lucifer Yellow-filled electrodes. Using the whole-cell patch-clamp technique, the waveforms of voltage-activated currents and action potentials are being examined. Preliminary results suggest that, although the time- and voltage-dependent properties of the currents through voltage-gated Ca^{2+} channels are similar, there do appear to be differences in action potential waveforms in these two cell types. Ongoing experiments are aimed at investigating these observations further. Support: NIH (T32-HL07275).

117.15

CHARACTERIZATION OF MEMBRANE CURRENTS FOUND IN VIVO IN ELECTROPHYSIOLOGICALLY IDENTIFIED NEURONS IN CAT MOTOR CORTEX. A. Baranyi, M.B. Szente, and C.D. Woody, UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Membrane conductances and currents of 486 neurons in the motor cortex of conscious cats were analyzed using bridge mode recordings and single electrode voltage clamp techniques. Pharmacologic separation and characterization of conductances were accomplished by intracellular pressure injection of drugs and cations. Five types of neurons were identified by their spike parameters, firing activity patterns, current-voltage relationships, and responses to ventrolateral thalamic and pyramidal tract (PT) stimulation: (i) fast PT, (ii) slow PT, (iii) bursting nonpyramidal tract (nPT), (iv) fast-spiking nPT, and (v) regular-spiking nPT neurons. Membrane currents were separated and characterized by their voltage-dependence, time course and pharmacologic sensitivity: (i) transient outward current (I_A), (ii) delayed rectifier current (I_K), (iii) Ca-activated fast (I_C) and slow ($I_{K(Ca)}$) potassium currents, (iv) persistent sodium current ($I_{Na(P)}$), (v) low threshold Ca current ($I_{Ca(T)}$), (vi) high threshold calcium current ($I_{Ca(L)}$), and (vii) inward anomalous rectifier current (I_R). The recorded combinations, magnitudes and parameters of activation and inactivation of these currents showed differences among cell types that contributed to their characteristic firing properties.
(Supported in part by AFOSR F49620-85-C-0100 and HD05958.)

117.16

INTRACELLULAR INJECTION OF APAMIN REDUCES SLOW AFTERHYPERPOLARIZATIONS AND IPSPs IN NEOCORTICAL NEURONS OF CATS. M.B. Szente, A. Baranyi, and C.D. Woody, MRRC, BRI, Depts. of Psychiatry and Anatomy, UCLA Med. Ctr., Los Angeles, CA 90024.

Electrophysiologic effects of intracellularly injected apamin, a Ca^{2+}-dependent K^+ channel blocker, were investigated in neurons of the motor cortex of awake cats. Single electrode voltage clamp techniques were used to measure membrane and synaptically activated currents. Changes occurred within 2-4 min after pressure injection of apamin with partial recovery observed within 8-15 min. Apamin selectively abolished an outward current that mediated a slow afterhyperpolarization (AHP) following depolarizing current pulses and action potentials without influencing the duration of the action potentials or their fast AHPs. In addition apamin increased the firing activity evoked by depolarizing current pulses and produced a small increase in the rate of background firing. The resting potential and input resistance were essentially unchanged by apamin. Apamin also diminished a late, slowly decaying component of inhibitory postsynaptic potentials and currents elicited by stimulation of the ventrolateral thalamus or the pyramidal tract. The results suggest that the late, slowly decaying component of these inhibitory postsynaptic responses is generated by an apamin sensitive, Ca^{2+}-dependent K^+ conductance which is also responsible for the slow AHP.
(Supported by AFOSR F49620-85-C-0100 and HD05958.)

ION CHANNELS: CELL FUNCTION II

118.1

VOLTAGE-DEPENDENT CALCIUM SIGNALLING IN SINGLE T LYMPHOCYTES. R.S. Lewis and M.D. Cahalan. Dept. of Physiology & Biophysics, University of California, Irvine, CA 92717.

A rise in intracellular free Ca^{2+} is believed to be an early triggering signal in the activation of T lymphocytes by mitogens. We have investigated the voltage dependence of this signal in single cells of the human T-cell leukemia line, Jurkat. Cells loaded with fura-2/AM were illuminated alternately at 350 nm and 385 nm, and their fluorescence measured with a photomultiplier attached to the microscope. The average response of a population of these cells to the lectin mitogen, phytohemagglutinin (PHA; 10 ug/ml), is a sigmoidal rise in $[Ca^{2+}]_i$ which peaks within several minutes at 22°C. Photomultiplier measurements show that the sigmoidal time course of this response results from an abrupt increase in $[Ca^{2+}]_i$ from ~100 nM to >500 nM in individual cells occurring with variable latencies after PHA addition, ranging from one to five minutes. After the initial rise, $[Ca^{2+}]_i$ in single cells oscillates with a period of minutes, suggesting positive and negative regulatory feedback. Membrane depolarization, induced by replacing Na^+ by K^+ in the external Ringer solution, inhibits the mitogen-stimulated Ca^{2+} rise, rapidly reducing $[Ca^{2+}]_i$ to values near the baseline level. This result contrasts markedly with the stimulatory effect of depolarization on $[Ca^{2+}]_i$ in most excitable cells. Depolarization may antagonize Ca^{2+} entry in T cells by directly inhibiting the entry mechanism or by reducing the electrical driving force on Ca^{2+} ions entering the cell. The effects of depolarization on $[Ca^{2+}]_i$ were studied further in Jurkat T cells voltage-clamped in the whole-cell mode, using patch pipettes containing K^+ aspartate and 100 uM fura-2 as the only Ca^{2+} buffer. Step depolarization to 0 mV does not detectably alter resting Ca^{2+} levels, consistent with previous evidence for a lack of voltage-dependent Ca^{2+} channels in T cells, and demonstrating that Ca^{2+} does not permeate to any significant degree through the T cell's voltage-dependent, depolarization-activated K^+ channels. (Supported by NIH grants NS 08021 and NS 14609 and the ONR)

118.2

SECOND MESSENGERS REGULATE CALCIUM INFLUX IN MAST CELLS. R. Penner*, G. Matthews+, and E. Neher*. *Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen, FRG and +Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Mast cells are non-excitable cells that secrete histamine in response to a variety of external chemical agonists. Agonist stimulation increases $[Ca]_i$ due both to IP_3-mediated release from internal stores (Neher, *J. Physiol.*, **395**:193, 1988) and to influx of external Ca^{2+}. In experiments using simultaneous patch-clamp recordings and fura-2 measurements of $[Ca]_i$, we have identified two possible mechanisms of Ca-influx. First, IP_3 can induce Ca-influx with increases in total whole-cell current less than 1-2 pA and without discernible channel activity; if this influx is via IP_3-gated channels, they must be of small-conductance and highly calcium-specific. Second, agonist stimulation activates cation channels with a reversal potential of 0 mV and a single-channel conductance of about 50 pS in Ringer and 16 pS in isotonic Ba^{2+}. Activation of the 50 pS channel can produce a whole-cell current of 2-15 pA at -50 mV. This channel can also be activated by internal GTP-γ-S, and its activation by agonists can be blocked by GDP-β-S, indicating that it is second messenger-gated rather than direcly agonist-gated. High $[Ca]_i$ can also block activity of the 50 pS channel. Although this channel resembles the IP_3-gated channel reported in lymphocytes by Kuno & Gardner (*Nature*, **326**:301, 1986), it is not gated by either IP_3 or IP_4, since high levels of IP_3 and IP_4 in the pipette solution do not activate the channel or prevent its activation by agonist.

118.3

REPETITIVE FIRING PATTERNS IN CAT NEOCORTICAL NEURONS DEPEND ON PRECEDING SUBTHRESHOLD MEMBRANE POTENTIAL W.J. Spain*, P.C. Schwindt, W.E. Crill. Dept. of Physiology & Biophysics, Univ. of Washington, Seattle WA 98195.

Activation of sufficiently slow ionic currents with subthreshold voltage dependence can alter the firing pattern evoked by a subsequent stimulus and in this way provide a postsynaptic mechanism for coding the preceding level of membrane potential. We have identified such coding and its ionic mechanisms in neocortical neurons (in slices). These neurons normally exhibit a monotonic decline of firing rate (adaptation) to a final level during depolarization from resting potential evoked by an injected current pulse. Preceding a test current pulse with a subthreshold depolarizing prepulse (using voltage clamp) reduced the initial firing rate. Hyperpolarizing prepulses usually resulted in a biphasic response. The initial firing rate increased up to 100%. After this burst, firing rate declined below, and accelerated to, the control level over periods up to 0.8s. Other cells responded to hyperpolarizing prepulses only with a prolonged increase in firing rate lasting up to 0.6s or only with a prolonged depression of firing rate lasting 1-2s. The effects in each cell were graded with the level and duration of the conditioning prepulse, and they persisted in Ca^{2+}-free perfusate or with 400 μM Cd^{2+} added. We hypothesized that the effects were mediated by two previously identified currents: a slowly inactivating K^+ current (IA) that is partially inactivated at resting potential, and the anomalous rectifier (IAR) which is activated by hyperpolarization. Pharmacological studies with Cs^+ (a specific blocker of IAR) and 4-aminopyridine (a specific blocker of IA) suggested that the postinhibitory behavior is determined by the balance between deactivation of IAR and the activation of IA following hyperpolarization. Postinhibitory excitation occurs in cells where IAR dominates, whereas postinhibitory inhibition occurs when IA dominates. Supported by NIH grants NS16972 & NS01166.

118.4

NERVE IMPULSES MODIFY VOLTAGE-GATED CHANNELS IN FROG NEUROGLIA. H.G. Marrero*, M.L.Astion*, J.A. Coles*, R. Blanco* and R.K. Orkand. Institute of Neurobiology UPR-MSC Old San Juan PR 00901

Using the loose patch clamp technique, macroscopic voltage-dependent TTX-sensitive sodium currents as well as 4-AP and sodium sensitive potassium currents were recorded from the surface of the intact optic nerve of Rana pipiens. The currents likely arise from the surface astrocytes because they are still recorded when most of the axons have degenerated and in the presence of 10 times normal potassium, which blocks axon excitability. In the intact nerve the currents are facilitated after an action potential in the underlying axons. The facilitation following a train of nerve volleys (10 Hz, 1 sec) decays to one-half in about 0.3 sec. This is 4-5 times more rapid than the decay of glial depolarization. The timecourse of glial depolarization reflects changes in extracellular potassium following its release from active axons. The peak of the facilitation occurs 30 msec after a nerve volley while the axons themselves are partially refractory. After adding 4-AP, the timecourse of the facilitation is slowed. The modification of the channel behavior by nerve impulses may represent a new type of nerve-glia interaction.

Supported by NIH Grants NS 07464, NS 12253, GM 07170 (MSTP Univ. of Pennsylvania) and NSF Grant RII 8705802.

118.5

INTRACELLULAR APPLICATION OF 4-AMINOPYRIDINE AND TETRAETHYLAMMONIUM DECREASES ACCOMODATION IN FROG MYELINATED AXON. M.O.Poulter and A.L. Padjen. Dept of Pharmacology and Therapeutics, McGill University, Montreal P.Q. Canada H3G 1Y6

The functional role of potassium conductances were studied by impaling myelinated axons of frogs with microelectrodes containing 4-aminopyridine (4-AP, 5-10mM; blocker of the fast potassium currents, G_{Kf1}, G_{Kf2}) and tetraethylammonium (TEA, 10-20mM; blocker of both the fast and slow potassium current, G_{Ks}). The after-hyperpolarizing potential (duration 2-5 ms following single action potential) disappeared and was replaced by an after-depolarizing potential lasting 50-100 ms within 5 minutes when either 4-AP and/or TEA were present in microelectrode. In response to intracellular current pulses (200 ms) accomodation of action potentials decreased more with TEA than with 4-AP. A computational model based on Hodgkin-Huxley equations, which included the three potassium and sodium currents, reproduced the results obtained with both blockers. These results imply that accommodation in amphibian myelinated axons is regulated by potassium channels and not function of sodium channel inactivation.

/Supported by the MRC/

118.6

EFFECTS OF THE SUBSTITUTION OF LITHIUM FOR SODIUM ON THE RESPONSE OF MOLLUSCAN SMOOTH MUSCLE TO ZERO POTASSIUM. R.B.Hill. Department of Zoology, University of Rhode Island, Kingston, R.I. 02881.

Part of the evidence for a contribution to resting potential by an electrogenic sodium pump is provided by a control experiment, prior to Li treatment. A radular protractor muscle (RPM) of Busycon canaliculatum had a resting potential of 72 mV, just before being subjected to 3 minutes in zero potassium. The immediate response was a 3 mV hyperpolarization, giving way to a 3 mV depolarization, and followed by a 4 mV after- hyperpolarization on readmission of K. It was hypothesized that depolarization was due to the stopping of Na/K exchange, and that after- hyperpolarization was due to stimulation of Na/K exchange by excess internal Na. When Na was replaced with Li, the response to 3 minutes in zero K was 10 mV hyperpolarization, followed by 20 mV depolarization on readmission of K. When the RPM was returned to Na, the muscle behaved as a K-electrode, responding to 3 minutes in zero K with a simple hyperpolarization. The effect of pre-treatment with Li is thus like that of low Na. The Na-channels may be blocked.

118.7

EFFECTS OF RYANODINE AND RUTHENIUM RED ON NEUROMUSCULAR TRANSMISSION IN CRAYFISH. R.N. Friedman* Dept. of Physiol., Meharry Med. Col. and Dept. of Physics, Vanderbilt Univ. and S. Fleischer* (SPON: J.P.Wikswo) Dept. of Molec. Biol., Vanderbilt Univ., Nashville, TN 37235.

In preliminary studies, we observed effects of ryanodine (Ry) and ruthenium red (RR) on short-term facilitation at neuromuscular junctions (NMJs) of crayfish (Procambarus clarkii) 1st and 2nd walking limbs. Facilitation of excitatory junctional potentials (EJPs) was evoked by 30 Hz, 10s stimulation of the nerve to the dactyl opener muscle. Ry, which produces concentration-dependent activation and inactivation of calcium channels in sarcoplasmic reticulum (SR), enhances EJP amplitude (Δ as great as 100%) and duration over a wide range of concentrations (1-100μM). The effective concentration varied with individual preparations, possibly because of diffusion barriers. RR has been shown to depress EJPs at a frog NMJ via a presynaptic mechanism and to antagonize Ry effects on SR. RR reduced crayfish EJP amplitude in a concentration-dependent manner over a range of 1 to 50 μM. RR also antagonized Ry-induced EJP enhancement. Neither Ry nor RR at the concentrations examined affected the muscle membrane resting potential. Ry decreased muscle input resistance only at high (100μM) concentrations. These studies may help to determine if certain calcium-regulating mechanisms modulating neurotransmitter release at NMJs are similar to those affecting calcium release in muscle SR.

Supported by NIH Grant NS24751.

118.8

IONIC CONDUCTANCES IN TYPE I CELLS FROM THE CAROTID BODY. EFFECTS OF LOW O_2 TENSION. C. Gonzalez[1], J.R. López-López*[1], J. Ureña* and J. López Barneo* Depto. Fisiología. Facultad de Medicina. Universidad de Valladolid(1) and Universidad de Sevilla(2). SPAIN

Rabbit carotid bodies (c.b.) were enzymatically dispersed and cultured in Minimum Essential Medium supplemented with 1 mM glutamine on poly-lysine coated cover-slips. Two to twenty four hours later the cover-slips were transfered to a superfusion chamber. Type I chemoreceptor cells were patch-clamped using a whole-cell configuration.

By adequate combinations of internal and external solutions it was possible to define on pulse depolarization the presence of voltage-dependent Na^+, Ca^{++} and K^+ channels in these cells. Na^+ and Ca^{++} currents are unaffected by lowering the pO_2 in the supperfusion fluid. K^+ currents were reversibly reduced being the reduction related to the environmental pO_2; the effect of hypoxia was similar in the presence or absence of internal Ca^{++} and ATP. The findings suggest that this O_2-sensitive K^+ current may have a primary role in the transduction process in the c.b.

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118.9

EFFECTS OF NH_3 AND CO_2 ON THE MAMMALIAN MUSCLE SPINDLES. Y. Fukami, Dept. Cell Biology & Physiology, Washington University School of Medicine, St. Louis, MO 63110.

The changes in impulse activity of the sensory endings of single isolated cat muscle spindles, as affected by NH_3 and CO_2 were analyzed. Muscle spindles in adult cat tail muscles were used. A single spindle isolated together with its innervating nerve was placed in an experimental chamber containing a normal Locke solution. To facilitate access of the bathing solution to the sensory endings the spindle capsule was nicked or partially resected. The pH of all solutions used was 7.4. Experiments were performed at room temperature of about 24°C.

Typically, NH_4Cl application resulted in an initial brief increase of impulse activity. This was followed by a reduction of the activity, usually to below the control level. Upon replacement of NH_4Cl solution with normal Locke, the activity further decreased, and only gradually returned toward the control level. Upon introduction of CO_2 solution impulse activities were immediately suppressed, which was followed by a gradual return to or by an increase even beyond the control level. Upon return to the normal solution the activity further increased and then gradually returned toward the control level.

In summary, we conclude that the effects of CO_2 and NH_3 on sensory impulse activity of the cat muscle spindle are most likely caused by changes in the pHi of the sensory ending.

Supported by NIH grant NS19661 and the Muscular Dystrophy Assn.

118.10

IMPULSE ENTRAINMENT AT TRIGGER ZONES WITH NON-UNIFORM EXCITATION AND GEOMETRIC STRUCTURES. P.N. Steinmetz* and J.F. Fohlmeister. Neuroscience Program, University of Minnesota, Minneapolis, MN 55455.

The impulse encoding process has been simulated on a CRAY II supercomputer. Our intent is to characterize all possible impulse encoding phenomena that result from cable and channel-density non-uniformities alone, employing only the most fundamental impulse kinetics. The trigger zone was modelled as a short axonal segment continuous with a cylindrically symmetric excitable cable of indefinite length (but with possible variations in cable diameter). All channel gating kinetics were restricted to those of the Hodgkin-Huxley model; non-uniform excitability was achieved by changes in $\bar{g}_{Na}$ and/or $\bar{g}_K$ (i.e., channel-density) only. Whereas the space-clamped membrane becomes spontaneously firing when $\bar{g}_{Na} \geq 2\bar{g}_{Na}(HH)$ (depending on temperature), an embedded hyperexcitable trigger zone of 15 μm length (10 μm diam. axon) remains silent for $\bar{g}_{Na}(TZ) > 100\bar{g}_{Na}(HH)$. With a hyperexcitable trigger-zone ($\bar{g}_{Na}(TZ) \gtrsim 10\bar{g}_{Na}(HH)$) the neuron maintains repetitive firing under constant current stimulation with current injected into the "cable" hundreds of microns distant from the TZ; this is in contrast to phasic behavior (single impulse initiated at site of current injection) for a uniformly excitable cable with standard values of $\bar{g}_{Na}$ and $\bar{g}_K$. Once initiated, impulses propagate normally.

118.11

MODELLING ELECTRICAL EXCITABILITY IN THE CELL BODY AND AXON OF TYPE B BULLFROG SYMPATHETIC GANGLION CELLS. W.Yamada, C. Koch, P. R. Adams. Div. of Biology 216-76, California Institute of Technology, Pasadena, Ca. 91125, U.S.A. and Howard Hughes Medical Institute, SUNY at Stony Brook, NY 11794.

Using conventional microelectrode techniques, seven voltage- , calcium-, and time- dependant currents have been characterized in type B sympathetic ganglion cells. Previously, we presented an electrical model of these cells based on voltage-clamp data (Koch and Adams, 1985). The model incorporates all seven ionic currents (I_{Na}, I_{Ca}, I_A, I_{AHP}, I_C, I_K, I_M) as well as calcium diffusion, buffering and pumping, and describes the electrical behavior of these cells under various protocals (e.g. current clamp).

Several outstanding problems remain. In particular, under the assumption of a spherical soma, the experimentally measured value of whole cell capacity corresponds to an anomalously high value of specific membrane capacity (8 $\mu F/cm^2$; Adams, Brown & Constanti, 1982). Furthermore, patch clamp data (Jones, 1987) supports the notion of two distinct populations of sodium channels; one in the cell body proper with a high voltage threshold for action potential generation and an axonal sodium current with a much lower threshold. Accordingly, we added an "n compartment" nonmyelinated axon to our cell body which includes the I_{Na} and I_K currents characterized by Frankenhaeuser & Huxley's (1964) frog node of Ranvier data. Timing of axonal and somatic action potentials was studied by varying the midpoint activation and inactivation values of both sodium currents as well as the geometry of the axon.

118.12

NEURON SIMULATIONS WITH SABER. N.T.Carnevale, T.B.Woolf, and G.M.Shepherd. Neurol.Dept. and Dept. of Neurobiol. and Behavior, SUNY, Stony Brook, NY 11794, and Sect. Neuroanat., Yale Univ. Sch. of Med., New Haven, CT 06510

Modeling is essential for testing hypotheses of neuronal function that involve time- and voltage-dependent ionic conductances. Commercial simulation programs have been useful tools for this work (e.g. SPICE--Segev et al., Biol.Cybern. 53:27, 1985; Bunow et al., ibid. 41). However, SPICE needed analog computer circuits to emulate active currents. This increased model complexity, slowed simulations, and hindered its widespread use for neuronal modeling.

We report here our experience modeling biophysically realistic membrane properties with SABER (Analogy Inc.), a new general purpose simulator. Like SPICE, it handles linear electrotonus easily (Flach et al. Soc.Neurosci.Abs. 13:159, 1987). One major advantage of SABER is that it allows construction of models with arbitrary properties: the empirically determined equations that describe ionic conductances, currents, and concentration shifts can be translated directly into model elements ("templates") written in C-like code.

We have used these features of SABER to: write templates for ionic currents with various kinetic schemes including the classical descriptions of Hodgkin and Huxley; simulate action potential initiation and propagation; model the effects of dendritic active sites on synaptic transmission (Shepherd et al. Soc.Neurosci.Abs. 1988); simulate the effects of the ionic concentration changes that accompany neuronal activity on membrane currents.

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118.13

THE SUPERNORMAL PERIOD IN NEURON MODELS. E. N. Yamoah* and N. Stockbridge* (SPON: R.E. Walley). Department of Surgery, University of Alberta, Edmonton, Canada T6G 2B7.

Supernormal excitability in nerves has been used to explain neural encoding, frequency dependent conduction at axonal branches and synaptic facilitation. However, the factors responsible for this phenomenon are unknown. We tested three neuron models for a supernormal period following a membrane action potential and investigated the basis for supernormality in the models that predicted it. A supernormal period was observed in the Hodgkin & Huxley (HH) model for the squid giant axon and the Connor, Walter & McKown (CWM) model of crustacean motor axons, while it was absent in the Frankenhaeuser & Huxley (FH) model of frog myelinated nerve fibres.

In the HH model, supernormal excitability results from undershoot of the potassium activation variable n during and following the after-hyperpolarization. Prevention of this undershoot in n removes supernormal excitability with no effect on the properties of the action potential except the after-depolarization. In the CWM model, inactivation of the A-current occurs during and following the after-hyperpolarization. We conclude that supernormal excitability, at least in the squid and crustacean axon models, is caused by the slow kinetics of potassium currents. The late reduction in potassium conductance persists after the membrane potential and inward current parameters have returned to their initial states and means that less depolarizing current is required to bring the membrane to threshold.

The absence of a supernormal period in the FH model shows one way this model is insufficient to account for all the observed properties of myelinated nerves.

(Supported by Alberta Heritage Foundation for Medical Research)

121

SYMPOSIUM. AT LAST! POTASSIUM CHANNELS: EXPRESSION AND REGULATION. N.C. Spitzer, U.C. San Diego (Chairperson); P.R. Adams, S.U.N.Y. Stonybrook; R.W. Aldrich, Stanford; L.Y. Jan, U. C. San Francisco; B. Rudy, N.Y. University.

Knowledge of potassium currents and channels has taken a great leap forward in the past few years, as a result of the fruition of physiological, biophysical, genetic and molecular biological approaches. Studies of nicotinic acetylcholine receptors, $GABA_A$ and glycine receptors, and sodium and calcium channels blazed the trail. Potassium channels are ubiquitous, and an understanding of their function will have broad implications. Here, we focus on new information of at least five different kinds.

We will first examine physiological and biophysical evidence that demonstrates that qualitatively different potassium currents participate in similar cellular functions, and that each current in turn is the result of contributions of qualitatively different channels (**Adams, Aldrich, Spitzer**). It has been possible to understand the changes occurring during early stages of differentiation of the excitable membrane at the whole cell and single channel levels; mechanisms that regulate these changes will also be proposed (**Aldrich, Spitzer**). The roles of these currents have been quantitatively evaluated with computer models that have allowed formal and rapid tests of different schemes (**Adams, Spitzer**). Genetic studies have furthered the understanding of potassium channel function (**Aldrich**), and molecular analyses have now achieved the cloning of what will no doubt be several potassium channels (**Jan, Rudy**). Use of the *Xenopus* oocyte injection expression system has confirmed the success of the molecular approaches and has allowed further analysis of the structure-function relationships of these channels (**Jan, Rudy**).

122

WORKSHOP: NEW APPROACHES TO THE FUNCTIONAL DEVELOPMENT OF THE NEOCORTEX. D.O. Frost, Yale Med. Sch. (Chairperson), J. Sanes, Washington Univ. Med. Ctr., S. McConnell, Stanford Med. Sch., D. O'Leary, Washington Univ. Med. Ctr., M. Bear, Brown Univ.

Different neocortical areas are associated with distinct functional systems. In each area, functionally related sets of neurons and afferent axons are segregated into radially oriented modules; efferent neurons projecting to different targets are segregated at different depths within a module. This symposium presents new approaches to studying the development of these features at the molecular, cellular and systems levels.

J. Sanes will demonstrate how retroviruses can be used to study neuronal lineage and test the hypothesis that neurons that are vertically distributed within a neocortical module are clonally related. S. McConnell will show how grafting techniques can be used to demonstrate that the laminar position and efferent connections of some cortical neurons are determined before or at the time of their last mitosis in the ventricular zone. D. Frost will demonstrate that in animals with surgically induced retinal projections to the somatosensory thalamus, somatosensory cortical neurons have visual response properties very like those of normal visual cortical neurons; thus, the visual and somatosensory cortices may use similar neuronal circuits to perform similar transformations on their inputs. D. O'Leary will present data from transplant experiments showing that the modality of afferents to a sensory cortex may determine which among its multiple, immature descending projections to different sensory systems are maintained and which are eliminated. M. Bear will show how receptor blockers are used to demonstrate the action of NMDA receptor-controlled Ca^{2+} channels in mediating the influence of electrical activity on the stabilization and elimination of immature cortical connections.

123.1

PROTEIN KINASE C - A SECOND MESSENGER RESPONSIBLE FOR THE NEUROTROPHIC ACTIONS OF PHORBOL ESTER AND EXCESS K
A. R. Wakade, S.V. Bhave,* R.K. Malhotra* and T.D.Wakade*. Department of Pharmacology, SUNY Health Science Center, Brooklyn, N.Y. 11203

Sympathetic neurons (SN) of the chick embryo are supported in culture not only by nerve growth factor (NGF) but also by excess K and phorbol esters. Since phorbol esters exert their effects through activation of protein kinase C (PKC), it was of interest to know if neurotrophic action of phorbol 12,13-dibutyrate (PDB), excess K and NGF was mediated by PKC. 150,000 SN derived from peripheral ganglia of 10-day-old chick embryos were supported in culture by either NGF (40 ng/ml), PDB (30 nM), or K (35 mM) for 3 days. PKC activity was estimated in cytosolic and membranous fractions. The respective values (pmol/mg prot.) were: 0.14, 0.09 for NGF-, 0.14 and 0.7 for 35 mM K-, and 0.14 and 0.47 for PDB-supported SN. PDB caused a dose-dependent (3 to 100 nM) increase in survival of SN and an increase in PKC activity. K produced dose-dependent (7.5 to 75 mM) increase in survival and activation of PKC. 35 mM K and 30 nM PDB did not produce additive effects on survival and PKC activity. PKC activity of SN suported by PDB for 3 days returned to basal level 30 min after washout and was increased by addition of PDB or excess K but not NGF. We conclude that PKC plays the role of a second messenger in the neurotrophic action of PDB and excess K.

123.2

NEUROTROPHIC ACTION OF FORSKOLIN ON CHICK SYMPATHETIC NEURONS IS INDEPENDENT ON cAMP FORMATION.
T.D. Wakade,* R.K. Malhotra,* S.V. Bhave,* and A.R. Wakade, (SPON: F. Scalia) Department of Pharmacology, SUNY-Health Science Center, Brooklyn, N.Y. 11203.

Sympathetic neurons (SN) obtained from 10-day-old chick embryos were cultured in a serum-free medium. The surival of SN was determined 3 days after the addition of either nerve growth factor (NGF) or forskolin (FORK). About 40% of plated SN survived in the presence of 10 μM FORK. This value was comparable to that obtained with NGF (40 ng/ml). Morphological appearance of SN supported by FORK was different than that of NGF-supported SN. The neurotrophic action of FORK was evident for at least 6 days. cAMP content of SN supported by NGF and FORK for 3 days was not significantly different (9.1 ± 1.0 vs. 11.3 ± 2.6 pmols/mg prot.). SN supported by NGF for 3 days and then exposed to FORK for 15 min showed a marked increase in cAMP (144.6 ± 17.9 pmols/mg prot.). However, SN supported by FORK for 3 days failed to show an increase in cAMP upon re-exposure to fresh FORK. SN exposed to FORK for 2 hr (in which period there is more than 10-fold increase in cAMP) and then returned to forskolin-free medium did not survive in culture. We conclude that the neurotrophic action of FORK is probably unrelated to adenylase cyclase-cAMP system.

123.3

FIBROBLAST GROWTH FACTOR MIMICS SOME OF THE EFFECTS OF NEURONAL GROWTH FACTORS ON CHICK PARASYMPATHETIC NEURONS IN CELL CULTURE.
R. Nishi and F.P. Eckenstein, Dept. of Cell Biology & Anatomy, Oregon Health Sciences University, Portland, OR 97201

Neurons of the chick ciliary ganglion (CG) have been shown to proceed through a period of neuronal cell death that coincides with synapse formation in the periphery. It has been suggested that neuronal cell death during development is controlled by the availability of trophic molecules released by the target tissues which the neurons normally innervate. We have previously shown that ciliary ganglion neurons destined to die can be maintained in cell culture if the medium is supplemented with soluble molecules derived from extract of eyes (which contains all the target tissues of the CG). We have identified two biologically active molecules in eye extract based upon a long- term bioassay : one molecule promotes survival and growth, whereas the other induces choline acetyltransferase. We are currently purifying these trophic molecules. Recently, it was reported that fibroblast growth factor (FGF) promotes survival of CG neurons for 24 hrs in cell culture. Because FGF has been isolated from both retina and vitreous humor, we have tested whether FGF also acts as a trophic molecule for longer times in cell culture. We have shown that basic and acidic bovine FGF stimulate survival long- term growth of cultured CG neurons. The dose- response curves for both FGFs were similar and reached half- maximal values at a concentration of 200pg/ml. The response to bFGF was not affected by the presence of heparin, whereas the response to aFGF required heparin. Neither FGF promoted the specific induction of choline acetyltransferase activity. FGF is not responsible for the growth promoting activity that we are purifying because the partially purified molecule does not bind to heparin. The precise function of FGF during neural development has yet to be determined.
Supported by the Medical Foundation of Oregon (RN & FE); Amyotrophic Lateral Sclerosis Association (RN); & ADRDA (FE)

123.4

BASIC FIBROBLAST GROWTH FACTOR PROMOTES THE SURVIVAL AND DEVELOPMENT OF MESENCEPHALIC NEURONS IN VITRO.
G. Ferrari*, M.-C. Minozzi*, C. Soranzo*, G. Toffano and S.D. Skaper (SPON: M.G. Nunzi). Fidia Research Laboratories, Abano Terme, Italy.

Basic fibroblast growth factor (bFGF) has been reported to act as a neuronotrophic factor for CNS neurons. Neuronal survival and neurite extension induced by bFGF have been described in hippocampal, cortical, spinal cord and granule neurons in vitro. These effects, however, have never been tested on fetal mesencephalic-derived neurons, nor associated with specific CNS neuronal cell types (e.g., in terms of expression of neurotransmitter-related properties). To address these questions, serum-free fetal rat mesencephalic cells were utilized to study the effects of bFGF on neuronal survival with time in culture. Addition of bFGF increased the number of surviving cells with a neuronal morphology. Due to the heterogeneity of the neuronal cells it was important also to determine if the effects of bFGF were associated with specific neuronal cell types. The survival of dopaminergic and GABAergic neurons was assessed by measuring their specific ^{3}H-DOPA and ^{14}C-GABA uptakes, respectively. bFGF (in the ng/ml range) was capable of increasing, in a dose-dependent manner, both ^{3}H-DOPA and ^{14}C-GABA uptakes. This effect was completely abolished by antibodies against bFGF. These results strengthen the possibility that bFGF may play a key role in normal nervous system development or function.

123.5

A POSSIBLE AUTOCRINE ROLE OF BASIC FGF FOR MAINTENANCE AND TRANSMITTER STORAGE OF CHROMAFFIN CELLS.
K. Unsicker, R. Westermann*, C. Grothe*, D. Gehrke*, and P. Böhlen*. (SPON: European Neuroscience Association), Dept. of Anatomy and Cell Biology, Univ. of Marburg, F.R.G.

Adrenal chromaffin cells cultured from young postnatal rat require trophic factors for their survival and maintenance of transmitters. Nerve growth factor (NGF) and ciliary neurotrophic factor (CNTF) have been shown to serve these needs, but their presence in the adrenal medulla and putative physiological relevance have not been proven. We show that basic FGF may have such a role in as far as this protein is stored in and released from chromaffin cells and enhances survival and transmitter storage of cultured chromaffin cells. Several lines of evidence suggest that bFGF or an immunologically related molecule is present in the adrenal medulla: (i) a protein obtained in bFGF-enriched fractions from bovine adrenal medulla extracts co-migrates with purified bFGF and is recognized by anti-bFGF IgG in immunoblots, (ii) the same antibody blocks the neurotrophic activities of (a) rat adrenal medullary homogenates, (b) bovine chromaffin vesicle proteins, (c) a conditioned medium obtained from carbachol-stimulated isolated bovine chromaffin cells, (iii) chromaffin vesicle proteins stimulate proliferation of cultured bovine aorta endothelial cells. Purified bFGF supported survival and prevented catecholamine losses of chromaffin cells cultured from 8 day old rats,but,in contrast to NGF and CNTF, did not induce neurite outgrowth on polyornithine or laminin substrates during a 4-day culture period. These results suggest a potential role of bFGF as a trophic factor for chromaffin cells.

Supported by a grant from the German Research Foundation (Un 34/11-1,2).

123.6

BASIC FGF IN MUSCLE AND ITS EFFECTS ON CULTURED MOTONEURONS AND SCHWANN CELLS.
Ken Vaca. Dept. of Neurology, Program in Neuroscience, Baylor College of Medicine, Houston, TX 77030.

A polypeptide has been purified from autopsied adult human skeletal muscle using an increase of choline acetyltransferase activity in cultured chick ciliary ganglion cells as bioassay. It has a mass of 18 kDa and elutes from a heparin affinity column at 1.5 M NaCl. Its activity is precipitated by antibodies to basic FGF and mimicked by recombinant human basic FGF. Similar bioactivity is found in muscle-conditioned medium and can be extracted from extracellular matrix of lysed cultured embryonic chick muscle by heparinase treatment or high salt treatment and heparin affinity purification. Besides enhancing cholinergic differentiation, basic FGF increases survival of ciliary neurons and Schwann cells, neurite outgrowth, Schwann cell motility and interactions of neurons and Schwann cells to form small ganglion-like aggregates and nerve-like fascicles. Neurons cultured prior to or during the cell death period are more acutely sensitive to basic FGF's effect on survival than cultures of older neurons. It is proposed that, in the adult, heparan sulfate proteoglycan localizes basic FGF in the endplate region to modulate cholinergic expression and that denervation-induced release of degradative enzymes liberates basic FGF to induce sprouting and reinnervation acutely or the extensive fibroblast proliferation seen in chronic denervation.

123.7

CHARACTERIZATION OF A HIPPOCAMPAL MEMBRANE-ASSOCIATED CHOLINERGIC NEUROTROPHIC FACTOR. B.C. Wise and M.J. Mastrangelo, Jr.* FGIN, Georgetown Univ., Washington, D.C. 20007

We have identified a CNS cholinergic neurotrophic factor (NTF) associated with hippocampal membranes. The NTF which increases choline acetyltransferase (ChAT) activity in primary cultures of fetal rat septal neurons is developmentally regulated and is unevenly distributed in brain regions (highest in hippocampus, negligible in cerebellum). The membrane-associated NTF is solubilized by incubation with 1 M NaCl and is inactivated by trypsinization. The NTF does not change the number of septal neurons immunostained for ChAT, GAD, or NGF receptor nor does it alter GAD activity in cultured cells indicating a specificity of action toward the cholinergic neuron which is not due to a survival effect. Gel filtration of the high salt membrane extract indicates a MW of 500 kD for the NTF. The protein has been partially purified by ammonium sulfate fractionation followed by phenyl- and DEAE-Sepharose chromatographies. The protein has an isoelectric point of about 6 and is not a glycoprotein since it does not bind to wheat-germ agglutinin or Con A matrices. By various criteria, the NTF is not identical to other neurotrophic proteins such as NGF or laminin and its relationship to the neural cell adhesion molecule (NCAM), remains to be determined.

123.8

SENSITIVITY OF SUBSTANTIA NIGRA NEURONS TO EPIDERMAL GROWTH FACTOR. R. Loy, D. Heyer* and P. DiStefano. Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642 and Neuroscience Research division, Abbott Laboratories, Abbott Park, IL 60064

Neurons in the CNS are sensitive to several identified growth factors, and loss of trophic support has been suggested as contributing to cell loss associated with neurodegenerative disorders, such as Alzheimer's disease and Parkinsonism. High levels of epidermal growth factor (EGF) have been seen in the rat substantia nigra (SN), although sensitive cells have been previously identified only in cortex, cerebellum and hippocampus. Using a monoclonal antibody specific to the EGF receptor (EGFR), 151-IgG, we find EGF sensitive cells both in the pars compacta and pars reticulata of the substantia nigra. These are small sized, round or fusiform cells, and medium-sized multipolar cells, whose distribution shows very little overlap with dopamine-containing cells. In material double stained for tyrosine hydroxylase (TH), only a few of the smaller EGFR-positive cells are also immunoreactive for TH. Adjacent sections stained for glutamic acid decarboxylase (GAD), show a very similar distribution to EGFR, although double labeling will be necessary to confirm the colocalization of GAD and EGFR. The presence of EGFR suggests a possible role of this growth factor in SN cell survival and neurite growth. Supported by NIH grant #NS25169.

123.9

NERVE GROWTH FACTOR INDUCES GENE EXPRESSION FOR THE ALZHEIMER'S β-AMYLOID PROTEIN. W.C. Mobley, F.M. Longo,* R.L. Neve, S.B. Prusiner, and M.P. McKinley,* Dept. of Neurology, University of California, San Francisco, CA 94143 and Harvard Medical School, Boston, MA 02115

β-amyloid is the principal protein in both cerebrovascular and neuritic plaque amyloid in the brains of Alzheimer's disease (AD) and elderly Down's syndrome (DS) patients. It is a small molecule which is derived from a precursor (β-PP) encoded on human chromosome 21 (Nature 325:733; Science 235:877,880). β-PP gene expression in the brain is principally within neurons. We report that β-PP gene expression is regulated during development and that nerve growth factor (NGF) induces a marked increase in the level of β-PP mRNA in developing basal forebrain.

Slot blots of total RNA were probed with a cDNA encoding β-PP (FB68L-Science 235:880). β-PP mRNA was found in all brain regions sampled; significant increases occurred during the early postnatal period. In septum, β-PP mRNA was not detected until postnatal day (PD) 9. A marked increase followed; levels greater than in the adult were found at PD 12 and 16. The increase in β-PP mRNA in septum was coincident with increasing activity for choline acetyltransferase (ChAT) and suggested that these markers may be coordinately regulated. NGF (30 μg) administered intraventricularly had a marked influence on ChAT activity and on the level of β-PP mRNA in septum. Three injections during the first postnatal week increased β-PP mRNA levels by more than 10-fold relative to vehicle-treated animals. The β-PP mRNA induced by NGF migrated at 3.2 kb, identical to that found in vehicle-injected animals. The effect of NGF was regionally selective in that NGF induced no increase in β-PP mRNA in the thalamus or brainstem. These data suggest that NGF acts to increase gene expression for β-PP in basal forebrain cholinergic neurons; they raise the possibility that expression of the β-PP gene in these neurons may normally be regulated by NGF.

123.10

NEUROTRANSMITTER RECEPTOR EXPRESSION AND NEURONAL TROPHIC FACTOR SECRETION BY AN UNINNERVATED TISSUE: AVIAN AMNION. C.W. Bowers. Dept. Physiol., UCSF Med. Ctr., San Francisco, CA 94143.

The smooth muscle of avian amnion is unusual because it is normally never innervated. However, based on contractile responses, I have shown that this uninnervated and avascular tissue expresses at least 11 different types of receptors for neurotransmitter substances including acetylcholine, norepinephrine, histamine, 5-HT, VIP, urotensin II, neurotensin and somatostatin-28. Three neurotransmitters, histamine, 5-HT and norepinephrine, each act via two separate and antagonistic receptors. The amnion also responded to prostaglandin E_2. On the other hand, the tissue did not respond to substance P or bradykinin, two peptides known to affect smooth muscle contractility in other systems. Studies with organ cultured amnion indicated not all of the responses develop in a defined medium. Explants from chick ciliary, dorsal root and Remak's ganglia attached and extended neurites on amnion in culture. Furthermore, the normally uninnervated amnion secreted a factor that was required for ciliary neuron survival in vitro. In contrast, sympathetic neurons did not survive on amnion, even in the presence of NGF. The results indicate that this novel smooth muscle preparation will be useful for identifying epigenetic factors that control the expression of functional receptors.

123.11

SYNTHESIS OF NEUROTROPHIC FACTOR FOR SYMPATHETIC NEURONS IN XENOPUS OOCYTES BY TRANSLATION OF mRNA EXTRACTED FROM RAT WOUNDED CEREBRAL CORTEX. T.T. Quach, A.M. Duchemin, B.K. Schrier, R.J. Wyatt. Lab. Developmental Neurobiol., NICHHD, Bethesda, MD and Neuropsychiatry Branch, NIMH, Saint Elizabeths Hosp., Washington, DC.

Injury to the rat cerebral cortex induces the appearance in the area around the lesion of trophic factors which promote survival of dissociated neurons in culture (Nieto-Sampedro et al., Science 217:860, 1982). We studied the expression of at least one of these factors in frog oocytes using the survival of sympathetic neurons from 12 day old chick embryos to detect the activity. Level of detection of the assay was enhanced 10 fold, as compared with the standard microassay, by miniaturization using 100 cells in 10 μl of culture medium per well in Terasaki plates.

Tissues enriched in neurotrophic factors were obtained from the walls of a 7-day post-lesion wound cavity of the cortex of 150 gm rats. Poly(A+)RNA was prepared from these tissues and from similar cortex areas of control rats, size-fractionated on a sucrose gradient and injected into oocytes. While oocytes produced only low trophic activity from translation of mRNA fractions from control cortex, significant trophic activity was produced and secreted by oocytes injected with 1.5-2.0 Kb mRNA fractions from lesioned cortex.

123.12

EVIDENCE FOR DISTINCT FACTORS AFFECTING TRANSMITTER AND NEUROPEPTIDE PHENOTYPES. H. Nawa* and P. H. Patterson. (SPON: N. Davidson) Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

The over thirty neuropeptides that have been identified thus far are found in an enormous variety of combinations with classical transmitters. The specification of these transmitter phenotypes poses a difficult developmental problem. An instructional signal that specifies one transmitter phenotype (acetylcholine) has been purified to homogeneity from heart cell conditioned medium (CM) (Fukada, PNAS 82: 8795, 1985). We find that unfractionated heart cell CM also induces the expression of several neuropeptides and their mRNAs in rat sympathetic neurons: substance P (SP), somatostatin (SOM), vasoactive intestinal polypeptide (VIP) and dynorphin, but not neuropeptide Y. A different pattern of peptide induction was observed for CM from skin fibroblasts. The inducing activities for SP, SOM and VIP are separable from each other and from the cholinergic activity by ammonium sulfate precipitation and ion exchange chromatography. These observations raise the possibility that a large number of distinct factors may influence the choice of transmitter phenotype in developing neurons.

123.13

THE EXPRESSION OF NERVE GROWTH FACTOR PROTEIN IN THE DEVELOPING HIPPOCAMPUS IN REAGGREGATE CULTURE.

J. D. Roback*+, B. H. Wainer+, and T. H. Large# (SPON: P. C. Hoffmann).
+The University of Chicago, Chicago, IL 60637, and #The University of California School of Medicine, San Francisco, CA 94143.

In the rat central nervous system, nerve growth factor (NGF) has been found to exert trophic actions on the septal cholinergic neurons during both development and adulthood. Recently, NGF mRNA and protein have been found at high levels in the hippocampus, the primary target of septal efferents. In addition, septal neurons retrogradely transport NGF from the hippocampus. In light of the putative trophic effect of hippocampally-derived NGF on septal cholinergic neurons, we are investigating the regulation of NGF expression in the hippocampus during development.

We previously demonstrated that dissociated fetal murine septal and hippocampal neurons grown under reaggregating culture conditions recapitulate many aspects of development observed *in situ* (Hsiang,J.,et al,Neurosci.,21:333,1987). In the present studies, we reaggregated dissociated hippocampus from E_{15} mouse embryos and cultured the aggregates for up to 21 days. At E_{15}, the hippocampus has not yet received septal afferents, and so our hippocampal reaggregates have never interacted with septal neurons. We harvested the reaggregates at selected time points (days 7,10,14,19, and 21 in culture) and quantitated their NGF protein content using a sensitive two-site enzyme-linked immunoassay (ELISA) (Weskamp,G. and Otten,U., J. Neurochem., 48:1179,1987).

Hippocampal reaggregates demonstrate a 12-fold increase in NGF protein content between days 7 (3.6±2.0 pg NGF/mg prot.) and 21 (44.2±12.0 pg NGF/mg prot.) in culture. This increase is similar to that seen in the hippocampus *in situ* during the corresponding period, postnatal days 0 to 14 (Large,T.H.,et al,Science,234:3552, 1986). These results suggest that, in reaggregate culture, hippocampal neurons, in the absence of any interaction with septal afferents, are able to recapitulate the developmental profile of NGF protein expression observed in the hippocampus *in situ*.

Supported by NIH grants 5-T32HD070009, NS-25787, and grants from The Alzheimer's Disease and Related Disorders Society, and the Brain Research Foundation of the University of Chicago.

CYTOSKELETON AND AXONAL TRANSPORT I

124.1

DISTRIBUTION OF MICROTUBULES IN CNS CELL BODIES PREPARED BY FREEZE-SUBSTITUTION. H. TATSUOKA and T.S. REESE. Lab. of Neurobiology, NINCDS, NIH, at MBL, Woods Hole, MA 02543.

In another abstract we report on the use of the chinchilla anteroventral cochlear nucleus as a preparation of CNS cell bodies amenable to direct freezing and freeze-substitution-here we report on the distribution of the myriad microtubules preserved in these cell bodies and analysed in stereo views of montaged serial reconstructions. Bundles of microtubules appear to originate near the concave face of the Golgi apparatus, between it and the nucleus, and run parallel to the nuclear envelope, in some instances toward the initial segment of the axon. Other microtubules originate close to nuclear pores. Vertically oriented microtubules cross the zone between the Golgi apparatus and rough endoplasmic reticulum but their concentration is lower than in the zone between the nucleus and Golgi apparatus. Microtubules run just beneath and parallel to the cell membrane in the outer cortical zone, between the endoplasmic reticulum and the cell membrane; some vertically oriented microtubules terminate very close to the plasma membrane. If microtubules in the cell body, like those in the axon, are pathways for organelle transport, then the characteristic distributions of microtubules in the cell body might represent transport pathways leading from one part of the cell body to another.

124.2

THE RETROGRADE TRANSLOCATOR IN SQUID AXOPLASM IS CYTOPLASMIC DYNEIN. B. J. Schnapp, Laboratory of Neurobiology, NINCDS, NIH, at the MBL, Woods Hole, MA. 02543.

We previously characterized two microtubule-based translocators in squid axoplasm that are candidates for the anterograde and retrograde vesicle motors responsible for rapid axonal transport (Vale et al, 1985, Cell, 43: 623). The anterograde translocator, kinesin, was purified while purification of the retrograde translocator proved elusive. I now report that HMW1 (Vale et al, 1985, Cell, 43:39), a high molecular weight (greater than 1000 kd) microtubule-associated protein in squid neural tissues is a microtubule translocator that promotes sliding of microtubules on glass at up to 2 um/sec and bead movement in the retrograde direction on centrosome-nucleated microtubules. This protein is indistinguishable from two-headed axonemal dynein and cytoplasmic dynein in bovine brain (MAP1C; Paschal et al, 1987; J. Cell Biol. 105:1273.) by sedimentation velocity (20s), electrophoretic mobility in SDS, and by its structure in molecular shadowed images. When axoplasmic supernatants exhibiting bidirectional bead movement are exposed to UV irradiation in the presence of vanadate, (specifically cleaves the dynein heavy chain; Eiford et al, 1986, J. Biol. Chem. 261:2337), retrograde bead movement is specifically blocked.

124.3

VESICLE TRANSPORT IN INTACT AND SEVERED GIANT AXONS OF CRAYFISH. T. A. Viancour. Biological Sciences, Univ. Maryland, Catonsville, MD 21228.

Vesicle fluxes and transport rates were studied using video microscopy techniques to view lateral and medial giant axons (LGAs and MGAs) which were either intact, or which were surviving an earlier transection. In the intact axons there were at least three transport-rate subclasses of vesicles moving by fast axonal transport. Rate distributions for anterograde moving vesicles were identical to those for retrograde moving vesicles. The fastest anterograde vesicles were optically distinguishable from retrograde vesicles. The results support the idea that elements of intact axoplasm impede vesicle translocations, possibly indicating a mechanism for targeting of vesicles.

Transection did not affect organelle fluxes in proximal segments of severed LGAs. Fluxes in distal segments of LGAs and MGAs declined more slowly than predicted from directly observed vesicle transport rates, taking 2-3 d to drop to 50% of normal. Fluxes fell to about 10% of normal by 7 days, and continued to drop toward zero in MGAs, but recovered in LGAs to about 50% of control levels. Both giant axons were physiologically normal for at least 28 days following transection.

124.4

3-D ULTRASTRUCTURE OF CYTOSKELETAL ELEMENTS IN BULLFROG OLFACTORY AXONS. P.R. Burton, M. Gurfinkiel*, and D.B. Treanor*, Department of Physiology and Cell Biology, The University of Kansas, Lawrence, KS 66045.

Images in serial micrographs of olfactory axons were examined, digitized, and subjected to computer-assisted 3-D reconstructions. Discontinuities in microtubules (MTs) and neurofilaments (NFs) are clearly shown. NFs are calculated to have an ave. length of 118 μm, are usually bundled, and the mean no. of profiles/axon is ~6.5 (range: 0-29); 8.5% of the axons lacked NFs in the regions examined. Axons show 2-3 MTs with an ave. length of 426 μm (Burton, Brain Res., 409:71). NFs and MTs tend to lie in their own domains in the axon, as does the smooth ER (SER) tubule. The typically single tubule is very long and is more often associated with NFs than MTs. Considering the calculated ave. lengths of NFs and MTs in olfactory axons, about 42 end-to-end NFs and 11 end-to-end MTs would be required to extend the length of a 5 mm long nerve. If NF and tubulin proteins are translocated from perikaryon to terminal as assembled polymers, it appears that they are moved into the axon within their own domains as trains of long segments with "free" ends. Extensive synaptic contacts occur between an axon terminal and many mitral cell dendrites with little evidence, to date, of axon branching. Typical asymmetric junctions show postsynaptic densities 250-400 nm wide, but a few junctions show an axon with a broader area of apparent synaptic activity partly surrounding a dendrite. Supported by NIH grant NS25518 and a grant from the KU Biomed. Comm.

124.5

ANALYSIS OF GENOMIC CLONES FOR THE 57KD NEURAL INTERMEDIATE FILAMENT PROTEIN (NIFP). L.M. Parysek* and C.A. Ley* (SPON: D.R. McCrimmon) Depts. Cell Biology & Anatomy and Neurology, Northwestern Univ., Chicago, IL 60611

Despite the exclusive neuronal distribution of the 57kD NIFP (Parysek and Goldman, J. Neurosci. 1988), cDNA's encoding this IF protein have indicated that it is more like Type III IF proteins than Type IV (neurofilament triplet) proteins (Leonard, et al, 1988, JCB, Parysek, et al., 1988, Neuron). We have isolated genomic clones representing the entire 57kD NIFP gene that have enabled us to obtain previously missing amino terminal coding sequence and begin an analysis of the promotor region of this gene. Sequence data indicates that from the ATG start codon, the region 453 bases upstream contains at least four GC boxes and two C-rich regions, one like that identified in the vimentin promotor. The sequence TATAA is located -82 bases upstream from the ATG start codon. There is no region of similarity to the 16mer GTAACGGGACCATG found in the genes of other Type III IF proteins, vimentin and desmin. Further analysis of this gene, particularly intron location, may provide clues to its neuron-specific expression.

Supported by the Les Turner ALS Foundation.

124.6

A 66KDa protein is a putative subunit of mammalian neurofilament. F.-C. Chiu* (SPON: W.T. Norton). Dept. of Neuro. and Neurosci. Albert Einstein Coll. of Med., Bronx, NY 10461.

We have purified a 66KDa protein from the triton-insoluble, cytoskeletal fraction of rat spinal cord. Upon removal of urea, this protein (IF66) formed 10 nm filaments. IF66 was biochemically distinct from NF-L in pI (5.5 for IF66, 5.1 for NF-L) and in amino acid composition. Antiserum to IF66 was raised and, on western blots of spinal cord homogenates, it stained a major immunoreactive band at 66KDa and a minor band at 140 KDa. On vibratome sections, this antiserum stained intensely axons in spinal cord and in cerebellum. Neuronal perikarya and nuclei, astrocytes and oligodendrocytes were not stained. Adsorption with purified IF66, but not NF-L, NF-M or NF-H, completely removed the immunoreactivity on western blots and in tissue sections. Using purified IF66 as standards, we estimated that IF66 constituted about 0.5% of the total protein in the homogenate of adult rat spinal cord, whereas NF-L constituted about 1.5%. IF66 was found only in nervous tissues, primarily in CNS with only trace amounts in the sciatic nerve. IF66 was not detectable in cultured astrocytes and extraneural tissues such as liver and kidney.

(Supported in part by USPHS NS23840)

124.7

CHARACTERIZATION OF PROTEIN KINASE ACTIVITIES IN A NEUROFILAMENT ENRICHED PREPARATION ISOLATED FROM BOVINE SPINAL CORD. HARISH C. PANT (SPON:K. KUSANO). LNC, NINCDS, NIH, BETHESDA, MD 20892

Protein kinase activities in a neurofilament enriched preparation from bovine spinal cord were examined using a variety of endogenous and exogenous substrates as well as specific activators and inhibitors. Incubations in the presence of [δ-^{32}P]-ATP and 10mM Mg^{2+} showed that all three neurofilament subunits (NF-L,M, and H) were phosphorylated under those conditions, but that the major labeled endogenous protein in this preparation was an 18KD protein. Neither activators or inhibitors of cAMP-dependent or Ca^{2+}/calmodulin dependent kinases altered this labeling pattern. However, staurosporine, a potent inhibitor of protein kinase C, selectively inhibited 32p incorporation into the 18KD protein, but not the neurofilament proteins (NF). Using various exogenous substrates (e.g., casein, H1 and H2A histones, and syntide-2) it was possible to demonstrate that this preparation did contain significant Ca^{2+}/calmodulin protein kinase II and PKC-like protein kinase activities which could be inhibited by mastoparan and staurosporine, respectively. High salt extraction of the NF preparation was used to separate the kinase activities. The kinase activity in the pellet, which contained virtually all the NF proteins, phosphorylated the 18KD protein and H1-histone but not the NF proteins or casein. The soluble fraction could phosphorylate all the NF proteins and casein, but not H-1 histone. These data indicate that the principal NF protein kinase activity in the bovine NF preparations appears to be due to casein-like protein kinase(s).

124.8

PHOSPHORYLATION OF SYNTHETIC PEPTIDES BY NEUROFILAMENT-ASSOCIATED PROTEIN KINASES. C.B. Caputo*, L.A. Sygowski*, & A.I. Salama. (SPON. J.B. Patel) Dept. of Pharmacology, ICI Pharmaceuticals Group, Wilmington, DE 19897.

Several protein kinases (PK) co-purify with neurofilaments (NF). We studied the peptide substrate & inhibitor specificities of these kinases. NFs with PKs were prepared from rat brain stem & spinal cord by an axonal flotation procedure & 77% of the PK activity was extracted with 0.8M KCl. PK phosphorylated 3 cAMP-dependent PK substrates, LRRASLG, LRRASLG-NH_2, & LRRASVA, with K_ms=2-5μM & V_{max}s=30μmol/min/mg. Neither cAMP nor cGMP enhanced these reactions. PK phosphorylated a Ca-calmodulin-dependent PK substrate, KKRPQRATSNVFS-NH_2, with calmodulin present (K_m= 17μM) & a segment of preproVIP, SSEGESPDFPEELEK (K_m=100μM), without cofactors. Peptide substrates for protein kinase C, casein kinase II, & tyrosine kinases were not phosphorylated by PK. A Walsh inhibitor peptide (aa 5-24) completely inhibited phosphorylation of cAMP-dependent PK substrates as 1μM (Ki=70nM). Trifluoperazine (Ki=30μM) & chlorpromazine (Ki=122μM) inhibited the calmodulin-dependent kinase. Caffeine inhibited phosphorylation of all peptides with a Ki=3.8mM for preproVIP & Ki=13.9mM for LRRASLG. At 1μg/ml heparin inhibited phosphorylation of preproVIP (by 89%) but not of LRRASLG. Thus, at least three types of PKs accompany NF proteins: one resembles the catalytic subunit of cAMP-dependent PK, the second is calmodulin-dependent, & the third is cofactor independent.

BEHAVIORAL PHARMACOLOGY

125.1

EEDQ-INDUCED INACTIVATION OF DOPAMINE D-1 OR D-2 RECEPTORS IN RATS DIFFERENTIALLY INHIBITS STEREOTYPIES INDUCED BY DA AGONISTS. J. Arnt and J. Hyttel. H. Lundbeck A/S, Ottiliavej 7-9, DK-2500 Copenhagen, Denmark.

Ex vivo D-1 or D-2 receptor binding in striatum was reduced by 65-78 per cent after treatment with EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) (20-40 µmol/kg, s.c., 24 hours before) in combination with either the D-2 antagonist raclopride (2.5 µmol/kg, s.c.) or the D-1 antagonist SCH 23390 (0.90 µmol/kg, s.c.), respectively. Parallel to a 65 per cent reduction in D-1 receptor binding there was a 51 per cent decrease in the production of cAMP in striatal homogenates. In rats with D-2 receptor inactivation we observed an inhibition of the stereotyped behaviour induced by the D-2 agonist quinpirole when given alone or in combination with the D-1 agonist SK&F 38393. The effect of the mixed D-1/D-2 agonist apomorphine was also inhibited. In rats with D-1 receptor inactivation we found no changes in the behavioural effects of quinpirole when given alone or in combination with one of the D-1 agonists SK&F 81297, SK&F 38393 or SK&F 75670. Likewise the effect of apomorphine was unchanged. The D-1 agonists did not induce stereotypies when given alone, although oral dyskinesias were observed. These were more intense in rats with reduced D-2 receptor density. These results indicate that a near normal density of D-2 receptors is critical for expression of the stereotyped behaviour induced by DA agonists. In contrast, there is a large surplus of D-1 receptors to enable the response to a D-2 agonist. This is illustrated in particular by the persistant behavioural effects of the partial D-1 agonist SK&F 75670.

125.2

COMPUTERIZED STUDIES OF BEHAVIOR REVEAL ALTERATIONS IN RESPONSIVITY TO D-1 AND D-2 AGONISTS FOLLOWING CHRONIC NEUROLEPTICS. G. Ellison, R. See, and J. Kim*. Dept. Psychol., UCLA, Los Angeles, CA 90024.

Using novel computerized methods, we have studied progressive changes in the behavior of rats administered chronic neuroleptics for very prolonged periods. Oral movement (OM) dyskinesias are directly quantified by a computerized TV video system whereby OMs are directly measured via UV-sensitive spots on the upper and lower lips of the rats. Animals which have been given chronic neuroleptics very gradually develop increased OMs of very small amplitudes; these are detected by computer but not by human observer. FFT analyses indicate that these oral movements are of the exact same frequency range reported in humans with TD. Animals given chronic neuroleptics also show develop a heightened reactivity to the ability of D-1 agonists to induce OMs. Motor activity, feeding, drinking, and rearing are also recorded by computer. Animals given chronic neuroleptics again respond more to a D-1 than to a D-2 agonist challenge. Thus, alterations in D-1 receptor tone may be important in chronic neuroleptic-induced side-effects.

125.3

REINFORCING AND DISCRIMINATIVE STIMULUS EFFECTS OF GBR-12909 IN RHESUS MONKEYS. M.S. Kleven, E.W. Anthony, E.B. Nielson, and W.L. Woolverton. The Drug Abuse Research Center, The University of Chicago, Chicago, IL 60637.

GBR 12909 (1-[2-[Bis(4-fluorphenyl)methoxy]ethyl]-4-(3-phenyl-propyl)-piperazine dihydrochloride) is a highly selective dopamine reuptake blocker. Since blockade of DA reuptake is correlated with reinforcing and discriminative stimulus (DS) properties of cocaine (COC), it is possible that GBR 12909 would share these effects of COC. Three rhesus monkeys were prepared with intravenous catheters and, under baseline conditions, allowed to self-administer COC in daily experimental sessions (0.03 mg/kg/inj; FR 10; 2-hr/day). When COC self-administration was stable, 0.9% saline was substituted for COC for several consecutive sessions until responding declined to low levels (<10 inj/session). The monkeys were then returned to baseline conditions and, when behavior was again stable, GBR 12909 (0.01-0.3 mg/kg/inj) was substituted for COC in the same manner as saline. GBR 12909 was self-administered at rates that exceeded those maintained by saline in all 3 monkeys with peak rates of self-administration of 60-80 inj/session in 2 of the monkeys. A second group of rhesus monkeys was trained in a 2-lever, food-reinforced, drug discrimination paradigm to discriminate cocaine (0.2 mg/kg; i.m., 10 min pre-session) from saline. When the discrimination was acquired, the monkeys were injected before test sessions with various doses of the training drug or GBR 12909 (0.1-1.6 mg/kg; iv, 5 min pre-session). COC engendered a dose-related increase in the percent of responses that occurred on the COC-appropriate lever. GBR 12909 produced a comparable dose-related increase in cocaine-appropriate responding with the highest dose (1.6 mg/kg) completely substituting for the training dose of COC . Thus, GBR 12909 functioned as a positive reinforcer and had COC-like DS properties in rhesus monkeys. These results are consistent with the hypothesis that blockade of DA reuptake is associated with COC-like behavioral effects. (Supported by DA-00250)

125.4

BEHAVIORAL EFFECTS OF (+)3-PPP MEDIATED THROUGH A DOPAMINERGIC MECHANISM. W.D. Essman* and J.H. Woods (SPON: R. Davis). Depts. of Pharmacol. and Psychol., Univ. of Michigan, Ann Arbor, MI 48109.

Various doses of three dopaminergic (DA) antagonists (haloperidol, spiroperidol, and (+)butaclamol) and the sigma ligand BMY 14802 were given 15 minutes before cumulative doses of 3-(3-hydroxyphenyl)-N-propylpiperidine ((+)3-PPP) in an observation procedure using male Sprague-Dawley rats. Each DA antagonist produced a dose-dependent reduction in (+)3-PPP-engendered behaviors (locomotion, sniffing, sideways walking), but not (+)3-PPP-induced convulsions. BMY 14802 reduced (+)3-PPP-induced behaviors, but only at a dose that induced changes in behavior by itself. In a cross-tolerance study between (+)3-PPP and the sigma selective compound 1,3-di-ortho-tolyl-guanidine (DTG), dose-effect curves for (+)3-PPP were determined before and after five days of i.c.v. administration of 100ug of DTG. While backward walking, forelimb extension, and piano playing induced by DTG diminished across days, the before and after dose-response curves for (+)3-PPP-engendered locomotion and sniffing did not differ statistically (although sideways walking did show a slight reduction). These results corroborate past findings of the importance of a DA mechanism in the behavioral effects of (+)3-PPP in this observation paradigm. Supported in part by USPHS Grant DA-5437.

125.5

DISCRIMINATIVE STIMULUS (DS) PROPERTIES OF *S*-(+)-N-METHYL-1-(1,3-BENZODIOXOL-5-YL)-2-BUTANAMINE (*S*-MBDB). R. Oberlender* and D.E. Nichols. Dept. of Medicinal Chemistry and Pharmacognosy, Purdue Univ. Sch. of Pharmacy, W. Lafayette IN 47907.

The synthesis and preliminary pharmacological evaluation of N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), the alpha-ethyl homologue of MDMA, were recently described (Nichols, et al., J. Med. Chem., 29: 2009, 1986). In a subsequent study (R. Oberlender and D.E. Nichols, Psychopharmacology, in press), MBDB exhibited DS properties in rats similar to MDMA but not (+)-amphetamine.

We now report the results of experiments with rats trained to discriminate the more active *S*-(+)-isomer of MBDB (1.75 mg/kg) from saline in a two lever food reinforced (FR-50) paradigm. Complete substitution and similar stereoselectivity was observed with MDMA and the primary amine, MDA. No substitution was observed when the hallucinogens LSD, DOM and mescaline, or the stimulants (+)-amphetamine, methamphetamine and cocaine were tested. By contrast, both (+)-amphetamine and cocaine completely substituted in MDMA-trained rats. The results indicate that *S*-(+)-MBDB is pharmacologically less complex than MDMA, and in particular seems to lack dopaminergic properties. These studies support the hypothesis that MBDB may be the prototype of a distinct drug class, to be called entactogens.

125.6

INTERACTION BETWEEN YOHIMBINE (ALPHA-2 ANTAGONIST) AND CLONIDINE (ALPHA-2 AGONIST) IN THE GELLER-SEIFTER CONFLICT TEST AND SHUTTLE-BOX AVOIDANCE. J.L. Howard and G.T. Pollard. Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709.

In man yohimbine is anxiogenic (e.g., Holmberg and Gershon, Psychopharmacologia, 2:93, 1961) and clonidine is anxiolytic (e.g., Hoehn-Saric et al., Arch. Gen. Psychiatry, 38:1278, 1981). These compounds have produced mixed results in animal models of anxiety. We reported (Howard and Pollard, FASEB Journal, 2:1384, 1988) that in rats both compounds increased responding in the conflict portion and decreased responding in the VI portion of a Geller-Seifter test; they also decreased avoidance responding in a two-way conditioned avoidance (CAR) test; effects were dose-related. Because of the similarity of the behavioral effects of the two compounds, we investigated their interaction in the Geller-Seifter and CAR tests by constructing dose-reponse curves for one compound in the presence of various doses of the other. Although the compounds produced similar behavioral effects, when given together their effects were not only not additive but were less than the effects of either alone. Isobolographic analysis confirmed infraadditivity (mutual antagonism) for the response rate increases in conflict and the response rate decreases in VI and avoidance.

125.7

TOLERANCE TO THE BEHAVIORAL EFFECTS OF THE CENTRALLY ACTIVE BETA ADRENERGIC AGONIST CLENBUTEROL. J.M. O'Donnell* (SPON: E.R. Flynn), Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

The centrally active beta adrenergic agonist clenbuterol reduced response rate and increased reinforcement rate of rats under a differential-reinforcement-of-low rate (DRL) 72-sec schedule, in a manner similar to that observed for anti-depressant drugs. However, in contrast to the effects of the tricyclic antidepressants imipramine and desipramine, tolerance developed to the effect of clenbuterol on DRL behavior. Prior to chronic treatment, clenbuterol reduced response rate and increased reinforcement rate with an ED_{50} value of about 0.1 mg/kg. The dose-response function for clenbuterol was redetermined after chronic treatment with clenbuterol for 1 week at 0.1 mg/kg/day followed by treatment for 4 weeks at 1 mg/kg/day. Following this chronic treatment regimen, clenbuterol, at doses up to 3 mg/kg, no longer affected DRL behavior. The density of beta adrenergic receptors was assessed in brain regions of rats administered clenbuterol chronically. It was found that chronic treatment with clenbuterol, using the regimen that resulted in behavioral tolerance, reduced the density of $beta_2$ adrenergic receptors. The differences between the behavioral effects of clenbuterol and desipramine following chronic treatment may be a result of their differing effects on the subtypes of the beta adrenergic receptor. (Supported by the Air Force Office of Scientific Research and USPHS Grant MH40694.)

125.8

BETA-ADRENOCEPTOR ANTAGONISTS AND CONFLICT BEHAVIOR IN THE RAT. D.J. Fontana*, T.C. McCloskey*, S.K. Jolly* and R.L. Commissaris* (SPON: T.E. Anderson). College of Pharmacy, Wayne State University, Detroit, MI 48202.

The beta-adrenergic antagonist propranolol is effective in treating phobic-type anxiety (i.e., stage fright). The present studies examined the effects of propranolol, diazepam, and phenobarbital, alone and in combination, on behavior in the Conditioned Suppression of Drinking (CSD) conflict paradigm, an "animal model" for the study of anxiety and/or anti-anxiety agents. In daily 10-minute sessions, water deprived rats were trained to drink from a tube which was occasionally electrified (0.5 or 0.125 mA), electrification being signalled by the presence of a tone. As expected, acute administration of diazepam (0.6-10 mg/kg) or phenobarbital (5-40 mg/kg) resulted in a marked and dose-dependent increase in punished responding at doses which did not alter background responding (water intake). In contrast, propranolol (0.5-8 mg/kg) did not affect CSD behavior. Moreover, pre-treatment with 2.0 mg/kg propranolol did not alter the anti-conflict effects of diazepam or phenobarbital. Finally, reduction of the shock intensity (i.e., decreased suppression) failed to alter the behavioral response to propranolol (1.5-5 mg/kg) or the interaction of 2.0 mg/kg propranolol with diazepam. These data suggest that the CSD paradigm may not be useful for the study of phobic-type anxiety or the agents used to treat it. (MH #42501-01) Protocol conforms with NIH Guide.

125.9

COMPARATIVE ACUTE AND CHRONIC EFFECTS OF PUTATIVE 5-HT2 AND 5-HT1A AGONISTS IN THE RAT: IS THERE MORE THAN ONE "SEROTONIN SYNDROME?" R. S. Pluchino* and M. R. Pranzatelli (SPON: R. Trifiletti). Department of Neurology, Columbia University, NY, NY 10032.

The phenylisopropylamine derivative DOI has been identified as a novel selective 5-HT2 receptor agonist, but the behavioral effects of DOI have not been reported. Acutely, in naive rats, both DOI and the 5-HT1A agonist 8-OH-DPAT induced behaviors of the "serotonin syndrome" but the two drugs could be differentiated. Only DOI evoked shaking behavior, body (skin) jerks, vertical head movements with sniffing, and hyperthermia. Only 8-OH-DPAT induced flat body posture, occasional hindlimb hyperextension, failure of habituation to acoustic startle, and reduction in rearing. Both drugs evoked forepaw tapping and lateral head weaving, but 8-OH-DPAT to a significantly greater extent. Both the 5-HT2 antagonist ritanserin (ID_{50}=0.39 mg/kg) and the 5-HT1A antagonist l-propranolol (ID_{50}=33 mg/kg) blocked the DOI syndrome, but DOI-induced body jerks were blocked only by ritanserin. The dose-response (0.5-40 mg/kg) for shaking behavior was biphasic (maximum at 3 mg/kg), but was monophasic for syndrome behaviors. The dose threshold and time course were similar, however. Chronic (21 d) treatment (3 or 9 mg/kg) with DOI prevented DOI-evoked shakes and other DOI-evoked behaviors but not behaviors evoked by 8-OH-DPAT. 8-OH-DPAT-induced behaviors, however, decreased signficantly after chronic 8-OH-DPAT treatment. These data suggest that putative selective 5-HT2 and 5-HT1A agonists both induce some behaviors of the serotonin syndrome but can be distinguished behaviorally. Supported by NIH grant 1-KO8-NS01158 and the Myoclonus Research Fund.

125.10

CONTRIBUTION OF MULTIPLE OPIOID RECEPTOR TYPES TO CONDITIONAL FEAR-INDUCED ANALGESIA. M.S. Fanselow*, D.J. Calcagnetti* and F.J. Helmstetter (SPON: H. C. Hughes). Psychology Dept., Dartmouth Coll., Hanover, NH 03755.

Pavlovian conditional fear stimuli elicit analgesia in rats. Since this analgesia is reversed by the opioid antagonists naloxone and naltrexone, it appears to be mediated by endogenous opioids. Using the formalin test as a measure of pain sensitivity, we examined the ability of three opioid antagonists to reverse conditional analgesia. Nor-Binaltorphimine (N-BNI), $Cys^2\ Tyr^3\ Orn^5\ Pen^7$-amide (CTP) and 16 methyl-Cyprenorphine (M80), are competitive opioid antagonists purported to be selective for k, μ and δ receptors, respectively. We found that CTP and M80 produced dose dependent reversals of conditional analgesia. However, N-BNI, even at doses that reversed the analgesia produced by the k agonist U50488H, had no impact on conditional analgesia.

NEUROENDOCRINE CONTROLS: PITUITARY II

126.1

ACUTE AND CHRONIC HYPERPROLACTINEMIA IN HUMANS: EFFECTS OF THE PRECURSOR AMINO ACIDS L-TYROSINE AND L-TRYPTOPHAN: H.Lehnert, J.Beyer*, U.Krause*, D.H.Hellhammer. Dept. of Endocrinology, II. Mediz. Universitätsklinik Mainz and Dept. of Clinical and Physiological Psychology, University of Trier, West-Germany.

Prolactin (PRL) secretion from the lactotroph cells of the anterior pituitary is under tonic inibitory control of the tubero-infundibular dopaminergic system. It has been shown that the dopmaine precursor amino acid l-tyrosine suppresses PRL secretion in chronically reserpinized rats, thus when DA neurons are activated. Tryptophan appears to stimulate PRL secretion at large doses.

We studied PRL secretion in healthy male volunteers (n= 12) and prolactinoma patients following intake of tyrosine and tryptophan (5 g orally) under basal and (in the volunteers) stimulatory conditions. Stimulation of PRL secretion was achieved by administration of metoclopramide (MCP, 10 mg i.v.) and TRH (200 μg i.v.).

Following administration of l-tyrosine we found an unexspected rise of basal PRL levels among the volunteers when compared with placebo (6,56+0,63 vs. 3,06+0,16 ng/ml; p 0,001). The drug-induced increase in PRL secretion was significantly attenuated. There was no effect of tryptophan. In prolactinoma patients, the administration of tyrosine appears to decrease PRL levels by about 15%, while tryptophan has no effect. Tyrosine administration may thus be effective in acute rather than chronic hyperprolactinemia.

126.2

MONOSODIUM GLUTAMATE LESIONS OF THE ARCUATE NUCLEUS INHIBIT PROLACTIN CELL ACTIVITY IN THE SYRIAN HAMSTER. D.E. Blask, C.A. Leadem* and D.B. Pelletier*. Dept. of Anatomy, Univ. Arizona Coll. Med., Tucson, AZ 85724.

Monosodium glutamate (MSG)-induced lesions of the arcuate nucleus destroy tuberoinfundibular dopamine neurons leading to a disinhibition of prolactin (PRL) secretion. In order to assess whether arcuate lesions in the hamster would have a similar effect on PRL synthesis, storage and secretion, we injected either MSG (8 mg/g BW) or saline into male and female hamsters on day 8 of the neonatal period. Fourteen and a half weeks later the pituitaries were removed for the analysis of PRL synthesis and storage via the incorporation of ^{3}H-leucine into PRL in vitro as well as by measuring the pituitary and media levels of PRL with a radioimmunoassay (RIA). Serum PRL levels were also determined by RIA as an index of in vivo PRL secretion. In both male and female hamsters, serum PRL levels were markedly decreased in response to MSG versus saline. MSG-induced arcuate lesions also caused a significant inhibition of PRL synthesis and storage in both sexes with the effect being more marked in female hamsters as compared with male animals. Therefore, unlike the rat, MSG-induced arcuate nucleus lesions in the hamster inhibit PRL cell activity suggesting that the neuroendocrine regulation of the PRL cell in these two species is markedly different, particularly with respect to the involvement of dopamine. Supported by PHS Grant 5R01 HD21212-02 from the NICHHD.

126.3

REGULATION OF THE TEMPORAL PROLACTIN SECRETORY RESPONSE TO INHIBITION OF DOPAMINE (DA). B.J. Arey* and M.E. Freeman* (SPON: P. Cancalon) Dept. Biol. Sci., Fla. State U., Tallahassee 32306.

We have recently reported that periodic pharmacologic depression of DA tone in ovariectomized (OVX) rats with the receptor blocker Domperidone (DOM) resulted in higher prolactin (PRL) secretory responses at 0300 h and 1700 h than at 1200 h. In this study we describe the role of serotonin (5-HT) or Vasoactive Intestinal Peptide (VIP) as possible stimulatory effectors of these heightened responses. Groups of OVX rats received either 2-days of pretreatment with p-chlorophenylalanine (PCPA, 250 mg/kg, s.c.) to block 5-HT synthesis or infusion of the VIP antagonist [D-4-Cl,Phe^6,Leu^{17}] VIP (VIP-A, 0.1 ug/kg/min) beginning 2 h before study. DOM (200 ug/kg,iv) injection at 1200 h in both PCPA-pretreated and control rats resulted in peak PRL values of approximately 120 ng/ml. Equivalent heightened patterns of PRL secretion were also evident at 0300 h in both control and PCPA-pretreated rats with peak levels of approximately 240 ng/ml. However, the heightened PRL secretory response to DOM at 1700 h was totally ablated by PCPA-pretreatment. There was no difference in the PRL secretory profiles of control or VIP-A infused rats during DOM challenge at 1200 or 1700 h. However, treatment of rats with VIP-A at 0300 h totally ablated the heightened PRL secretory response to DOM at this time. These data suggest that the temporal endogenous stimulatory rhythm on PRL secretion is differentially regulated. At 0300 h PRL is regulated by VIP, while the 1700 h component is driven by 5-HT. Supported by NIH, HD-11669

126.4

IN VIVO AND IN VITRO EVIDENCE FOR PROLACTIN FEEDBACK ACTIONS ON HYPOTHALAMIC OXYTOCIN, VASOACTIVE INTESTINAL PEPTIDE AND DOPAMINE SECRETION. D.K. Sarkar. Dept. of Reproductive Medicine, Univ. of Calif. at San Diego, La Jolla, CA 92093.

The feedback action of prolactin (PRL) on oxytocin (OT), vasoactive intestinal peptide (VIP) and catecholamine release was determined in ovariectomized rats and in fetal hypothalamic cells in primary culture. Ovariectomized rats, when transplanted with 4 anterior pituitaries (APs) to the kidney capsule for 2-3 weeks, had elevated plasma PRL levels (3.8-fold) and showed decreased in situ AP weights (.62-fold) and PRL concentrations (.63-fold). The levels of dopamine (DA) and OT in pituitary portal plasma of hyperprolactinemic rats were increased 1.7- and 1.9-fold, respectively. However, pituitary portal plasma levels of VIP of these rats were decreased .31-fold. The secretion of DA, dihydroxyphenylalanine (DOPA) and OT was increased whereas VIP secretion from cultured hypothalamic cells was reduced in a dose-dependent fashion following PRL treatment. These data suggest that PRL stimulates OT release and inhibits VIP release and that a subtle imbalance between the hypothalamic secretion of a PRL-inhibiting factor (DA) and PRL-releasing factors (VIP and OT) during elevated systemic levels of PRL is responsible for decreased lactotrophic function. (Supported by an NIH grant AG05454).

126.5

EFFECT OF HYPERPROLACTINEMIA (HYPERPRL) ON PRL AND LH SECRETION AS ASSESSED BY THE REVERSE HEMOLYTIC PLAQUE ASSAY. M.A. Sortino, I.R. Cohen and P.M. Wise. Dept. of Physiology, Univ. of Maryland, Baltimore, MD 21201.

HyperPRL, induced by exogenous administration of PRL, is associated with diminished secretion of endogenous PRL and LH. We undertook this study to better understand whether HyperPRL influences PRL and/or LH secretion via a direct action at the pituitary level. Rats were ovariectomized (day 0) and treated with PRL (4 mg/kg, sc., 3 times daily) from days 4-6. Pituitaries were collected 1 h after the final injection and prepared for the reverse hemolytic plaque assay. After 1 h incubation, PRL release from single lactotrophs was lower in HyperPRL rats (1295 ± 58 vs 794 ± 66 μm^2 for control and HyperPRL, respectively). Incubation with TRH (10^{-7} M) for 1 h stimulated a larger PRL release in control (158 ± 6.9%) compared to the HyperPRL animals (124 ± 14.4%). By 2 h basal and TRH-stimulated PRL secretion was the same in the 2 experimental groups. Under basal conditions the percent of PRL-secreting cells was similar in control and HyperPRL rats after 1 or 2 h of incubation. TRH stimulated an increase in the percent of PRL-secreting cells in controls at 1 and 2 h and in PRL-treated rats at 2 h only. In vivo treatment with PRL did not affect in vitro release of LH under basal or GnRH-stimulated conditions. Thus, HyperPRL influences PRL but not LH secretion at the pituitary level. HD-15955

126.6

DOPAMINE INDUCES OPENING OF VOLTAGE-OPERATED POTASSIUM CHANNELS IN RAT LACTOTROPHS. M. Memo, A. Valerio*, L. Castelletti*, C. Missale, A. Forgione·*, P.F. Spano. Inst. Pharmacol. Brescia Univ. Sch. of Med., Brescia. ·Ravizza Res Lab.s, Muggio', Italy.

The effects of dopamine (DA) on K fluxes in membrane of rat lactotrophs were studied by a radiochemical approach which consists of a manual rapid-quench method for measurement of 86Rb efflux. Rb efflux from Rb-preloaded lactotrophs into nominally Ca-free solution containing 5 mM K was linear from 1 to 60 s, with a calculated rate of 0.2 %/s. Raising the concentrations of K to depolarize the cells stimulated the Rb efflux (0.6 %/s) within 1 s. DA increased the efflux of Rb in a calcium-free medium containing 5mM K. This effect was statistically significant 15 s after exposure of the cells to 10 nM DA. Increasing K concentrations to gradually depolarize the cells enhanced the rate of increase of Rb efflux induced by DA, being evident in the initial 2-5 s of incubations. These effects were 1) pharmacologically characterized as D2-receptor mediated and 2) unaffected by treatment of the cells with forskolin. Our results indicate that DA promotes opening of K channels. This effect appear to be both calcium- and cyclic AMP-independent.

126.7

CYCLIC AMP-INDEPENDENT STIMULATION OF PRL RELEASE INDUCED BY VIP. M.Pizzi*,M.O. Carruba*, E. Simonazzi*, M. Benarese*, M. Memo,P.F. Spano Inst. Pharmacol., Brescia Univ., Sch. of Med., Brescia - Italy.

Vasoactive intestinal peptide (VIP) has been reported to stimulate prolactin (PRL) release from anterior pituitary cells by activation of adenylate cyclase activity. In the present study the calcium-dependence of VIP responce in rat anterior pituitary cells was evaluated. We observed that VIP dose- and time-dependently increased (10-1000 nM) PRL release from cells incubated both in a Ca-containing buffer (EC50 50 nM) and in Ca-free medium (EC50 500 nM). Concomitant measurement of cyclic AMP production in the cells indicated that the presence of Ca was necessary for VIP to elicit the activation of adenylate cyclase. Moreover we observed that the peptide (200 nM) strongly activated 45Ca influx in the cells at 5 sec of incubation. The present data indicate that VIP production of cyclic AMP in the cells is Ca-dependent. One of the earlier events produced by VIP is the entry of external Ca in the cell. In Ca-free condition high concentration VIP stimulates PRL release without producing changes in cyclic AMP levels. The involvement of a second messenger different from cyclic AMP or external Ca is proposed.

126.8

LACTATION INDUCED INHIBITION OF PLASMA OXYTOCIN AND CORTICOSTERONE, AND HYPOTHALAMIC CRF VASOPRESSIN AND ENKAPHALIN mRNA RESPONSES TO STRESS. S.L. Lightman and W.S. Young III Laboratory of Cell Biology, NIMH, Bethesda, MD 20892

In spite of efficient episodic release of oxytocin in response to nipple stimulation, lactating rats lose their oxytocin response to stress (Carter & Lightman, Neuroendocrinology 46: 543, 1987). We have now investigated whether lactation alters hypothalamic mRNA as well as hormonal responses to stress.

Control female rats or lactating rats 7 days post partum were injected i.p. with either 0.15M (controls) or 1.5M saline (1.8ml/kg body weight) and returned to their home cages. For the hormonal studies the rats were killed after 30 min and their trunk blood collected whilst for the mRNA studies the animals were killed at 4 hours and the brains rapidly removed into dry ice for subsequent quantitative in situ hybridisation histochemistry.

In the control rats i.p. hypertonic saline caused a 480pmol/l increase in oxytocin, a 54pmol/l increase in vasopressin and a 314ng/ml increase in corticosterone. In the lactating rats there was a considerable diminution in the responses of oxytocin (148pmol/l) and corticosterone (51ng/ml), but little change in that of vasopressin (41pmol/l). Stress also caused a marked accumulation of CRF vasopressin and enkephalin mRNAs in the control rats. In the lactating rats, however, this increase in CRF and vasopressin mRNAs was not seen and their enkephalin mRNA response was also markedly diminished.

126.9

IN VIVO CATECHOLAMINERGIC ACTIVITY (CA) IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVN) AFTER HEMORRHAGE IN CATS. K.V. Thrivikraman, D.A. Bereiter and D.S. Gann Depts. Surgery & Neurobiology, R.I. Hospital/Brown Univ. Providence, RI 02902

Although catecholaminergic inputs from hemodynamically-responsive areas in the brain stem affect PVN neural activity, the local CA response within the PVN to cardiovascular stimuli is not known. This study examined temporal changes in CA in the PVN after moderate hemorrhage (20% blood loss over 3 min), a physiological stimulus known to alter the activity of PVN neurons and to evoke pituitary hormone secretion. Cats were anesthetized with chloralose, paralyzed with gallamine triethiodide. An *in vivo* voltammetric technique (multistep chronoamperometry) was used to record oxidation current (OC) with carbon microelectrodes (Graphpoxy PX) and the OC at +250mV was used as an index of CA. The OC was sampled every min beginning at 4 min prior to onset of hemorrhage and during the hemorrhage paradigm. At each recording site the response during blood loss was compared by unpaired t-test. Of a total of 60 recording sites, 48 were identified histologically within the PVN. Hemorrhage caused a fall in the mean arterial pressure in all cats. The CA response to hemorrhage was dependent on the site of recording: an increase at 8 sites located in the caudal PVN and a decrease at 12 sites located anterolaterally to the above 8 sites. At the remaining 28 sites in the PVN, CA did not change during hemorrhage. Of the 12 sites outside PVN, CA decreased at 2 sites and did not change at 10 other sites. In the PVN, the response to blood loss was significant (> ± 2 SD) by 1 min at all sites. Reinfusion of the shed blood reversed the increases in CA at 6 of the 8 sites and reversed the decreases in CA at 7 of the 12 sites. The data indicate that sites at which CA increased after hemorrhage were confined to the caudal PVN, in an area shown previously to elicit pituitary hormone release. The data are consistent with the hypothesis that an increase in the release of catecholamines within the caudal PVN in response to hemorrhage is facilitatory for pituitary release of vasopressin and/or adrenocorticotropin. Supported by NIH grant DK-26831.

126.10

DEHYDRATION INDUCES FOS IMMUNOSTAINING IN HYPOTHALAMIC MAGNOCELLULAR NEURONS. S.M. Sagar and F.R. Sharp§, Depts. of Neurology and §Physiology, University of California, and VA Medical Center, San Francisco, CA 94121.

Fos, the protein encoded by the proto-oncogene c-fos, or a closely related antigen, is rapidly and transiently expressed in neurons in response to synaptic input, neurotransmitters and growth factors. In order to study the response of CNS neurons to a normal physiological stimulus, Fos immunostaining was performed in the brains of adult male rats after overnight water deprivation or three hours after i.p. injection of 1-4 ml 1.5M NaCl. Control rats received free access to water or 1 ml 0.15M NaCl, i.p., respectively. Affinity purified polyclonal antisera raised to synthetic Fos peptides were used.

Both stimuli selectively induce Fos immunostaining in nuclei of the magnocellular neurons of the hypothalamic-neurohypophysial system. The response to hypertonic saline is dose-related. Control animals have no detectable staining of the magnocellular neurons, and nuclear staining is blocked completely by adsorption of antisera with 100 ng/ml peptide antigen.

These observations add to the evidence that Fos, or a related protein, is expressed in neurons in response to normal stimuli and that Fos immunostaining offers a method of functional metabolic mapping at the cellular level of resolution.

126.11

GABA-CONTAINING AFFERENTS TO THE SUPRAOPTIC NUCLEUS FROM THE DIAGONAL BAND OF BROCA. L. D. Wilkin, L. Sherman* and E. Anton*. Dept. of Cell Biology & Anatomy, Chicago Medical School, Chicago, IL 60064.

Gamma-amino butyric acid (GABA) is a central nervous system inhibitory neurotransmitter that has been proposed to have a role in baroreflex inhibition of vasopressin secretion. The diagonal band of Broca (DBB) has been proposed as a potential inhibitory baroreflex relay center. However, specific cardiovascular-related inhibitory feedback pathways onto neurons controlling vasopressin secretion have not been demonstrated. In this study, GABA pathways between the septum/DBB region and the supraoptic nucleus (SON) of the hypothalamus were investigated using retrograde neural pathway tracing and glutamate decarboxylase (GAD) immunohistochemistry. Following discrete injections of the fluorescent dye fast blue into the SON, retrogradely labeled cells were found in the vertical limb of the DBB. Immunohistochemical analyses revealed GAD-immunoreactive neurons in the horizontal limb of the DBB and the ventral portion of the vertical limb. Some of the retrogradely labeled cells in the ventral portion of the vertical limb of the DBB were also immunopositive for GAD. These results provide anatomical evidence for potential inhibitory inputs from the ventral DBB to the SON which may play a role in baroreflex inhibition of vasopressin secretion.

126.12

NEUROTRANSMITTER ACTIONS ON NEUROHYPOPHYSIAL PITUICYTES. D.M. Burnard, B.A. MacVicar and Q.J. Pittman. Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada. T2N 4N1

The neurohypophysis consists primarily of hypothalamic neurosecretory endings and glial cells known as pituicytes, which may regulate neurohypophysial hormone release. Pituicytes receive synaptoid contacts and have receptors for opiates and other neurotransmitters. In order to determine the actions of neurotransmitters on these cells, intracellular recordings were obtained from pituicytes (n=26) in the isolated pituitary maintained in vitro. Pituicytes were identified by an average resting membrane potential (RMP) of -38.2 ± 0.8 mV (5 mM K^+), an average membrane input resistance (R_{in}) of 9.0 ± 1.3 mΩ and an absence of action potentials in response to depolarizing current injection. Occasionally neurosecretory endings were impaled which exhibited spiking. Bath application of dynorphin (1-13)(1-10 μM) increased pituicyte R_{in} by 17.5 ± 4.1 mΩ and methionine-enkephalin (10μM) increased R_{in} by 9.7 ± 1.9 mΩ. No significant changes in RMP were observed. Isoproterenol (100 μM) decreased R_{in} of pituicytes, with no obvious changes in RMP. These findings may suggest a functional link between pituicytes and neurosecretory nerve fibres and a role for pituicytes in the regulation of neurosecretion.

SUBCORTICAL VISUAL PATHWAYS II

127.1

INHIBITION BY ACETYLCHOLINE OF IDENTIFIED INTERNEURONS IN THE CAT LATERAL GENICULATE NUCLEUS, *IN VITRO*

H.-Ch. Pape* (SPON: P.R. Adams) and D.A. McCormick, Section Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

Gating of visual information through the dorsal lateral geniculate nucleus (LGNd) is controlled by ascending cholinergic brainstem neurons. In the present study we investigate ionic mechanisms of acetylcholine (ACh) action in the cat LGNd that may underlie cholinergic modulation of visual geniculate response properties. Intracellular recordings from cells in lamina A or A1 of the cat LGNd maintained *in vitro* revealed two distinct classes of neurons, which we term P- and I-neurons. P-cells displayed electrophysiological properties similar to those of thalamocortical relay cells (Crunelli et al., J.Physiol., 390: 243, 1987). I-neurons possessed on the average action potentials of a shorter duration (I: 0.34 +/-0.07 ms, mean +/ S.D., n=7; P: 0.50+/-0.20 ms, n=20), higher input resistances (I: 89+/-15 MΩ;P: 51+/-20 MΩ), more linear membrane potential to current relationships, and lacked the typical low threshold Ca^{++}spike of P-cells which underlies burst discharges. Local application of ACh to P-cells caused depolarizing responses (McCormick & Prince, J.Physiol.,392: 147, 1987), whereas I-neurons were inhibited by ACh due to a membrane hyperpolarization associated with an increased apparent input conductance. The muscarinic agonist acetyl-beta-methylcholine elicited a similar hyperpolarization of I-neurons, which reversed to a depolarization at an average membrane potential of -102+/-4.8 mV (n=3). Intracellular labeling with Lucifer Yellow revealed morphological features of P-cells typical for either type 1 or type 2 relay cells, whereas I-neurons displayed somatic and dendritic morphologies of type 3 GABAergic interneurons.

We conclude that the brainstem cholinergic system facilitates the relay of visual information through the LGNd during periods of increased alertness not only by depolarizing relay cells, but also by hyperpolarizing intrageniculate interneurons through a muscarinic receptor mediated increase in potassium conductance.

127.2

NEURONAL ACTIVITIES RELATED TO THE PONTO-GENICULO-OCCIPITAL (PGO) WAVES IN THE LATERAL GENICULATE (LG)-PERIGENICULATE (PG) COMPLEX. M. Steriade, D.Paré, D. Bouhassira*, M. Deschênes, and G. Oakson*. Lab. Neurophysiol., Sch. Med., Univ. Laval, Québec, Canada.

We have examined the discharges of LG and PG cells in relation to PGO waves in chronically-implanted, naturally sleeping cats. LG and PG neurons were identified by their responses to optic tract stimulation. PGO focal waves were recorded by the microelectrode used for action potential recording. One or two minutes before all other signs of REM sleep, PGO waves appear as single high-amplitude events over the background of a fully synchronized EEG. During REM sleep, they are grouped in wave clusters of much lower amplitudes.

Most PG neurons discharged short, high-frequency bursts followed by silenced firing in relation to single or clustered PGO waves. The PGO-related activity of LG neurons was different. (a) During the pre-REM stage, PGO waves correlated with a high-frequency discharge burst of LG cells, followed by a long (0.2-0.4 s) train of single spikes. In view of the low spontaneous firing rates of LG neurons during EEG synchronization, the PGO-related activity leads to a high signal-to-noise ratio during this transitional epoch. (b)During REM sleep, the spontaneous firing of LG neurons is tonic. The PGO-related activity lacks the initial burst (see a) and consists of a spike train that only slightly exceeds the high-rate of spontaneous discharges. These data explain the high amplitude of single PGO waves during the pre-REM period (likely due to synchronous bursts in a pool of LG neurons), indicate that PGO are not merely corollary discharges of eye movements as they appear well before the saccades of REM sleep, and suggest that the high signal-to-noise ratio in the visual thalamocortical system during the pre-REM stage may underly vivid imagery during this epoch. Supported by MRC Grants MT-3689 and MT-5781.

127.3

THRESHOLD REDUCTION IN CAT LGN NEURONS OF LAMINA A1 AT A LOCATION CORRESPONDING TO THE BLIND SPOT OF THE OTHER EYE. F.Schmielau*,M.F.Schmielau-Lugmayr* and U.Eysel* (SPON:ENA).Inst.for Med. Psychology, D-2400 Lübeck (Fed.Rep.of Germany).

In adult anaesthetized (Nembutal®)and paralyzed cats recordings were obtained from on- and off-center neurons of lamina A and A1 of the lateral geniculate nucleus (LGN) with receptive fields (RF) at various eccentricities in the visual field. Special interest was dedicated to neurons with RF eccentricities between 10 and 20° including the location of the blind spot (LBSP). For each neuron post stimulus time histograms were obtained to stationary stimulation of the RF center with small spots of light of various luminance values down to the threshold (Th). In lamina A neurons with RFs close to LBSP showed an increase of Th by more than 1 log unit within few degrees of visual angle. In contrast in neurons of lamina A1 a decrease of Th was observed when the RFs were localized at LBSP. This relative reduction of threshold in lamina A1 neurons at a location corresponding to the site of the blind spot is considered as a mechanism compensating for the lack of photoreceptors at the blind spot in binocular vision. A model including the perigeniculate nucleus is proposed.

127.4

SUBLAMINAR VERSUS COLUMNAR ORGANIZATION IN THE LATERAL GENICULATE NUCLEUS OF THE CAT. D.B. Bowling and J.I. Caverhill*. Department of Medical Physiology and the Lion's Sight Center, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

A question has recently arisen as to whether ON and OFF cells in the A layers of the cat LGN are arranged in vertical columns or in sublaminae. Whether the organization is columnar, sublaminar or both is important for understanding how the visual image is represented in the LGN and how signals from the nucleus are utilized in the synthesis of cortical receptive fields.

The hypothesis that ON and OFF cells form vertical columns was tested in two ways. First, data from 184 electrode tracks made parallel to the laminar borders (orthogonal to the hypothetical columns) were analyzed for ON/OFF periodicities. The results were compared with computer simulations based on different column sizes and mosaics. Second, a direct search for ON and OFF columns was made by making multiple, closely spaced vertically oriented electrode tracks.

Both tests were negative for columns but confirmed the sublaminar organization of ON and OFF cells in the layers.

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127.5

RESPONSES TO ABRUPT VELOCITY CHANGES IN CAT LGN DISCRIMINATE Y-CELLS FROM X-CELLS. B.W. Van Dijk[+*]; The biophysics laboratory; Rockefeller University; 10021 N.Y., N.Y. (spons.: ENA)

In this study the responses to two different sets of stimuli were compared. One set of stimuli consisted of random dot patterns which abruptly started and stopped moving. The random dot size and the velocity were chosen such that the stimulus would exactly repeat only after 32 cycles. In the other set of stimuli identical random dot patterns were counterphase modulated in one phase and steadily presented in another phase. The modulation rate was chosen to match that for the moving stimuli; again the stimulus would only repeat exactly after 32 cycles. Responses to both types of stimuli were averaged over 32 or 64 cycles. The two sets of stimuli result in nearly identical local luminance modulations; they differ in the global correlations of the local luminance modulations.

Data from 52 cat LGN cells recorded from 6 animals showed that all 18 X-cells did not respond differently to the two types of stimuli, while all 34 Y-cells responded much more vigorously to the moving stimuli.
Optimal responses from the Y-cells could be obtained at an equivalent local luminance modulation rate of approx. 8Hz for cells at all eccentricities (from 1.4^{o} to 43^{o}); although both the temporal and the spatial tuning of the responses were rather broadband and wider than those obtained using moving sine wave gratings.

The data can be described in terms of the Hochstein and Shapley model of the Y-cell receptive field, by assuming that the non-linear subunits have a gain which is smaller than unity and show saturation at low contrast levels.

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127.6

BRANCHING OF X AND Y FUNCTIONAL PATHWAYS IN CAT LATERAL GENICULATE NUCLEUS. D. N. Mastronarde. Dept. of MCD Biology, University of Colorado, Boulder, CO 80309.

The cat LGN does not simply relay X and Y information from retina to cortex in two parallel streams. Drawing on direct recordings of retinal inputs to LGN cells in the A layers, I have previously described distinct classes of cells with excitatory X-input: single-input with lagged (X_L) and non-lagged (X_S) visual responses, multiple-input (X_M), mixed input (X/Y), and interneurons (X_I). Here I report analogous heterogeneity among Y-cells. By comparing the arrangement of Y-inputs to Y-cells with that of X-inputs to X_S- and X_M-cells, Y-cells can be divided into predominantly single input (PSI) cells and those that clearly have multiple input (MI). PSI and MI Y-cells have some consistent differences, such as in receptive-field center size, but do not seem to fall into distinct classes as X_S and X_M do. There is some evidence for separate classes of Y-interneurons (Y_I) and lagged Y-cells (Y_L). Each cell category mentioned here is estimated to provide at least as much coverage of the visual field as do retinal X- or Y-cells, indicating that each is competent to provide a complete representation of some aspect of visual information. Thus, from only two classes of retinal input, the LGN generates cells with fast responses at a series of spatial resolutions, cells at both low and high spatial resolution with delayed, relatively sustained responses, and X- and Y-interneurons for intrageniculate inhibition.

	higher ⟸ Spatial resolution ⟹ lower			
fast relay cells	X_S	X_M	X/Y	PSI Y ··· MI Y
lagged relay cells	X_L			Y_L
interneurons		X_I		Y_I

127.7

ORIENTATION SENSITIVITY IN THE CAT'S LATERAL GENICULATE NUCLEUS (LGNd). T. Shou* and A.G. Leventhal (SPON: J. Conlee) Dept. Anat., Univ. Utah Sch. Med., Salt Lake City, Utah 84132.

Most cat retinal ganglion cells and LGNd relay cells are weakly orientation sensitive. While some studies suggest that the biases of LGNd cells reflect those of their retinal inputs (Soodak et al., J. Neurophysiol. 58:267-275, 1987), others indicate that LGNd cells are more sensitive to orientation than are their retinal counterparts (Vidyasagar and Urbas, Exp. Brain Res. 46:157-169, 1982).

We studied the physiological orientation biases of over 400 cells in the cat's LGNd using the same visual stimuli (drifting sinusoidal gratings) used previously to study retinal orientation sensitivity (Levick and Thibos, J. Physiol. (Lond.) 329:243-261, 1982). The strength of the orientation biases and the distributions of the preferred orientations of LGNd cells subserving different parts of the visual field were compared with the physiological and anatomical orientation biases (Leventhal and Schall, J. Comp. Neur. 220:465-475, 1983) of ganglion cells in the appropriate parts of the retina.

We find (Soodak et al., 1987) that LGNd cells exhibit the same degree of orientation bias as do retinal ganglion cells. Also, the overall distribution of preferred orientations varies in different regions of the LGNd and reflects the overall distribution of preferred orientations of ganglion cells in topographically corresponding parts of retina. Unlike in the retina, however, LGNd cells having similar preferred orientations are clustered. It is known that cells having inputs from the two eyes, on, off, x, y, and w center ganglion cells are distributed nonrandomly in the LGNd. If cells having similar preferred orientations are also distributed nonrandomly then the initial sorting of all properties segregated in visual cortex may begin in the LGNd.

127.8

LATERAL GENICULATE NUCLEUS NEURONS IN AWAKE BEHAVING PRIMATES: I. RESPONSES TO B&W 2-D PATTERNS J. W. McClurkin, T. J. Gawne*, B. J. Richmond, L. M. Optican*, and D. L. Robinson. Lab of Neuropsychology, National Institute of Mental Health, and the Lab of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

We have shown that neurons in both inferior temporal and striate cortex convey stimulus-related information with temporal modulation. Is this temporal modulation also present in lateral geniculate nucleus (LGN) neurons?

We recorded the responses of 12 parvocellular X-like units from the lateral geniculate nucleus of two fixating rhesus monkeys. The stimulus set consisted of 224 orthogonal B&W Walsh patterns at 7 luminance combinations (0.4 - 62 cd/m2), and squares and annuli. The stimuli were based on either a 4x4 or an 8x8 rectangular grid. Every stimulus was presented to each cell a minimum of five times. The receptive fields were between 1 and 8 degrees eccentric to the fixation point. Whether the response was excitatory or inhibitory was governed by the luminance of the pixel stimulating the receptive field center. However, different stimuli elicited different temporal patterns in the response. For example, transient responses could have 1 or 2 peaks, and sustained responses could have rising or falling slopes. Two observations suggest that the variety of temporal patterns was not due solely to erroneous fixations or eye-movements. The responses to squares and annuli were similar to those typically found in anesthetized preparations. Also, a statistical analysis showed that the responses were stimulus dependent.

The responses of LGN neurons to complex visual patterns consist of temporal modulation as well as variations in strength. This temporal modulation depends on the pattern of luminance in the surround, as well as the luminance in the receptive field center.

127.9

LATERAL GENICULATE NUCLEUS NEURONS IN AWAKE BEHAVING PRIMATES: II. TEMPORAL MODULATION IS MORE IMPORTANT IN LGN NEURONS THAN GANGLION CELL FIBERS B. J. Richmond, J. W. McClurkin, T. J. Gawne*, and L. M. Optican*. Lab of Neuropsychology, National Institute of Mental Health, and the Lab of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

The preceding abstract showed that the stimulus pattern elicited temporally modulated responses from neurons in the LGN. We also recorded from 5 fiber-like units, which had low-amplitude spikes of very short duration (<0.25 msec), and were found directly above the LGN, where the optic tract spreads out over the nucleus. We hypothesize that these were ganglion cell fibers. How much information about the stimulus was conveyed by the temporal modulation?

The temporal modulation was quantified by decomposing the response waveforms into their principal components (PCs), which are waves of excitation and inhibition. For the LGN neurons the information transmitted by the three most powerful PCs (0.99 bits) was 1.9 times the information transmitted in the spike count (0.54 bits). For the ganglion cell fibers, the information transmitted by temporal modulation (0.99 bits) was 1.4 times that in the spike count (0.72 bits). This ratio was significantly smaller than that for the LGN ($P<0.02$).

We have previously determined that this ratio is 1.9 in striate cortex, and 2.25 in inferior temporal cortex. The changes in the ratio reflect a change in the amount of information in spike count, since the total amount of information in all areas was about 1 bit. We conclude that temporal modulation assumes a significant role in encoding stimulus-dependent visual information, beginning as early in the visual system as the LGN.

127.10

LATERAL GENICULATE NUCLEUS NEURONS IN AWAKE BEHAVING PRIMATES: III. SUCCESSFUL PREDICTIONS OF A MULTI-CHANNEL MODEL. T. J. Gawne*, J. W. McClurkin, L. M. Optican*, and B. J. Richmond (SPON: J. Carl). Lab of Neuropsychology, National Institute of Mental Health, and the Lab of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

We have shown that the temporally-modulated responses of striate cortical neurons can be represented by a model with parallel spatial-to-temporal filters. We tested the ability of a similar model to predict temporally modulated responses of LGN neurons to arbitrary patterns.

Using responses described in the preceding abstracts, a similar model was used to predict the first three principal components (PCs) of the temporal pattern of the response. These three PC's accounted for more than half of the total variability of the responses. In the model, each PC was regarded as the output of a separate spatial-to-temporal filter. Each channel consisted of a compressive input non-linearity cascaded into a linear spatial filter. Using parameters derived only from the Walsh patterns, the model successfully predicts the temporally modulated responses to "center-surround" annuli of different luminances. The first PC is driven by a spatial filter that had a dominant excitatory or inhibitory center pixel, with an anisotropic surround. The second PC is driven by a spatial filter that was dominated by a bipolar pair of pixels with an anisotropic surround. This model's third filter failed to predict the third PC.

The ability to predict the temporally modulated responses of LGN neurons to arbitrary, 2-D, B&W patterns suggests that they are encoding a richer description of visual stimuli than has previously been recognized. Because the proportion of information transmitted by temporal modulation is substantially greater in the LGN neurons than in ganglion cells, we hypothesize that one function of the LGN is to encode multiple stimulus features as temporal messages.

127.11

MODELING CHROMATIC CONTRAST DETECTORS AT THE OUTPUT OF THE PRIMATE LATERAL GENICULATE NUCLEUS. P. Gouras. Columbia Univ., Dept. Ophthalmology, 630 W. 168 St., NYC 10032

Color vision requires neurons to detect spectral contrast independently of the receptor quantal catch on either side of a boundary. We have examined each functionally distinct neuron in the cynomolgus lateral geniculate nucleus for its ability to do this (8 parvo and 2 magnocellular on- and off-center varieties) from a sample of 367 neurons. Only 3% of the cells, the short-wave subserving parvo on- and off-center cells, can do this but to a limited degree. In order to construct hypothetical detectors that can distinguish certain spectral (chromatic) contrast unequivocally from this output, the following strategy was used. The outputs of four varieties of on- and off-center parvo cells were linearly added or subtracted; off-centers were essential for detecting spectral contrast as a decrement of energy contrast. This implies that both on- and off-center cone opponent cells are essential for color vision. The receptive field size of the chromatic contrast detectors had to subserve a relatively large number of cones to reduce ambiguity arising from simple achromatic contrast. This implies that the grain of the chromatic detecting system must be larger than the achromatic one and could explain why orientation selectivity is more developed in the latter. The hypothetical chromatic contrast detectors are single opponent cells but antagonism between them could make them double opponent.

127.12

MORPHOLOGY OF RETINOGENCIULATE AXON ARBORS IN MONOCULARLY DEPRIVED PRIMATES. E.A. Lachica*, M.W. Crooks* and V.A. Casagrande. Depts. Psychol. & Cell Biol., Vanderbilt Univ., Nashville, TN 37232. (SPON: G. Condo)

In both primates and cats monocular pattern deprivation (MD) results in amblyopia in the deprived eye. In cats this behavioral change may be the result of the selective loss of retinogeniculate Y axons and physiological loss of Y-LGN cells in the A layers. The cause of these changes in the primate is less clear. In the prosimian primate galago, both X-like and Y-like LGN cells are smaller; W-like LGN cells appear to be unaffected. Physiologically, however, X-like, Y-like and W-like LGN cells are normal. To determine what affect MD has on retinogeniculate axon morphology, nearly 100 bulk HRP-filled axons from MD galagos were reconstructed and compared to normal adult arbors. Our main results show that retinal axons innervating X-like (parvocellular) and Y-like (magnocellular) LGN layers are smaller, abnormal in shape, have absolutely fewer boutons, but more boutons per unit area than normal and nondeprived counterparts. Axons innervating W-like (koniocellular) LGN layers appear unchanged. Hence, in primate, unlike cat, MD does not selectively eliminate or compromise retinal information conveyed by any one visual channel. Presumably, the comparable behavioral changes found in MD primates result from changes that occur central to the LGN. (Supported by EYO1778 to VAC & T32 EYO7007 to EAL)

NEUROETHOLOGY II

128.1

SITES OF MULTIPLE CONTROLS OF EXCITABILITY IN THE ESCAPE CIRCUIT OF THE COCKROACH PERIPLANETA AMERICANA. J. M. Camhi. Dept. of Zoology, Hebrew University Jerusalem, Israel

When a cockroach runs or flies, there is strong depression of the wind-evoked responses of its ventral giant interneurons (vGI's)--abdominal first order interneurons of the escape circuit. Are other controls of escape excitability also expressed at this early point in the circuit? Since dissected preparations show at best weak escape behavior, cuff electrodes were implanted on a thoracic (T1-2) connective, the body was closed, and one to two days later recordings were made during wind-evoked escape behaviors in tethered, but normally behaving, animals. In this T1-2 recording location, the small spikes of vGI axons are readily discriminated from the large spikes of postsynaptic, thoracic interneurons (TI's).

The strength of the escape behavior decreased owing to habituation, and to depression while the animal groomed its antennae or while it stood in a "quiescent" state. In all three of these depressed situations, the vGI responses to wind were unaltered; but the TI responses became strongly depressed. Thus, the vGI-TI connections appear responsible for these forms of behavioral depression.

In several other escape systems (e.g. crayfish), at some point following the main site of habituation, a single action potential in one giant neuron, easily excited by hook electrodes, evokes in a full the initial phase of escape. In the cockroach, it is already known that no one vGI by itself evokes escape. I stimulated through the T1-2 cuff in search of such an all-or-nothing excitation of escape behavior. Behavior exactly like the escape response to wind was evoked. However, the strength of the behavior was continuously graded over a wide range with the number, the frequency, and the voltage of the stimulus pulses. This suggests that, unlike most other escape systems tested, there is no one giant interneuron in which one spike--or even a burst of spikes--can evoke a full cockroach escape behavior.

128.2

EFFECTS OF LESIONING INDIVIDUAL GIANT INTERNEURONS ON THE DIRECTION OF THE ESCAPE TURNING RESPONSE IN THE ADULT COCKROACH, Periplaneta americana. C.M. Comer and G.T. Stubblefield. Dept. Biological Sciences & Committee on Neuroscience, Univ. of Illinois, Chicago, IL 60680

The wind-elicited escape response of the cockroach is believed to be mediated by giant interneurons (GIs). Gross lesion studies indicate that the direction of the turn which initiates escape (a turn away from the wind) may be based upon a comparison of wind-evoked neural activity in GIs on the two sides of the nerve cord. We have been testing this idea by lesioning specific GIs individually or in various combinations by intracellular injection of Pronase.

There are 7 bilaterally paired GIs, separated into distinct dorsal (dGI) and ventral (vGI) subgroups on each side of the nerve cord. We found that unilateral deletions of single vGIs from the cord can alter the direction of the turning response to wind. Comparable deletions of dGIs have not produced changes in turn direction.

Unilateral ablation of vGIs #1 or #3 (highly directionally selective cells) caused animals to turn toward winds from the ipsilateral side and the effect of ablating #3 was greater than that of ablating #1. Unilateral ablation of GI #2 (an omnidirectional cell) did not shift turn direction. Lesioning specific combinations of vGIs concomitantly greatly enhanced the shift in turning. These results suggest different roles for vGIs and dGIs in the escape response and they allow the formulation of models of the way activity in the GIs influences the direction of the motor response.

(Supported by NSF grant #BNS-8617393 to C.M.C.)

128.3

MULTIPLE FEEDBACK LOOPS AND DELAY LINES IN THE FLYING COCKROACH. F. Libersat and J. M. Camhi. Dept.of Zoology, Hebrew University, Jerusalem, Israel.

The wind sensitive giant interneurons (GI's) of the cockroach Periplaneta americana send their axons from the last abdominal ganglion (A6) to the thoracic locomotory ganglia. These cells comprise a dorsal group (dGI's) whose stimulation can evoke flight and a ventral group (vGI's) that mediate running escape behavior evoked by wind stimulation of the cerci (two posterior wind-sensitive appendages). We now show that during flight, the dGI's are activated rhythmically at the wingbeat frequency; by contrast, all the vGI's remain silent, in spite of strong flight winds and their wind sensitivity is decreased. The rhythmic activation of the dGI's involves several feedback loops. (1): A descending rhythmic activity of small diameter axons excites in A6 the dGI's whose spikes return to the thorax within one flight cycle. (2): This descending rhythm also produces, via motoneurons, a cercal vibration at the wingbeat frequency. This vibration, in turn, excites rhythmically cercal receptors known to excite the dGI's. The duration of this entire loop is two flight cycles. (3): the rhythmic wind gusts produced by the wings constitute a third feedback loop. The flight inhibition of the vGI's might prevent activation of escape movements of the legs by the flight winds. The dGI's, activated at the wingbeat frequency, could participate in flight maintenance.

128.4

COURTSHIP SUCCESS IN THE CRICKET *GRYLLUS BIMACULATUS*: DIFFERENTIAL CONTRIBUTION OF CARRIER FREQUENCY AND HARMONICS IN COURTSHIP SONG. L.A. Murray* and R. R. Hoy. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

Male field crickets produce long-range calling songs to attract conspecific females to their territories, and short-range courtship songs to facilitate copulation. The courtship songs do not have the stereotyped, species-specific, pulse temporal patterns that characterize calling songs, but instead have a more complex harmonic structure. In the calling song of *G. bimaculatus*, the carrier frequency (4.5 kHz) is also the dominant frequency, but in the courtship song, the third harmonic (13.5 kHz) is the dominant frequency, although there is significant energy in the fundamental.

When male crickets are muted by wing surgery, their ability to successfully court females is diminished at least three-fold. However, when tape recordings of their pre-mute courtship songs are played back during active courtship, their copulatory success was restored by this "replacement therapy," to virtually normal levels. The behavioral effect of courtship song can be fractionated into its constituent harmonic elements. Specifically, we found that playback of artificial courtship songs composed of the third harmonic (13.5 kHz) component alone, was sufficient to restore the courtship success of muted males to normal levels. However, playback of artificial songs containing only the fundamental (4.5 kHz) component alone did not increase courtship success above the very low levels achieved by mute males. Thus, the third harmonic (dominant frequency) in courtship song appears to be both the necessary and sufficient frequency component to elicit copulation behavior in the female cricket.

An identified auditory neuron in the cricket, HF_1AN, is strongly excited by 13.5 kHz sound pulses, but much less so by 4.5 kHz sound. This same neuron has been implicated in avoidance steering during flight behavior. It is possible that this same neuron is sufficient for both copulation behavior and acoustic startle, switching its role depending upon the behavioral context of the animal.

(Research funded by NINCDS and a Javits Neuroscience Investigator award).

128.5

ULTRASONIC NEURONS IN THE BRAIN OF CRICKETS. P.D. Brodfuehrer, M.L. May and R.R. Hoy. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

Tethered flying crickets perform evasive steering movements in response to ultrasonic stimuli such that they fly away from the sound source. The neuronal circuitry controlling negative phonotaxis is partially understood. In particular, a sensory interneuron, Int-1, has been shown to be necessary and sufficient for eliciting this behavior. Int-1 is located in the prothoracic ganglion and sends its axon anteriorly into the brain where it terminates. Ultrasound, however, only elicits negative phonotaxis in flying crickets. On the other hand, Int-1's sensitivity to ultrasound is the same in flying and non-flying crickets. Thus it appears that the flight oscillator gates the ability of Int-1 to produce evasive steering movements associated with negative phonotaxis. We were interested in first, determining whether gating occurs in the brain and secondly, in identifying brain neurons which receive ultrasonic auditory input.

We found that first, descending auditory activity from the brain is graded with respect to ultrasonic stimulus intensity even in non-flying preparations, and secondly, the strength of this activity is increased during flight. Thus input from the flight oscillator and from Int-1 are gated at the level of brain neurons. We have also identified three classes of brain neurons which receive ultrasonic auditory input. These brain neurons include descending interneurons which are excited by ultrasound, and local circuit neurons within the brain which are either inhibited or excited by ultrasound. In general, these brain neurons are broadly tuned (5-30 kHz) and, the strength of their responses is graded with stimulus intensity. The role these neurons play in controlling negative phonotaxis is presently being investigated. Supported by NIH grant NS11630 to R.R.H.

128.6

A THREE-DIMENSIONAL MODEL OF THE ULTRASOUND-INDUCED NEGATIVE PHONOTACTIC RESPONSE IN THE AUSTRALIAN FIELD CRICKET (*TELEOGRYLLUS OCEANICUS*). M.L. May, B.R. Land*, P.D. Brodfuehrer and R.R. Hoy. Sect. of Neuorbiology and Behavior, Cornell University, Ithaca, NY 14853.

The ultrasound-induced negative phonotactic response in flying Australian field crickets is believed to be an escape behavior or, more specifically, a bat avoidance response. If crickets steer away from ultrasonic stimuli to avoid predation then the nature of the induced flight path is of primary importance in assessing the adaptive significance of the behavior. Moreover, an analysis of the flight path may produce cause and effect relationships between various body movements and the resultant steering effects.While several kinematic factors -- abdominal ruddering, forewing tilt, and increased wingbeat frequency -- and aerodynamic rotations -- pitch and roll -- have been examined in detail, there has been no attempt to deduce the three-dimensional flight path following an ultrasonic stimulus. Ideally, an analysis of the flight path should come from free-flight in the field or a wind tunnel but both options pose formidable experimental problems. As such, modeling offers a good first step in assessing the three-dimensional flight path. Further, crickets show stimulus intensity-dependent linearity of response magnitude in most kinematic and aerodynamic factors involved in negative phontaxis and this predisposes the system to modeling.

At present, our model incorporates the effects of ultrasound-induced pitch and roll rotations relative to a stimulus located in the horizontal plane of the cricket and 90° to the long axis of the body. From this model and empirically determined pitch and roll rotations, a 25 dB suprathreshold stimulus induces a 14 cm downward and 35 cm lateral deviation from the normal flight path. While this is a significant alteration in the flight path, it is only a conservative estimate of the total course deviation. A more accurate three-dimensional model will require the incorporation of additional effects due to yaw rotation and possible changes in flight speed.

128.7

SENSORY CUES CONTROLLING SONIC COURTSHIP IN *POLLIMYRUS ISIDORI* (MORMYRIDAE). J.D. Crawford* and C.D. Hopkins (SPON: O. Hamill). Neurobiology & Behavior, Cornell University, Ithaca, NY 14853

Pollimyrus isidori is an electric fish in which males court females with an acoustic song. Experiments showed that male singing was evoked by conspecific females but not by males. In play-backs, EODs were effective releasers of sonic courtship. Sexual dimorphism was pronounced in *Pollimyrus* EOD waveforms while the fish were breeding. However, when sequential presentations of male and female EODs were made, males did not show discrimination: male and female electric models were courted with equal vigor. Recognition of natural EOD sex-differences was examined further using a habituation-dishabituation paradigm. One EOD type (M or F), was presented at natural inter-EOD intervals, for 15 S. Bouts were repeated once each min until a male's vocal response fell below 80 % of his initial response. The other EOD type was then introduced and the male's response was compared with 4 reponses bracketing this EOD. Males showed no evidence of recognizing the introduction of a new waveform. Male responsiveness to a broader set of electric stimuli was explored by presenting EOD-like single period sinusoids ranging from 20 to 800 μS in duration (peak power from 50 KHz to 1.25 KHz). The vocal response showed a broad high-pass characteristic with a corner of about 7 KHz (143 μS). Other behaviors showed that males detected all presented stimuli.

In *Pollimyrus isidori*, the EOD plays a role in courtship communication but males do not extract sex information from waveform cues. This difference from *Brienomyrus brachyistius* (Hopkins & Bass, 1980, Science, 212) may be related to the importance of sound communication in *Pollimyrus*. Our field observations of breeding *Brienomyrus* have revealed no sonic component to courtship. Acoustic sex-recognition in *Pollimyrus* is under study. NIMH 5T3215793 (JDC) & MH37972 (CDH).

128.8

CONVERGENT EVOLUTION OF SONIC MOTOR SYSTEMS IN TELEOST FISHES: TEMPERATURE DEPENDENCE OF SYNCHRONOUS AND ASYNCHRONOUS SYSTEMS. A. H. Bass, M. Weiser*, and R. G. Baker. Section of Neurobiology & Behavior, Cornell University, Ithaca, N.Y. 14853; Department of Physiology & Biophysics, New York University Medical Center, N.Y., N.Y. 10016; and Marine Biological Laboratory, Woods Hole, Mass. 02543

Temperature was used as a physiological variable to compare the functional organization of the sonic motor system in four species of marine teleost fishes: *Porichthys notatus* and *Opsanus tau* (Order Batrachoidiformes); *Prionotus carolinus* and *Myoxocephalus scorpius* (Order Scorpaeniformes). In *P.n.* and *O.t.*, a midline sonic motor nucleus synchronously activates a bilateral set of muscles surrounding the swimbladder. In *P.c.* and *M.s.* the sonic motor nucleus occupies a ventrolateral position bilaterally. Sonic muscles fire asynchronously in *P.c.*, but in *M.s.* the sonic muscles are cranioclavicular and fire synchronously like in *P.n.* and *O.t.*. Sonic activity was evoked with either natural or brain stimulation. Discharge frequency was measured intra-cranially from sonic motor nerves over temperature ranges of 4-32°C. In *O.t.*, maintaining animals at temperatures of 18°, 16°, or 11° for periods of 2-4 months did not affect the discharge frequency over similar temperature ranges. At the same temperature, the fundamental frequency recorded from each sonic motor nerve was similar among all four species. Thus, the central pacemaker driving each system responds similarly to temperature change, even in *P.c.* where the discharge is asynchronous and effectively doubles the fundamental frequency at any temperature.

In conclusion, despite differences in central or peripheral anatomy, the functional organization of the sonic motor system is remarkably similar across different taxonomic levels. Supported by NSF, NIH and a Steinbach Fellowship from the MBL.

128.9

THE NUCLEUS PRAEEMINENTIALIS: PROPERTIES OF NEURONS PROVIDING DESCENDING INPUTS TO THE ELECTROSENSORY LATERAL LINE LOBE OF WEAKLY ELECTRIC FISH. J. Bastian and B.O.Bratton. Dept. of Zool., Univ. Oklahoma, Norman, OK 73019.

The nucleus praeeminentialis (NP) recives input from the electrosensory lateral line lobe (ELL) and provides a major descending input to this same structure. We studied the responses of NP neurons to stepwise increases in EOD amplitude, to sinusoidal EOD AMs of various frequencies, and to moving electrolocation targets. Some neurons responded to the rising phase of the AM (On cells), others responded to the falling phase (Off cells), and some responded to both (On-Off cells). These types produced very phasic responses and were non-spontaneous. Cells giving sustained responses were also non-spontaneous. Spontaneously active cells were inhibited by increased EOD amplitude. NP neuron responses to moving electrolocation targets show less decrement with increasing fish-target distance as compared to the ELL neurons, some show directional selective responses, and some show maximum responses at distances other than closest to the fish. NP cells were also marked with Lucifer yellow to allow morphological identification of physiological categories. Supported by NIH Grant NS12337, and OCAST #1669.

128.10

CONSPECIFIC EOD DETECTION IN A WEAKLY ELECTRIC FISH. L.J. Fleishman and H.H. Zakon. Dept. Zoology, Univ. Texas, Austin, TX 78712

Sternopygus produces a quasi-sinusoidal electric organ discharge (EOD) which differs in frequency between individuals and sexes (females roughly an octave higher). EODs are perceived by electroreceptors which have a V-shaped tuning curve, with a best frequency nearly equal to the EOD frequency. Males find mates by detecting female EODs, but this ability would appear to be limited by electroreceptor tuning.

We investigated the possibility that the addition of a fish's EOD to that of a conspecific results in nonlinear filtering, allowing greater detectability of two signals combined than could be predicted from the response to either alone.

We determined behavioral tuning curves using conditioning. We then eliminated the EOD by lesioning the pacemaker nucleus, and retested the fish. EOD elimination caused no change in relative threshold to different frequencies, indicating that there is no nonlinear interaction. Threshold at all frequencies was elevated by 20 dB, because the presence of the EOD placed the combined signal at an amplitude where the electroreceptors are most sensitive to small changes. Microelectrode recordings from electroreceptor units stimulated with the sum of two sine waves yielded comparable results.

Peripheral filtering is linear for added sinusoids, and must limit the ability of males to detect female EODs.

128.11

AN ELECTROSENSORY MAP IN THE SKATE TECTUM LIMITED TO THE VISUAL FIELD. D. Bodznick, M.C. Ronan and E.J. Schneider*. Dept. Biology, Wesleyan University, Middletown, CT 06457.

Visual and electrosensory maps in the tectum of the little skate, Raja erinacea, were studied using multiple and single unit recordings. Visual stimulus was a pulsed LED (560 nm, .5^0 diam.) approx. 30 cm from the eye; electric stimulus was a dipole field presented through an electrode pair 30 cm from the center of the fish or handheld electrodes scanned along the body surface. Visual responses were recorded from the superficial tectum with rostral (0^0) to caudal (180^0) azimuths mapped uniformly from rostral to caudal tectum. Visual elevation from directly overhead to the pectoral fin edge mapped medial to lateral, with an overrepresentation of the 20^0 span of field at the horizon. The electrosensory map in deep tectum has a similar uniform representation of azimuth from rostral to caudal levels. Elevation of electrosensory fields mapped medial to lateral with a similar large representation of horizontal fields near the fin edge. Roughly 80% of electroreceptors in little skates are located ventrally and have no corresponding visual field. Ventral receptors are mapped not in tectum but in the adjacent lateral mesencephalic nucleus (LMN). Electrosensory maps in tectum and LMN are aligned so that a continuous representation of electrosensory elevation extends from medial tectum to ventolateral LMN.

128.12

A KINEMATIC COMPARISON OF MAUTHNER AND NON-MAUTHNER C-STARTS IN GOLDFISH. R.C. Eaton, R. DiDomenico and J. Nissanov. Neuroscience Group, Campus Box 334, Univ. of Colorado, Boulder, CO 80309.

This study utilizes digitized kinematic data and lesions of individual Mauthner (M-) cells, large medial reticulospinal command neurons, to examine their role in goldfish C-starts elicited by displacement stimuli. Our results show a major difference in response lateralization in animals with only one M-cell compared to those with both cells intact, or both cells absent. Animals with one M-cell responded by turning to the side opposite the remaining M-cell in 94% of the trials, whereas those both M-cells intact responded with equal probability to both sides. In addition, M-responses were on the average 4 ms shorter in latency than those without the M-cell present. This difference may confer a behaviorally significant advantage to the M-cell in blocking other networks that can trigger C-starts. Nevertheless, with the exception of latency, the central program producing the escape behavior adapts automatically to the absence of both M-cells: animals with bilateral M-cell lesions continued to produce the full spectrum of response types and kinematic performance levels seen in intact animals. [Supported by NIH grant NS22621].

128.13

WHAT DIFFERENCE DOES IT MAKE HAVING A MAUTHNER AXON? S.M. Van Horne*, R. C. Eaton, J.R. Fetcho and J. Nissanov. Neuroscience Group, CB 334, Univ. of Colorado, Boulder, CO 80309, Dept. Physiol., SUNY at Buffalo, NY 14214, Neurobiology Unit, Scripps Inst. Oceanography, UCSD, La Jolla, CA 92093.

The lack of motor differences between C-starts with and without the Mauthner (M-) cell, questions the functional significance of M-axon size relative to other motor axons in the spinal cord. We analyzed spinal cords for the size distribution of axons to compare with the M-axon. At the mid-trunk level for two goldfish, 9.3 and 10.2 cm standard length, the M-axon diameters were 40 and 46 um. The next largest fibers were 25 um, a difference of 1.7 times, which should reflect the relative conduction velocities (CVs). If the M-axon conducts at 80 m/s, the next largest fibers would conduct at 47 m/s. Thus, the M-axon depolarizes along its length in 1.25 ms compared with 2.12 ms for the next largest fibers. This 0.88 ms difference would not be seen using present cinematic methods but it should cause a performance advantage of 0.43 to 1.3% of a body length more distance covered, depending on elapsed time during the C-start. The MLF occupies no more than 0.77 sq. mm and the M-axons occupy only about 0.4% of this. Thus, the M-axon CV gives only a small difference in performance, but this has little cost in terms of area occupied by the fibers. [Supported by NIH grant NS22621].

128.14

THE MOTOR PATTERN GENERATOR FOR SNAPPING IN TOADS. J.-P. Ewert and A. Weerasuriya*. (SPON: C. H. Hockman) Dept. of Neuroethology, University of Kassel, Kassel, Federal Republic of Germany.

The hypothesis that a neuronal network interposed between the output elements of the optic tectum, and hypoglossal and other cranial motoneurones subserves snapping in anurans is critically examined. Morphological and electrophysiological data have not revealed a direct monosynaptic connection between the optic tectum and hypoglossal motoneurones. On the contrary, the available evidence supports the presence of interneurones mediating impulse traffic from the tectum to cranial motoneurones. Since snapping is a ballistic stereotyped behaviour, it is argued that these interneurones are part of a motor pattern generator for snapping (MPG) in anurans. In order to localise these interneurones, HRP was iontophonetically deposited in the medial medullary reticular formation (MRF) of toads, resulting in retrograde cell labeling in the lateral tectum and anterograde terminal labeling in the hypoglossal nucleus. Bilateral tectal and toral inputs to MRF suggest that the MPG consists of two unilateral subunits having independent access to the relevant motoneurones. This is consistent with the observation that toads with unilateral tectal lesions are still capable of snapping. These results in conjunction with previous electrophysiological data from the medulla strongly support the concept of a MPG for snapping.

SEROTONIN RECEPTORS I

129.1

EFFECT OF ACUTE AND LONG-TERM ADMINISTRATION OF SM-3997 ON 5-HT NEURON FIRING ACTIVITY AND ON RESPONSIVENESS OF POST-SYNAPTIC NEURONS TO SEROTONIN AND NOREPINEPHRINE. R. Godbout, Y. Chaput and C. de Montigny. Dept. of Psychiatry, McGill University, Montréal, Canada H3A 1A1.

The anxiolytic SM-3997 is a selective 5-HT$_{1A}$ ligand, and its metabolite 1-PP is an antagonist of α_2-adrenoceptors (Chaput et al., this meeting).

Acute intravenous SM-3997 depressed dorsal raphe 5-HT neuron firing activity with an ED$_{50}$ of 9.1±1.1 µg/kg. In rats treated for 2, 7 or 14 days with SM-3997 (10 mg/kg, s.c.), there was a marked reduction of 5-HT neuron firing activity after 2 days, a partial recovery after 7 days and a complete one after 14 days of treatment. At this latter point in time, the effect of LSD on 5-HT neuron firing activity was markedly reduced, indicating a desensitization of somatodendritic 5-HT autoreceptors.

Microiontophoretic application of SM-3997 onto dorsal hippocampus pyramidal neurons blocked the effect of 5-HT, but not that of NE, indicating that SM-3997 is a partial agonist of postsynaptic 5-HT$_{1A}$ receptors. However, the responsiveness of these neurons to 5-HT, NE and SM-3997 was not modified by the 14-day treatment with SM-3997.

These data indicate that long-term treatment with SM-3997 desensitizes somatodendritic 5-HT autoreceptors, but not postsynaptic 5-HT$_{1A}$ receptors, and that, at the dose administered, neither postsynaptic 5-HT$_{1A}$ nor postsynaptic α_2-adrenoceptors are blocked by SM-3997 and 1-PP.

129.2

EFFECT OF 1-PP ON α2-ADRENOCEPTORS: ELECTROPHYSIOLOGICAL STUDIES IN THE RAT CENTRAL NERVOUS SYSTEM IN VIVO. Y. Chaput, O. Curet and C. de Montigny. Dept. of Psychiatry, McGill University, Montreal, Canada H3A 1A1.

1-PP (1-pyrimidinylpiperazine) is the common metabolite of the anxiolytic 5-HT$_{1A}$ agonists SM-3997, buspirone, gepirone and ipsapirone. The present in vivo studies were undertaken to determine the effect of 1-PP on pre- and postsynaptic α_2-adrenoceptors in male Sprague-Dawley rats.

1-PP applied by microiontophoresis (10 to 30 nA) or administered intravenously (4 to 8 mg/kg) blocked the effect of microiontophoretically-applied norepinephrine (NE) on CA$_3$ dorsal hippocampus pyramidal neurons, but not those of 5-HT and GABA. Since the effect of microiontophoretically-applied NE on these neurons has been shown to be mediated by postsynaptic α_2-adrenoceptors, these results provide evidence that 1-PP is an effective antagonist of postsynaptic α_2-adrenoceptors.

1-PP (4 mg/kg, i.v.) increased the effectiveness of the stimulation of the locus coeruleus in suppressing CA3 hippocampus pyramidal neuron firing activity. This data suggests that 1-PP blocks terminal α_2 autoreceptors.

1-PP reversed the suppressant effect of clonidine (20 µg/kg, i.v.) on the firing activity of NE neurons of the locus coeruleus (ED$_{50}$: 80 µg/kg, i.v.). This suggests that 1-PP blocks somatodendritic α_2-adrenergic autoreceptors.

In conclusion, these data demonstrate that 1-PP is an antagonist of both pre- and postsynaptic α_2-adrenoceptors.

129.3

MOUSE BRAIN SEROTONIN RECEPTOR-MEDIATED RESPONSES IN *XENOPUS* OOCYTES: IONIC MECHANISMS OF CALCIUM-ACTIVATED CHLORIDE CURRENTS. L. Yu, N. Davidson and H.A. Lester. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Serotonergic responses can be induced in *Xenopus* oocytes injected with mouse brain RNA, as a result of the expression of the mouse 5-HT_{1C} serotonin receptors. The response is in the form of a two-phase Ca^{++}-activated Cl^- current. The first phase is a fast transient inward current, followed by a sustained plateau in the second phase. The Cl^- current in mRNA-injected oocytes can be induced under three conditions: 1) addition of serotonin in low Ca^{++} (<1μM) solution; 2) addition of serotonin in normal Ca^{++} (1.8mM) solution; and 3) exposure to extracellular Ca^{++} at 1.8mM in the presence of serotonin. The latency of the Cl^- current varies depending on the activation condition. The addition of Ca^{++} in the presence of serotonin gives an immediate response. 5-HT induced response has a 10 to 15-second delay in the presence of normal extracellular Ca^{++}, while the delay time is doubled in the low Ca^{++} solution. These data suggest that the three types of Cl^- responses are the results of activation by Ca^{++} from different sources. The duration of the plateau phase depends on extracellular Ca^{++}.

129.4

^{125}I-Azido-PAPP, A SELECTIVE PHOTOAFFINITY LABELING PROBE FOR 5-HT_{1A} RECEPTORS. W. Yang*, C.H. Chen*, S. Chumpradit*, H.F. Kung*, and J. Shih. School of Pharmacy, Univ. of South. Calif., L.A., CA 90033, and Dept. of Radiology, Univ. of Penn., Phila., PA 19104

Previous work from this laboratory has shown that 1-[2-(4-aminophenyl)ethyl]-4-(3-trifluoromethylphenyl) piperazine (LY 165163, PAPP) is a selective ligand for 5-HT_{1A} receptor and ^{3}H-azido-PAPP is a specific photoaffinity labeling probe for 5-HT_{1A} receptor (for review, Shih et al. Psychopharm Bulletin 22:818-824, 1986; J. Neurochem. 49:1361-1366, 1987). Recently, we have synthesized Iodo-PAPP, Iodo-azido-PAPP and ^{125}I-azido-PAPP. In vitro binding studies show that both I-PAPP and I-azido-PAPP displaced about 60% of the total ^{3}H-5-HT bindings in bovine hippocampal membranes. At 2 nM ^{3}H-5-HT, the IC_{50} for I-azido-PAPP was 35 nM whereas IC_{50}'s for 5-HT, PAPP and azido-PAPP were 5.6, 8.1 and 5.0 nM, respectively. At 1 nM ^{3}H-DPAT, the IC_{50} for I-azido-PAPP was 40 nM, whereas the IC_{50} for 5-HT, PAPP and azido-PAPP were 6.0, 10.0 and 3.1 nM, respectively. Upon photoirradiation of ^{125}I-azido-PAPP with brain membranes, two proteins with molecular weight of 55K and 38K daltons were labeled. The photoincorporation of ^{125}I-azido-PAPP could be blocked by 5-HT_{1A} specific ligands 5-HT, DPAT, spiperone and LSD. These results suggest that ^{125}I-azido-PAPP is a selective photolabeling probe for 5-HT_{1A} receptors. (Supported by NIMH Grant No. MH 37020)

129.5

PHOTOAFFINITY LABELING OF RAT BRAIN 5-HT_{1B} RECEPTORS WITH [^{125}I]-IODOCYANOPINDOLOL DIAZIRINE. M.W. Hamblin, P.I. Adriaenssens*, G.L. Tan*, K. Ariani*, and R.D. Ciaranello. Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.

[^{125}I]-iodocyanopindolol diazirine ([^{125}I]ICYPD) was originally developed as a beta adrenergic photoaffinity label (Bergermeister, et al, BBA 729:219, 1983). We now decribe its use as a photoaffinity label for rat brain 5-HT_{1B} receptors.

[^{125}I]-ICYPD (free concentration approximately 100-200pM) irreversibly photolabeled a number of rat hippocampus and substantia nigra membrane proteins. Specificity in labeling for two protein bands could be demonstrated. Isoproterenol (3-30uM) blocked photolabeling of a protein of Mr=64000, consistent with specific labeling of beta adrenergic receptors. Radiolabeling of a second protein of Mr=52000 could be blocked by inclusion of compounds with high 5-HT_{1B} affinity, such as RU 24969 (250nM), dihydroergotamine (50nM), or 5-HT (1000nM), but not by isoproterenol (3-30uM) or the 5-HT_{1A} specific compound DPAT (250nM). Inclusion of several protease inhibitors had no effect on the observed Mr. These studies were limited by considerable non-specific labeling related to the low abundance of 5-HT_{1B} receptors in these tissues. Nonetheless these studies provide the first estimate of molecular weight for the 5-HT_{1B} receptor and show that its size is typical for the G-protein linked receptor family of which it may be a member.

129.6

A PINDOLOL DERIVATIVE SELECTIVELY INTERACTS WITH 5-HT_{1A} RECEPTORS IN THE RAT HIPPOCAMPUS. L.M. Liau, J.W. Pitha*, and S.J. Peroutka. Dept. of Neurology, Stanford University Medical Center, Stanford, CA 94305.

A pindolol derivative with a high affinity for beta-adrenoceptors was synthesized (ISOBIM, N^1-(Bromoacetyl)-N^8-[3-(4-indolyloxy)-2-hydroxypropyl]-(Z)-1,8-diamino-p-methane). Radioligand binding studies indicated that the drug is extremely potent (<1.0 nM) at 5-HT_{1A} receptors labeled by ^{3}H-8-OH-DPAT. Moreover, the drug was also selective in that it was more than ten-fold more potent at beta-adrenergic receptors. ISOBIM (10^{-10} M to 10^{-3} M) alone had virtually no effect on baseline or forskolin-stimulated adenylate cyclase activity. By contrast, ISOBIM was able to significantly ($p<0.001$) reverse 8-OH-DPAT-induced inhibition of forskolin-stimulated activity. 8-OH-DPAT, a 5-HT_{1A} agonist, inhibited forskolin stimulation by 25-35%. In the presence of nanomolar ISOBIM, the amount of inhibition produced by 8-OH-DPAT was completely reversed and occassionally stimulation rates were brought above controls. Therefore, ISOBIM is a potent antagonist of 5-HT_{1A} receptors and may serve as a useful tool in the analysis of 5-HT_{1A} receptors and function.

129.7

PHARMACOLOGICAL CHARACTERIZATION OF THE SEROTONIN (5-HT) AUTORECEPTOR MODULATING THE ELECTRICALLY-EVOKED RELEASE OF [^{3}H]-5-HT FROM SLICES OF HUMAN FRONTAL CORTEX. A.M. Galzin*[1], P. Blier[1], J.P. Chodkiewicz*[2], M.F. Ppirier*[3], H. Lop*[3], F.X. Roux*[2], A. Bedondo*[2], A. Lista*[1], R. Ramdine*[1] and S.Z. Langer[1]. [1]Laboratoires d'Etudes et de Recherches Synthelabo (L.E.R.S.), 75013 Paris, France; Services de Neurochirurgie[2] et de Psychiatrie[3], Hôpital Sainte-Anne, 75014 Paris, France

In the rat brain, the presynaptic 5-HT autoreceptors located on 5-HT terminals belong to the 5-HT_{1B} subtype. Although there is controversy as to the existence of 5-HT_{1B} binding sites in the human brain, the presence of a terminal 5-HT autoreceptor of an uncharacterized pharmacological type has been documented (Schlicker, E. et al., Brain Res., 331:337, 1985). We studied the effects of 5-HT receptor agonists and antagonists on the electrically-evoked release of [^{3}H]-5-HT from preloaded human frontal cortex slices. Specimens of normal cerebral cortex (which had to be removed in order to gain access to deep-seated brain tumors) were obtained from seven neurosurgery patients. Two periods of electrical stimulation (3 Hz, 30 mA, 2 ms x 2 min) were applied 90 min (S_1) and 134 min (S_2) after the onset of superfusion. The fractional release evoked by the electrical stimulation (S_1) was 1.37±0.20% of the total radioactivity with a S_2/S_1 ratio of 1.40±0.12. The 5-$HT_{1A/1B}$ agonist 5-carboxamidotryptamine (5-CT) produced a potent inhibition (77% at 30 nM) of the release of [^{3}H]-5-HT which was blocked by 5-HT antagonists with the following efficacy: methiothepin>metergoline=methysergide >propranolol. The selective 5-HT_{1A} agonist 8-OH-DPAT at 100 nM did not modify the release of [^{3}H]-5-HT whereas the 5-HT_{1B} agonist RU 24969 produced a maximal inhibition of 65% at 100 nM. In contrast, a 100% inhibition of [^{3}H]-5-HT release was obtained in rat frontal cortex slices with 100 nM RU 24969. These results suggest that the release of 5-HT in the human frontal cortex is modulated by an inhibitory 5-HT autoreceptor which is different from the 5-HT_{1A} or $_{1B}$ subtypes. The relative potencies of 5-HT receptor antagonists to block the inhibition produced by 5-CT together with the difference in potency of RU 24969 between rat and human frontal cortex slices indicate that this receptor has a pharmacological profile that closely resembles that of the 5-HT_{1D} subtype described by Heurting, R.E. and Peroutka, S.J. (J. Neurosci., 7:894, 1987).

129.8

G-PROTEINS RECONSTITUTE BINDING TO NEM-INACTIVATED HIGH AFFINITY [^{3}H]5-HT BINDING SITES IN BRAIN. C. A. Stratford, G. L. Tan*, M.H. Hamblin, and R.D. Ciaranello. Laboratory of Developmental Neurochemistry, Dept. of Psychiatry, Stanford University Medical School, Stanford, CA 94305.

N-ethyl maleimide (NEM) inactivates high affinity [^{3}H]DPAT binding to 5-HT_{1A} and [^{3}H]5-HT binding to 5-HT_{1B}, and 5-HT_{1D} sites in rat and bovine brain. Antagonist binding to these sites is relatively insensitive to this treatment. Binding to 5-HT_{1C} and 5-HT_2 sites is not affected by NEM treatment (Soc. Neurosci. Abs.13(2): 1236). In the present study we show that binding to NEM-inactivated sites can be reconstituted by addition of purified G-proteins to the membrane preparations.

Binding to NEM inactivated membranes is reconstituted by addition of a mixture of G_i and G_o proteins purified from bovine brain in the presence of sodium cholate (.005%; final conc.), sonication and subsequent washing. Reactivation is maximal at a ratio of approximately 1000 G-protein molecules to 1 binding site. Binding of [^{3}H]DPAT to 5-HT_{1A} sites in rat brain and of [^{3}H]5-HT to 5-HT_{1D} sites in bovine brain is reactivated by addition of the G-proteins. The reactivated binding is sensitive to addition of 5'-guanylyl imidodiphosphate (GppNHp) to the binding assay. When membranes are treated with NEM, the apparent K_i for 5-HT displacement of [^{125}I]iodocyanopindolol binding to 5-HT_{1B} sites is increased from 2 nM to 57 nM and is no longer sensitive to GppNHp. Addition of G-proteins to the mixture decreases the apparent K_i (3-5nM) and restores GppNHp sensitivity of 5-HT displacement. These results suggest that 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} sites are endogenously linked to G_i and/or G_o-like proteins.

129.9

5-HYDROXYTRYPTAMINE$_{1A}$ RECEPTORS ARE LINKED TO A G_i ADENYLATE CYCLASE COMPLEX IN RAT BRAIN. M.A. Harrington and S.J. Peroutka. Department of Neurology, Stanford University School of Medicine, Stanford, CA 94305.

5-Hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptors can be radiolabeled with [^{3}H]-8-hydroxy-2-(di-n-propylamino)tetralin (^{3}H-8-OH-DPAT), are sensitive to inhibition by GTP and GDP, but not GMP, and have been shown to mediate the inhibition of adenylate cyclase following stimulation by forskolin. ADP-ribosylation of GTP-binding proteins by pertussis toxin was found to abolish both 5-HT- and 8-OH-DPAT-induced inhibition of forskolin-stimulated adenylate cyclase activity in rat hippocampal membranes, indicating that 5-HT$_{1A}$ receptors are linked to the G_i protein mediating the inhibition of adenylate cyclase.

To further characterize the interaction between 5-HT$_{1A}$ receptors and G proteins, rat cortical membranes were preincubated with nonhydrolyzable GTP analogues GTPgammaS and GDPbetaS, resulting in an increased K_D and lowered B_{max} of subsequent ^{3}H-8-OH-DPAT binding in a dose- and time-dependent manner. These findings indicate a decrease in receptor affinity and density of ^{3}H-8-OH-DPAT binding of up to 60%. 5-HT$_{1A}$ receptors have also been shown to be directly linked to a potassium channel. These data suggest that the receptors may be linked to two different effector systems.

129.10

LIMITATIONS TO USING THE INHIBITION OF FORSKOLIN-STIMULATED ADENYLATE CYCLASE TO SCREEN FOR COMPOUNDS ACTING THROUGH SEROTONIN$_{1A}$ RECEPTORS. L.J. Cornfield, P.J. Monroe, E.W. Taylor*, S.S. Nikam* and D.L. Nelson.. Dept. of Pharmacology & Toxicology, College of Pharmacy, University of Arizona, Tucson, Arizona 85721.

Numerous studies have shown that serotonin$_{1A}$ (5-HT$_{1A}$) receptors are negatively coupled to adenylate cyclase. Thus, this assay has potential as a screening tool for evaluating the functional activity of new putative 5-HT$_{1A}$ agonists and antagonists. The forskolin-stimulated adenylate cyclase assay was performed for this study using Sprague-Dawley rat hippocampal membranes. The potencies of known 5-HT$_{1A}$ agonists (such as buspirone, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and 5-HT itself) in this system were consistent with those at 5-HT$_{1A}$ sites defined by [^{3}H]8-OH-DPAT binding. Examination of some novel 5-HT$_{1A}$ ligands revealed several compounds (including spiroxatrine and three aralkylamines) that had potencies that did not correlate with 5-HT$_{1A}$ binding, despite exhibiting potential 5-HT$_{1A}$ agonistic activity in the cyclase system. Consequently, results from this functional assay must be interpreted with caution when screening compounds for 5-HT$_{1A}$ activity, due to the possible inhibition of adenylate cyclase via non-5-HT$_{1A}$-mediated mechanisms. (Supported by NIH Grants MH39530, NS16605 and NS01009).

129.11

BEHAVIORAL, DISCRIMINATIVE STIMULUS AND NEUROCHEMICAL ACTIONS OF DRUGS ACTING AT SEROTONIN (5-HT) RECEPTOR SUBTYPES: EFFECTS OF 5-HT$_{1A}$ AGONISTS AND 5-HT$_2$ ANTAGONISTS. J.E. Barrett, S. Gleeson* and M.A. Nader*. Dept. of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Effects of compounds believed to be agonists and\or antagonists at the 5-HT$_{1A}$ or 5-HT$_2$ receptor were studied in pigeons under procedures known to be indicative of anxiolytic activity. Key pecking was maintained under a schedule where each 30th response in the presence of a red or white keylight produced food; when the keylight was red, each 30th response also produced a mild (2-4 mA) electric shock. Keylight colors alternated every 3 min throughout a 30 min session. Ipsapirone (0.1-10 mg/kg), MJ-7378 (0.01-5.6 mg/kg), 2-mPP (0.3-10.0 mg/kg), and spiroxatrine (0.03-3.0 mg/kg), i.m. - compounds with high affinity for the 5-HT$_{1A}$ receptor - all increased punished responding at doses that did not affect or that decreased unpunished responding. The 5-HT$_2$ antagonists ritanserin (0.03-10 mg/kg) and ketanserin (0.1-10 mg/kg) also increased punished responding. In pigeons trained to discriminate spiroxatrine or buspirone from saline, all 5-HT$_{1A}$ compounds produced drug-appropriate responding, whereas the 5-HT$_2$ compounds produced responding on the key correlated with saline administration. Although these compounds produce their discriminative stimulus effects through different receptor subtypes, results with the punishment procedure indicate that all should be effective anxiolytic compounds.

129.12

5-METHOXY-LUZINDOLE (N-0745):A PARTIAL MELATONIN RECEPTOR AGONIST. M.L. Dubocovich, J.M. Fang* and L. Yousif*. Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Luzindole (N-0774, 2-benzyl-N-acetyltryptamine, LUZ) is a competitive melatonin (MEL) receptor antagonist (Dubocovich, M.L., J. Pharmacol. Exp. Ther., in press, 1988). Here we report the pharmacological properties of 5-methoxy luzindole (5-M-LUZ) on MEL receptors. In the rabbit retina labeled in vitro with ^{3}H-dopamine (DA), 5-M-LUZ (0.1 nM-1 μM) inhibited (IC_{50} = 1.4 nM) the calcium-dependent release of DA, with a maximal inhibitory (35-40 %) effect at 100 nM. By contrast, the inhibitory effect of MEL (IC_{50} = 33 pM) was antagonized by 1, 10, 100 and 1,000 nM of 5-M-LUZ (IC_{50} = 0.95, 2.4, 15.2 and 118 nM, respectively). The equilibrium dissociation constant (K_B) of 5-M-LUZ calculated from the Schild plot was 0.12 nM (slope 0.93). In the C3H/HeN mouse 5-M-LUZ (30 mg/kg, i.p., 30 min) administered in vivo reversed the α-CH_3-p-tyrosine (α-MpT, i.p., 2 h)-induced depletion of norepinephrine (NE) (ng/ mg tissue) in the hypothalamus [Control: 2.85 $\pm$ 0.13 (6); α-MpT: 2.15 $\pm$ 0.1 (6); α-MpT plus 5-M-LUZ: 2.66 $\pm$ 0.12 (4), $p < 0.05$)]. 5-M-LUZ also blocked the reversion of the α-MpT induced depletion of NE by the agonist 6-Cl-MEL (30 mg/kg, i.p., 30 min) [α-MpT plus 6-Cl-MEL plus 5-M-LUZ: 2.24 $\pm$ 0.08 (8)]. In conclusion, 5-M-LUZ acts as a partial MEL receptor agonist, i.e., it shows pharmacological properties of a MEL receptor agonist and antagonist both in in vitro and in vivo models. Supported by MH 42922 and Nelson Research.

129.13

2-[125I]-IODOMELATONIN LABELS TWO DIFFERENT MELATONIN (MEL) BINDING SITES IN CHICKEN BRAIN MEMBRANES. (SPON: S.F. Holloway). G. Shankar* and M.L. Dubocovich. Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago 60611.

We have characterized a 2-[^{125}I]-iodomelatonin (2-[^{125}I]-I-MEL) binding site in the chicken brain with pharmacological characteristics similar to the functional MEL receptor in the retina. The affinities (nM) of MEL analogs at this binding site were : 6-Cl-MEL (8) > 2-I-MEL (16) $\geq$ MEL (17) > 2-CH_3-6,7-diCl-MEL (50) > 6-OH-MEL (180) >> 5-HT (2,000). Here we report a second 2-[^{125}I]-I-MEL binding site with high affinity for compounds inactive at the functional MEL receptor. Chicken brain membranes were incubated with 2-[^{125}I]-I-MEL (100 pM) and various drugs at 0^{o}C. Monophasic dose response curves of 5-HT agents yielded the following pharmacological profile (affinity, nM): methysergide (70) $\geq$ spiperone (80) > RU-24969 (380) > spiroxatrine (480) > ketanserine (600) > ICS-205930 (1,050) > metergoline (3,600) > 5-carboxamidotryptamine (6,600) > 8-OH-DPAT (7,500) > methiothepine (7,800) >> gepirone (>100,000). Various α-and β- adrenergic compounds were inactive . This profile does not correspond to any 5-HT site. Further, the 5-HT analogs are inactive in chicken retinal membranes. It appears that 2-[^{125}I]-I-MEL labels two sites in the chicken brain: one site with affinity for compounds affecting MEL-induced inhibition of ^{3}H-dopamine release from chicken retina and a second site with affinity for compounds with no known functional response and which do not exist in the retina. Supported by MH 42922.

130.1

GABA FACILITATES AFFERENT NICOTINIC SYNAPSES IN THE INFERIOR MESENTERIC GANGLION. W. H. Stapelfeldt and J. H. Szurszewski. Dept. of Physiology and Biophysics, Mayo Medical School, Rochester, MN 55905.

Recent immunohistochemical studies demonstrated the presence of GABA immunoreactive material in afferent fibers of colonic origin projecting to the inferior mesenteric ganglion (IMG). To determine the functional role of this pathway the effects of GABA receptor/Cl-ionophore modulating drugs were studied in isolated guinea pig IMGs receiving ongoing afferent synaptic input from attached segments of distal colon using standard intracellular recording techniques. In the presence of bicuculline or picrotoxin (10-50 μM) depolarizing post-synaptic responses to exogenous GABA were reduced or abolished and ongoing nicotinic fast EPSPs were decreased in amplitude and frequency by 40%. Addition of diazepam (5 μM) facilitated the depolarizing response to exogenous GABA and caused a 40% increase in amplitude and frequency of ongoing asynchronous nicotinic fast EPSPs. Clamping of the postsynaptic depolarizing response revealed a presynaptic facilitatory action of GABA on afferent nicotinic terminals. Central preganglionic nicotinic synapses were not affected by GABA. These findings suggest that GABA is released in the IMG during ongoing colonic motor activity and functions as a presynaptic facilitatory modulator of nicotinic mechanosensory afferent synapses. (Supported by DK 17632.)

130.2

CENTRAL AND PERIPHERAL SYNAPTIC INPUTS TO THREE TYPES OF SYMPATHETIC NEURON IN THE GUINEA PIG CELIAC GANGLION. R.L. Meckler and F.M. McLachlan*, School of Physiol./Pharmacol., Univ. of New South Wales, Kensington, NSW 2033, AUSTRALIA.

Electrophysiological criteria were used to classify neurons into three types according to the ion channels in their membranes (Cassell & McLachlan, J. Physiol., 394:331-349, 1987). Two types of neurons discharge phasically during maintained depolarization; one of these has a long afterhyperpolarization (LAH). The third type discharges tonically. Excitatory synaptic potentials (e.s.ps) were evoked by stimulation of splanchnic (SPL) and celiac (CN) nerves. Phasic neurons receive <10 e.s.ps of splanchnic origin, one of which is usually suprathreshold; very rarely small e.s.ps are evoked from CN stimulation. LAH neurons generally receive fewer SPL and occasionally more CN inputs. In contrast, tonic neurons receive only a few e.s.ps from SPL, but up to 30 e.s.ps from CN stimulation. Thus activity of tonic neurons is dominated by synaptic inputs of peripheral origin, whereas phasic and LAH neurons receive predominant input from the central nervous system. The spatial distributions of neurons of different types within the ganglion correspond with the distribution of 3 types of neurochemically identified neurons that regulate different gastrointestinal functions: vasoconstriction, inhibition of motility and inhibition of secretion (Macrae, Furness & Costa, Cell Tiss. Res., 244:173-180, 1986).

130.3

CO-REGULATION OF NPY AND NE IN SUPERIOR CERVICAL GANGLION CULTURE. R.E. Mains and K.L. Marek. Dept. Neuroscience, Johns Hopkins Univ., Baltimore, MD. 21205.

The co-regulation of Neuropeptide Y (NPY) and Norepinephrine (NE) synthesis has been investigated in rat superior cervical ganglion (SCG) neuronal cultures. NPY synthesis was measured after incubation in medium containing [^{3}H]tyrosine followed by immunoprecipitation and SDS-PAGE. NE synthesis was determined from the same extract by RP-HPLC. NPY mRNA levels were quantitated in SCG cultures by Northern analysis.

In previous studies we have shown that the synthetic rates of NE and NPY were independently regulated in response to heart cell derived cholinergic inducing factor. In these experiments SCG cultures were maintained for 10 days in serum free medium containing specific adrenergic pharmacologic agents, dexamethasone, dBcAMP or phorbol myristate acetate (PMA). Synthesis and accumulation of NE decreased following treatment with reserpine (20-fold), clonidine (2-fold) and desipramine (3-fold), but NPY synthetic rate was unchanged. Dexamethasone and cAMP caused a coordinate increase and PMA a coordinate decrease in NE and NPY synthetic rate. Preliminary evidence indicates that NPY mRNA levels show corresponding changes. These data provide strong evidence for independent control of synthesis and release of NPY and NE in SCG neurons.

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130.4

VAGAL CONTROL OF INSULIN SECRETION: FURTHER EVIDENCE FOR GASTRIC AND HEPATIC BRANCHES PLAYING THE MAJOR PHYSIOLOGICAL ROLE.

H.-R. Berthoud and T.L. Powley. Laboratory of Regulatory Psychobiology, Purdue U., W. Lafayette, IN, 47907.

Although vagal control of insulin secretion has classically been attributed to the celiac branches of the abdominal vagus, we have recently demonstrated that the gastric and hepatic branches mediate the bulk of electrical stimulation-induced (Soc. Neurosci. Abstr. 12, p 644) and cephalic phase (Soc. Neurosci. Abstr. 13, p. 332) insulin secretion. However, because the magnitude of the insulin response elicited by electrical stimulation is much larger than that produced by the cephalic phase of food intake or any other known neurogenic stimulus, it could be artifactual, due to the antidromic excitation of vagal afferents (Delbro et al. Acta Physiol. Scand. 118, 1983 and 122, 1984). It would thus not be affected by a ganglionic blocker. We performed, therefore, bilateral electrical cervical vagal stimulation in rats with only the gastric plus hepatic or only the hepatic abdominal branches intact, first in the absence and then in the presence of hexamethonium (HEX) (5-10mg/kg i.v. at -15min). HEX abolished the early (1 min) increase of peripheral insulin levels (control: +117±22% of basal; HEX: -4±8%) and suppressed the integrated 10 min response by more than 90%. Pancreatic glucagon secretion was similarly affected.

This suggests that vagal gastric and hepatic branch mediated stimulation of insulin and glucagon secretion is likely to be due to excitation of preganglionic motor fibers, and not antidromic excitation of afferents. (NIH Grant DK27627)

130.5

Light and Electron Microscopic Analysis of the Accessory Celiac Branch of the Vagus Nerve. J.C. Prechtl and T.L. Powley, Lab. of Regulatory Psychobiology, Purdue Univ., W. Lafayette, IN 47907

The accessory celiac branch (AC) is the least studied of the abdominal vagal branches in the rat. It separates from the ventral vagal trunk and courses on the left gastric artery towards the celiac plexus. Although the conclusion that the AC and the dorsal celiac branch form a symmetrical pair is supported by several observations (i.e. similar peripheral paths, preganglionics originating from homotypic columns in the dorsal motor nucleus, similar numbers of vagal efferents), the smaller size and less extensive branching of the AC suggests that it may not be a complete homotype of the dorsal celiac branch (Prechtl & Powley 1985, JCN; also 1987, Neurosci. Abst.). In order to characterize more thoroughly the AC, we prepared the abdominal vagal complexes from six rats for electron microscopy. From cross-sectional montages of the branches (X10,000), all axons were counted and their dimensions were determined (OASYS Image Analyzer). To evaluate the possibility of extrinsic fibers (non-vagal origin, see Prechtl & Powley, Anat. Embryol. 1987) in the AC, as well as to examine the intraneural organization of the branch point, 21 osmium-stained nerve whole mounts were prepared for light microscopy.

The AC branch contained 3103±404 unmyelinated fibers and only 5.5±2 myelinated fibers; two of the specimens contained no myelinated fibers at all. The mean diameter of the unmyelinated axons was 0.64±.01um, and that of the myelinated axons was 1.63±.1um. Finally, the whole mounts revealed that in at least 81% of the cases, the AC did contain extrinsic fascicles like those we have found in other vagal branches. (NIH grant DK27627)

130.6

DENDRITIC FIELDS AND MORPHOLOGY OF IDENTIFIED NEURONS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS. E.A. Fox and T.L. Powley, Lab. of Regulatory Psychobiology, Purdue U., West Lafayette, IN 47907.

The dorsal motor nucleus of the vagus is composed of five primary, topographically separate, parallel, longitudinal columns of neurons; each is associated with a different subdiaphragmatic vagal branch (i.e. one of the two celiac, two gastric, or unpaired hepatic branches). Physiological and behavioral studies have shown that different columns and their respective branches mediate different (and in some cases multiple) functions. In the present ongoing study, the neurons of the different columns were compared in terms of their dendritic fields and morphology to characterize the substrate(s) for differential control of vagal responses. The preganglionics in individual columns were labeled by retrograde transport of fast blue (Brain Res. 341(1985) 269-282). Then the processes of cells randomly selected from these identified neurons (n=173) were stained by intracellular iontophoresis of lucifer yellow (Brain Res. 436(1987) 143-147). The coronal profiles of neurons in each of the celiac columns were more extensive than those in each of the gastric columns on several measures (no. primary dendrites, highest branching order, no. total dendritic segments and percentage of neurons with dendrites extending into the adjacent column). Celiac neuronal profiles were also more extensive than those of the hepatic column, which were similar to those of gastric neurons. The morphological characteristics of the different columns correlate with their different function specializations (Neurosci. Abstr. 12(1986) 644). Within all columns there were also rostrocaudal gradients in the above measures as well as in soma profile area, dendritic orientation, and percentage of neurons with extranuclear dendrites. Horizontal and sagittal views are being studied. (NIH grant DK 27627).

130.7

COLOCALIZATION OF CHOLINERGIC AND CATECHOLAMINERGIC (DOPAMINERGIC) RELATED PHENOTYPES IN NEURONES OF THE DORSAL MOTOR NUCLEUS OF THE VAGUS IN THE ADULT WISTAR RAT. C. FEUERSTEIN, M. MANIER*, S. MOUSSAOUI*, P. MOUCHET*, N. MONS*, M. GEFFARD and J. THIBAULT* Lab. Physiology sect. Neurophysiology, INSERM U 318, Fac. Neurology, CHU of Grenoble, BP 217 X, 38043 Grenoble cedex, France.

Neuronal cell bodies containing Tyrosine Hydroxylase (TH) like immunoreactivity have been recently detected in the cervical spinal cord of Wistar rats (Dietl et al., Histochemistry, 1985, 82 : 385-389 ; Mouchet et al., Brain Res. Bull., 1986, 16 : 341-353), some of them being located dorsally to the central canal where they represent the caudal extension of the catecholaminergic cell group of the dorso medial medulla. Some of these cells contain also Choline Acetyl Transferase (Manier et al., Neurosci. Lett., 1987, 80 : 141-146) and they belong to the caudal portion of the dorsal motor nucleus of the vagus nerve (DMNV). To test whether the TH related immunoreactive material present in these cholinergic cells might have functional catecholaminergic properties, we have used immunohistochemical methods with specific antibodies raised against aromatic L-aminoacid decarboxylase, dopa, dopamine and norepinephrine. Our results evidence the presence of both dopa and dopamine related immunoreactivities, without detectable noradrenergic related ones. Both cholinergic and dopaminergic characteristics appear then colocalized in these DMNV cells, their selective target organs being yet unknown.

130.8

CATECHOLAMINE DISTRIBUTION IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS IN THE CAT: EVIDENCE FOR A ROLE OF NOREPINEPHRINE IN MODULATION OF GASTRIC MOTILITY. P.J. Hornby* D.H. Kuhn* W.P. Norman and R.A. Gillis* (SPON: S.N. Pradhan). Depts of Pharmacology and Anatomy, Georgetown University, Washington, DC.

Catecholamine (CA)-containing cell bodies in the dorsomedial medulla have been well documented; however, the precise organization of CA-containing terminals within nuclei of this region, and a functional role of this innervation remain to be elucidated. Use of antibodies to the CA-synthesizing enzymes and immunocytochemistry reveals striking differences in the distribution and density of fibers immunoreactive (-ir) to tyrosine hydroxylase (TH) and dopamine ß-hydroxylase (DBH). Specifically, a moderate density of TH-ir fibers and a very dense innervation of DBH-ir fibers and terminals is localized in the dorsal motor nucleus of the vagus (DMV). This DBH innervation is most dense in the region of the DMV (1-3mm rostral to the obex) where cell bodies are labelled with HRP-WGA after application of the tracer to the pyloric region of the stomach. Microinjection of norepinephrine (30-100ng) or clonidine (30-175ng) into the DMV results in a marked increase in gastric motility (preinjection minute motility index (MMI) is 0.70 ±0.37; post-injection MMI is 4.81±1.09), but no change in heart rate or blood pressure. The effect of microinjection of clonidine is abolished following i.v. yohimbine (1mg/kg). These data suggest that activation of noradrenergic neurons which synapse in the DMV leads to an increase in gastric motility via stimulation of α2-adrenoceptors. Supported by NIH grant AM 29975.

130.9

AREA POSTREMA: GASTRIC VAGAL INPUT FROM PROXIMAL STOMACH AND INTERACTIONS WITH NUCLEUS SOLITARIUS. W.D. Barber and C.S. Yuan*. Department of Anatomy, College of Medicine, University of Arizona, Tucson, Arizona, 85724.

This study was conducted on anesthetized cats to electrophysiologically evaluate the response of neurons in area postrema (AP) to electrical stimulation of 1) gastric vagal afferent fibers which serve the proximal stomach and 2) neuronal populations in nucleus solitarius (NTS) containing neurons which receive gastric vagal input. Small gastric branches of the ventral and dorsal vagal trunks were stimulated with single pulses, 0.3 msec duration, 300 uA at a frequency of 0.5 Hz. One hundred-sixty orthodromic unitary responses to gastric vagal stimulation were recorded bilaterally in AP. The majority of the units recorded in AP received input from gastric afferent fibers traveling in both the ventral and dorsal vagal trunks. The mean latency of the gastric vagally evoked response in AP was 272 ±56 msec. Orthodromic and antidromic unitary responses, evoked by electrical stimulation of NTS were recorded bilaterally in AP. These physiological data demonstrated 1) bilateral reciprocal connections between AP and localized areas of NTS which receive gastric vagal input and 2) unitary responses in AP to input from stimulation of vagal afferent fibers which serve the proximal stomach. These neuronal populations in NTS and AP, activated by gastric vagal afferents, may be functionally involved in the intake of food and/or nausea. (Supported by USPHS Grant DK 31804 and DK 35434).

130.10

ROLE OF THE RAT AREA POSTREMA IN SELECTIVE FACILITATION OF BAROREFLEXES BY VASOPRESSIN. J.D. Peuler*, G.L. Edwards, and A.K. Johnson (SPON: P.G. Schmid). CVC VAMC, Depts Int Med and Psych, U of IA, Iowa City, IA 52242.

Reflex inhibition of heart rate (HR) and renal sympathetic neural activity (RSNA) are facilitated in normal but not area postrema lesioned (APL) rabbits when arterial pressure (MAP) is increased by iv infusions of vasopressin (AVP) compared to phenylephrine (PE) (Circ Res 56:410-417, 1985). Reflex inhibition of HR but not lumbar SNA is facilitated in rats when MAP is increased by AVP vs PE (Vasopressin, Raven Press, NY, 1985, pp 11-18). To determine if effects of AVP on baroreflexes in rats are mediated through the area postrema, we monitored RSNA and HR in APL (n=8) and normal (sham-operated, n=8) rats under chloralose anesthesia during slow increases in MAP (<0.3 mmHg/sec; 3 min) induced iv by AVP (2-20 mU/kg/min) and by PE (1-10 µg/kg/min). Reflex inhibition of RSNA (-%Δ/ΔmmHg) was similar during AVP vs PE in normal (-3.8±0.6 vs -3.4±0.3) and APL rats (-4.4±0.6 vs -3.9±0.4). However, reflex inhibition of HR (-Δbpm/ΔmmHg) was facilitated when MAP was increased by AVP vs PE in normal rats (-2.9±0.5 vs -1.6±0.1, p<0.05) and this facilitation was absent in APL rats (-2.2±0.3 vs -2.2±0.4). Thus, unlike the rabbit, facilitation of baroreflexes by AVP in the rat is limited to HR but not RSNA. However, as in the rabbit, facilitation of reflex bradycardia by AVP is mediated through the area postrema.

130.11

ENHANCED EXCRETION OF SODIUM BY AREA POSTREMA-LESIONED RATS AFTER INTRAGASTRIC SALINE LOADS. G.L. Edwards, T.G. Beltz* and A.K. Johnson. Depts. of Psychology, Pharmacology and the Cardiovascular Center. Univ. of Iowa, Iowa City, IA 52242.

Previous studies indicate that rats with lesions of the area postrema and the adjacent nucleus of the solitary tract (AP-lesions) exhibit a marked increase in intake of concentrated saline solutions ad libitum (Brain Res. 211: 355, 1981). In this study we have examined the ability of rats with AP-lesions to excrete loads of concentrated saline solution. Rats received either sham lesions (n=14) or AP-Lesions (n=13) and were allowed to recover for at least 1 month. At this time AP-lesioned rats consumed significantly greater amounts of 3% saline solution ad libitum than sham operated rats (7.1 ± 1.4 ml/3h vs .4 ± .2 ml/3h). AP-lesioned and sham rats were gavaged with 10 ml 3% saline and urinary volume, sodium, potassium and osmolality were monitored for 6 h. AP-lesioned rats excreted significantly greater quantities of sodium during the first 2 h after the load than did sham operated rats (2.49 ± .22 meq vs 1.69 ± .12 meq). The enhanced excretion was accompanied by an increased urine volume in the AP-lesioned group during the first 2h after the load (9.2 ± .9 ml vs 6.5 ± .5 ml). These data suggest that AP-lesioned rats can excrete sodium loads more efficiently than control rats. Thus, it is possible that the enhanced sodium excretion by AP-lesioned rats contributes to the increased intake of concentrated sodium solutions. (Supported by NIH HL14388)

130.12

INTRACELLULAR ANALYSIS OF EXCITATORY NTS INPUTS TO THE NUCLEUS PARABRACHIALIS. A.R.Granata,S.Afshapour and S.T.Kitai. Dept. of Anat. and Neurobiol.,The Univ. of Tenn., Memphis. Memphis TN. 38163.

The nucleus parabrachialis (PBN) is interconnected with the mediocaudal nucleus tractus solitarius (NTS) . Ascending NTS projections to PBN may relay visceral and cardiovascular inputs to the forebrain. In addition, these autonomic signals can be integrated at the level of PBN. Therefore, it was of interest to study the synaptic actions of NTS inputs to the PBN using an intracellular recording technique. Rats were anesthetized with urethane plus ketamine. Excitatory postsynaptic potentials (EPSP) were evoked by ipsilateral NTS stimulation on 38 neurons. EPSP were considered monosynaptic because their latencies did not change with either variations in stimulus intensity or high frequency repetitive stimulation . A short EPSP onset latency ($\bar{X}$: 3.7 ms; S.D.: 0.4) and a long EPSP onset latency ($\bar{X}$: 6.1 ms; S.D.: 0.6) were observed. Six neurons, were antidromically(mean axonal conduction velocity : 4.6 m/sec, S.D.: 0.5) as well as orthodromically (EPSP) stimulated by NTS, indicating that PBN is reciprocally connected with the NTS. EPSPs were evoked by either central amygdaloid nucleus (Ac) or lateral hypothalamic area (HL) stimulation,in some neurons which were excited by NTS stimulation,denoting input convergence in a single neuron between the NTS and the HL or Ac The recorded neurons labeled with HRP were located in the ventrolateral and Kölliker-Fuse subdivisions of PBN. It is concluded, that PBN is monosynaptically excited by two different projections originated in the NTS. Some of these PBN cells in turn, project to the dorsal medulla. (Supported by AMER. HEART ASSOC. GRANT #861294 to A.R.G. and NIH GRANT NS20702 to S.T.K.).

131.1

CHARACTERIZATION OF PREPROENKEPHALIN DNA-BINDING PROTEINS
E.F. LaGamma, G. Weisenger, and N. Goldstein. Depts. of Peds. and Neurobiology, SUNY @ Stony Brook, NY 11794-8111

Recent work has shown that several receptor-linked, signal transduction, phosphoprotein mechanisms may act in concert to regulate the transcription of preproenkephalin mRNA in the rat adrenal medulla (La Gamma, Br. Res. 441:292, 1988). To identify relevant gene regulatory proteins, we are exploiting gel retardation assays to initially characterize this system. We have shown binding of medullary (cytosolic and nuclear proteins) to a 299 bp 32P labelled DNA probe representing a key portion of the 5' regulatory region of the preproenkephalin gene. All binding is reduced by increased K+ concentration (10mM). Elevated Na+ concentration (50 mM) appears to enhance binding of certain bands in the absence of K+. A K+ enhancement effect is also evident for some bands in low Na+. Moreover, retained bands are eliminated by proteinase K and are not competed away by nonspecific DNA (poly dA/dT or poly dI/dC). The 299 bp fragment contains a cAMP and phorbol ester conserved site and other regions having over 80% homology with SV40, GCN4, SP1, and AP1 protein binding and regulatory sequences. If binding to these sequences is confirmed by footprint analysis and transfection studies, the evolutionary significance of the apparent presence of shared gene regulatory mechanisms in these diverse systems would be intriguing. Supported by the National Science Foundaiton and Dysautonomia Foundation.

131.2

MODULATION OF HUMAN PRO-ENKEPHALIN GENE EXPRESSION: INTERACTIONS OF RECEPTOR SYSTEMS.
B. C. Beaubien* and J. Douglass* (SPON: E. Hanneman). Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, Oregon, 97201

The experiments reported in this communication provide evidence that induction of gene expression and stimulation of secretion are differentially regulated in neuroendocrine systems. In addition, the ability to inhibit induction of the cAMP-responsive element (CRE) in the human pro-enkephalin gene by G_i-associated receptors is shown to be dependent on cell type; not all receptors which inhibit cAMP synthesis reduce the increase in transcription observed in response to G_s-associated events.

The 5' sequence of the human pro-enkephalin gene has been studied with reference to cAMP responsivity and found to contain a region sensitive to cAMP induction. Attaching this nucleotide sequence upstream from a reporter gene, chloramphenicol acetyl transferase (CAT), allows cAMP-mediated events to be monitored. This system was used to investigate the association between stimulation of secretion and gene expression. Interestingly, in a comparison of two types of cells bearing a similar complement of receptors, the GH_4C_1 and AtT-20 lines, the sensitivity of cAMP-stimulated transcription to inhibition by somatostatin (SST) varied. AtT-20's exhibited the expected inhibition of CRF-stimulated transcription with SST, while GH_4C_1's showed enhanced transcription in cells exposed to medium containing vasoactive intestinal polypeptide (VIP) and SST, over those exposed to VIP alone.

This result is compared to other stimulatory modalities; in particular this divergence from the more simple secretion model is related to the role of Ca^{2+} in the control of cAMP catabolism.

131.3

ISOLATION AND STRUCTURAL CHARACTERIZATION OF THE BOVINE TYROSINE HYDROXYLASE GENE
S.R. D'MELLO*, L.M. TURZAI*, A.E. GIOIO*, and B.B. KAPLAN Molecular Neurobiology Program, Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Investigations into the structure and regulation of expression of the genes involved in catecholamine biosynthesis has led to the isolation of the entire bovine tyrosine hydroxylase (TH) gene. A bovine TH cDNA probe was used to screen a Charon 28 genomic library. Screening of approximately one million recombinant phage resulted in the identification of one positively hybridizing clone. Preliminary results using restriction endonuclease mapping, Southern blot and sequence analysis reveal that the bovine gene contains 13 exons spanning approximately 8kb of genomic DNA. Determination of the transcription initiation site indicates that the TH gene has a 5' untranslated region of 27 bp. A TATA-box sequence is located between positions -29 and -24 from the transcription initiation site and a cyclic AMP regulatory element between -45 and -38. Genomic Southern blot analysis reveals only a small number of positively hybridizing bands with each restriction enzyme suggesting that TH is a single-copy gene in the cow. Comparison of the bovine gene with the TH genes from rat and human reveals striking structural and sequence similarities.

131.4

COMMON TRANS-ACTING FACTORS ARE INVOLVED IN REGULATION OF NEUROTRANSMITTER GENE EXPRESSION BY CYCLIC AMP.
S. E. Hyman, M. Comb* and H. M. Goodman*. Department of Molecular Biology, Massachusetts General Hospital., Boston, MA 02114

Gene expression can be regulated trans-synaptically through the actions of second messengers. Transcription of genes encoding proenkephalin (ENK), vasoactive intestinal polypeptide (VIP), somatostatin (SST), and tyrosine hydroxylase (TH) can be stimulated by cAMP acting through inducible enhancer-like DNA elements. With the discovery that neurons contain multiple transmitters it has become important to understand the mechanisms of coregulation of transmitter synthesis. We have compared the structure and function of cAMP-inducible elements within the ENK, VIP, SST, and TH genes, and have found that they all utilize at least one common trans-acting factor that is required for cAMP induction, as shown by competition for this factor in vivo (cotransfection competition) and in vitro (DNAse I footprint competition). This factor may be identical to CREB (Montminy, M. R. Nature 328:175, 1987). A second factor, AP2, (Mitchell, P. J. Cell 50:847, 1987) binds to the ENK and TH genes and is required for maximal stimulation of ENK transcription by cAMP. SST has a similar binding site. This analysis suggests a mechanism of transcriptional coregulation of cAMP-responsive, neuronally expressed genes, based on competition for binding of limiting trans-acting factors.

131.5

EXPRESSION AND CHROMOSOMAL LOCATION OF THE HUMAN LIVER MONOAMINE OXIDASE A AND B.
N.C. Lan*, C. Heinzmann*, I. Klisak*, E. Lai*, R. Sparks* and J. Shih (SPON: J. Rho). Sch. of Pharm., Univ. of South. Calif., L.A., CA 90033, Dep. of Med. UCLA, L.A., CA 90024, Div. of Biol., Calif. Instit. of Tech., Pasa., CA 91125.

Monoamine oxidase (MAO) is the central enzyme responsible for the degradation of neurotransmitters. Based on substrate and inhibitor specificities, two types of MAO, A and B, have been described. MAO A and MAO B from several tissues are shown to consist of two subunits with mol. wts. of 63,000 and 60,000 Kd, respectively.

Using oligonucleotide probes designed from the protein sequences, we have recently isolated two full length cDNA clones encoding human liver MAO A and MAO B. In this report, we have cloned these cDNA inserts into a mammalian expression vector containing the SV40 origin of replication and transfected them independently into COS cells. The enzymatic activities and inhibitor specificities of the expressed proteins obtained from these transfected cells are similar to that of the native proteins, suggesting that both enzymes consist of two identical subunits.

Using somatic cell hybrids, in situ hybridization, and field-inversion gel electrophoresis, we assigned the MAO A and B genes to be located on the x chromosome at xp11.23 and they appear to be closely linked. (Supported by Grant No. MH 39085 and MH 37020)

131.6

STRUCTURAL FEATURES OF HUMAN MONOAMINE OXIDASE A ELUCIDATED FROM PEPTIDE AND cDNA SEQUENCES.
Y-P. P. Hsu*, W.Weyler*, S. Chen, K.B. Sims*, W.B. Rinehart*, M. Utterback*, J.F. Powell* and X.O.Breakefield: E.K. Shriver Ctr., MA; VA Med. Ctr., CA; Beckman Res. Inst., CA; Clinical Res. Ctr., UK; Mass. General Hospital, MA; Harvard Med. Sch., MA

Monoamine oxidase (monoamine:O_2 oxidoreductase; EC 1.4.3.4; MAO) is a crucial enzyme in the metabolism of neurotransmitters such as dopamine, norepinephrine and serotonin. The primary structure for one form of the enzyme, MAO-A, has been determined through cloning and sequencing of a full length 2.0 kb cDNA from human liver. It codes for a 60 kd protein whose deduced sequence is highly homologous to tryptic peptides of MAO-A purified from human placenta. Two MAO-A messages from human placenta and one MAO-B message from human lymphocytes are recognized by this cDNA. Structural features which may be involved in binding of the FAD cofactor and targeting of the enzyme to outer mitochondrial membranes will be discussed.

131.7

STRUCTURE AND EXPRESSION OF A SEROTONIN 5-HT$_{1C}$ RECEPTOR GENE AND ITS cDNA B.J. Hoffman[1], M.H.T. Nguyen*[3], M.H.T. Le*[3], P.R. Hartig[1], H.A. Lester[2], N. Davidson*[2] and H. Lubbert*[3]. Dept. of Env. Health Sci., [1]Johns Hopkins University, Baltimore, MD 21205, [2]Divs. of Biology and Chemistry, Caltech, Pasadena, CA 91125 and [3]Preclinical Research, Sandoz AG, Ch-4002 Basel.

We have recently developed a strategy for the cloning of neurotransmitter and ion-channel genes using electrophysiological assays of mRNA-injected Xenopus oocytes (Lübbert et al. PNAS 84, 4332 (1987)). Using this strategy, we have isolated cDNA clones for the serotonin 5-HT$_{1C}$ receptor from a mouse choroid plexus papilloma. The receptor expressed in oocytes injected with hybrid-selected RNA is fully functional and displays appropriate pharmacology, indicating that a single 5-kb RNA encodes the 5-HT$_{1C}$ receptor. cDNA clones were used to identify mouse genomic clones. This serotonin receptor is homologous to muscarinic and adrenergic receptors, but has an additional hydrophobic region at the amino-terminus which may serve as a signal peptide. Like bovine rhodopsin, the gene of the serotonin receptor contains several introns in the protein coding region while those of adrenergic and muscarinic receptors are unspliced. In addition, we will present evidence for a loss of splicing specificity in choroid plexus tumors.

131.8

GENES INVOLVED IN MPTP NEUROTOXICITY. M.M.S. Lo, M.J. Kadan, S.G. Carlson*, R.C. Douglas*. NIDA, ARC, Baltimore, MD 21224.

Pheochromocytoma (PC12) cells were mutated by infection with a recombinant retrovirus, and selected with 500μM MPP$^+$ (an active metabolite of MPTP). Many different colonies surviving 2 weeks of treatment on MPP+ were isolated. Analysis of the proviral positions in 26 different mutants showed that 5 different loci in the PC12 genome suffered repeated viral mutation. A particular locus was found to contain integrated virus in four different MPP$^+$ selected mutants. This shows that only a few genes are involved in MPP$^+$ neurotoxicity. The provirus and flanking genomic DNA downstream to the virus were cloned from one mutant (M39). At least two distinct messages were detected in normal PC12 cells when the rescued genomic DNA was used as a probe. Three distinct areas in the rescued genomic DNA contained coding sequences. These coding sequences were used to isolate cDNA clones from a normal PC12 cDNA library. Two genes were identified and cloned in this manner. They were found to be unrelated. One gene was found to be induced by more than 20 fold in NGF treated PC12 cells compared to normal PC12 cells. The other gene was unstimulated. These two genes are specifically expressed in PC12 cells. The functional relevance to MPP+ neurotoxicity will be determined by expressing these cloned genes in MPP$^+$ resistant cells Support in part by a grant from Johns Hopkins C.A.A.T.

131.9

ISOLATION OF CDNAS ENCODING HUMAN ACETYLCHOLINESTERASE. C. A. Prody* and H. Soreq, Dept. of Biochemistry, Hebrew University, Jerusalem, Israel.

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) differ in their substrate specificity and sensitivity to different inhibitors. To study the structure-function relationships between human cholinesterases, oligodeoxynucleotide probes were synthesized according to amino acid sequences in evolutionarily conserved and divergent peptides from electric fish AChE compared to human serum BuChE. The screening of a cDNA library from human brain basal ganglia with one of these probes resulted in the isolation of a clone encoding human AChE. This was then used to screen cDNA libraries from human fetal brain, liver and muscle, resulting in the isolation of several considerably longer clones. The peptide sequence deduced from the nucleotide sequence displayed an overall similarity of 51% to human BuChE, 56% to Torpedo AChE, and 31% to Drosophila AChE. In mRNA blots from fetal brain, muscle and liver, these cDNAs hybridized with two bands of 2.5 and 4.5 kb, probably corresponding to the two types of cDNAs that were isolated. Southern blot analysis demonstrated that AChE is encoded by distinct gene(s) from those encoding BuChE. These findings imply that variations in the primary sequence of cholinesterase genes and cDNAs may be implicated in differences in the substrate specificity and sensitivity to inhibitors of their resultant enzyme products.

131.10

IDENTIFICATION OF AN ENHANCER IN THE CHICKEN SKELETAL MUSCLE RECEPTOR ALPHA SUBUNIT GENE. **Y. Wang*#, H.-P. Xu*#, X.-M. Wang*#, M. Ballivet*&, and J. Schmidt#. #Dept. of Biochemistry, State University of New York at Stony Brook, Stony Brook NY 11794, and &Dept. of Biochemistry, Sciences II, University of Geneva, 1211 Geneva, Switzerland.**

The function of the upstream flanking region of the chick muscle acetylcholine receptor alpha subunit gene was investigated. A sequence extending from -1919 to +55 (with respect to the start site) and Bal31-generated deletions thereof were joined to the rabbit beta globin coding region, and the resulting constructs transfected into C2C12 mouse myogenic cells before and after differentiation, and into 3T3 fibroblasts. The reporter gene was transcribed only in C2 myotubes and only when the alpha-derived upstream region was at least 116 nucleotides long. The -116 to -41 segment, in combination with the SV40 early promoter, was functional regardless of orientation even when inserted 1.3 kb downstream of the cap site. Additional tests revealed that a 36-nucleotide fragment (-116 through -81), combined with either the alpha-subunit or the SV40 promoter, was sufficient for tissue- and stage-specific expression. This element retains the ability to stimulate correct transcription upon transposition and inversion, and thus fulfills the criteria of an enhancer. Considering the strength of this enhancer we propose that it is involved in the pronounced transcriptional activation of the alpha subunit gene during differentiation and, possibly, after denervation of mature muscle.

131.11

MOLECULAR MECHANISMS OF NGF GENE TRANSCRIPTION **M. Zheng and G. Heinrich. Lab. Molecular Endocrinology, Dept. Medicine, Howard Hughes Medical Institute, Mass. Gen. Hosp. and Harvard Med. School, Boston, MA 02114.**

NGF production is regulated at the level of transcription and mRNA stability. To study NGF gene transcription, the mouse NGF gene promoter region was analyzed by 5' deletions. In transient expression assays in L929 cells the segment from -36 to -76 was associated with increased, and the segment from -170 to -250 with decreased basal transcription, suggesting opposing regulatory elements are present. In gel retardation assays, a synthetic double-stranded oligonucleotide representing the segment -91 to -139 bound a nuclear factor with high affinity and specificity. Southwestern blots revealed bands at 30 kD and 31 kD that specifically bound the same oligonucleotide. For several other segments from +8 to -250 only low affinity binding patterns were found including in regions that contain the putative opposing regulatory elements. DNase-1 footprinting assays are being carried out in the 5' flanking region to map both high and low-affinity binding sites, and experiments are under way to measure the effects of protein binding on the secondary structure of specific segments of the 5' flanking region. These data indicate that basal NGF gene transcription involves multiple DNA/protein interactions and is functionally complex. Stimulation of NGF gene transcription by regulators such Il-1 as well as developmental and cell-specific regulation may be mediated through related mechanisms.

131.12

BIND AND CLONE: LIGAND-AUTORADIOGRAPHIC RECEPTOR EXPRESSION SCREENING. G.R. Uhl. Gene Nsci. Unit, ARC/NIDA & Depts. of Neurol.& Nsci. Johns Hopkins Med. Sch., P.O. Box 5180, Baltimore, MD 21224.

Study of neurotransmitter receptor genes can facilitate understanding of these important molecules and their regulation, yet classical purification and cloning approaches can be difficult. The high affinity, specificity, sensitivity and spatial localization afforded by receptor autoradiography are desirable properties for identifying receptor binding sites in eukaryotic expression libraries and could allow direct selection of cDNA or gene sequences encoding the receptor.

We have employed a polyester cloth replica-filter technique that: a) Samples cell membranes from eukaryotic cell colonies growing on culture dishes, b) Provides low nonspecific binding with ^{125}I-labeled ligands c) Retains membrane fragments and DNA through receptor autoradiographic incubations and d) Can be aligned with the master plates to recover DNA and/or cells from the regions of the culture plate where receptor expression appears.

We have used this system to screen: a) L-cells transfected with genomic DNA, and b) COS cells transiently expressing cDNA libraries in the eukaryotic expression vector pCDM8. Each of these approaches has yielded apparently-positive DNA-mediated expression of high-affinity ^{125}I-neurotensin binding sites in previously-nonexpressing cells.

132.1

MARKERS FOR THE EARLY SYMPATHOADRENAL LINEAGE. J. Carnahan* and P. H. Patterson (SPON: E. A. DeYoe). Biol. Div., 216-76, Calif. Inst. of Tech., Pasadena, CA 91125.

Adrenal chromaffin cells, sympathetic neurons and small intensely fluorescent (SIF) cells are all derived from the neural crest, but they each have distinctive phenotypes. In order to isolate and study the precursors for this lineage, it would be advantageous to have highly specific monoclonal antibodies (mAbs) directed against their surfaces. Since there is evidence that the precursors share properties with SIF and chromaffin cells, we used the immunosuppression method to obtain such mAbs. Mice were tolerized to neurons by injection of membranes from adult rat sympathetic ganglia and cyclophosphamide and then injected with membranes from neonatal adrenal medullae. Six mAbs were isolated that bind to postnatal chromaffin cells, but not to SIF cells or neurons. These mAbs bind to at least three independent, cell surface epitopes. Most importantly, all of the mAbs stain most, if not all, tyrosine hydroxylase-positive cells in early sympathetic ganglia. Staining is first apparent with the end of crest cell migration, disappears from ganglia by E15, but is maintained in adrenal chromaffin cells. Thus, these mAbs define a putative sympathoadrenal precursor that shares several traits with chromaffin cells.

132.2

SOME PERIPHERAL NEURONS AND PANCREATIC ISLET CELLS OF MAMMALS MAY ARISE FROM A COMMON SOURCE. G. Teitelman. Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Mammalian embryos contain cells that express several neuronal antigens but do not originate in the ectoderm. One of these cell types is pancreatic islet cells which stem from the endoderm. The question is raised as to whether endoderm and/or mesoderm, isolated prior to the arrival of migrating neural crest cells, can give rise to cells that express not only neuronal antigens but also a neuronal morphology. The timing of neural crest cell migration was determined using immunohistochemical techniques in a line of transgenic mice harboring a hybrid insulin gene (Hanahan, Nature 1985). In transgenic mouse embryos, neural crest cells express the insulin gene construct during migration. Migrating neural crest cells are first seen at E10. Therefore, at day 9 of development (E9; 4-10 somites) peripheral tissues lacked stained neural crest cells. To test the potentiality of the endoderm (and/or mesoderm), the neural tube was removed from mouse embryos at E9 (smaller than 10 somites) and the remaining tissues were examined after several days in culture. The embryonic explants contained endocrine cells that expressed pancreatic hormones, tyrosine hydroxylase (TH) and Neuronal Specific Enolase (NSE), two neuronal specific enzymes. The explants also contained cells that displayed a characteristic neuronal morphology and also stained with antisera against TH and NSE. These findings indicate that the endoderm and/or mesoderm contains cells able to express typical neuronal traits in culture and that these cells may differentiate into endocrine cells or neurons.

132.3

PROLIFERATION AND DIFFERENTIATION OF EMBRYONIC CHICK SYMPATHETIC NEURONS: EFFECTS OF CILIARY NEUROTROPHIC FACTOR (CNTF). H. Rohrer*, U. Ernsberger* and M. Sendtner* (SPON: Y.-A. Barde). Max-Planck Institute for Psychiatry, D-8033 Martinsried, FRG

Chick paravertebral sympathetic ganglia contain at early developmental stages (embryonic day 7) a cell population which divides in culture while expressing various neuronal properties. In the attempt to identify factors that control neuronal proliferation we found that CNTF specifically interferes with the proliferation of those cells expressing neuronal markers.

In addition, CNTF affects the differentiation of sympathetic ganglion cells by inducing the expression of VIP-immunoreactivity (VIP-IR) and by increasing the activity of choline acetyltransferase, which are markers for cholinergic sympathetic neurons. Whereas tyrosine hydroxylase immunoreactivity and VIP-IR was expressed by 85±5% and 0% of the cells respectively after 1 day in culture, after 4 days 50-60% of the cells expressed VIP-IR in the presence of CNTF. These results indicate that VIP-IR is induced in cells which initially express tyrosine hydroxylase immunoreactivity.

These findings suggest a role for CNTF as a factor affecting the proliferation and differentiation of developing sympathetic neurons.

132.4

COMMON PROGENITOR CELL FOR SENSORY AND AUTONOMIC NEURONS IN THE QUAIL NEURAL CREST. Maya Sieber-Blum. Dept. of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, WI 53226.

In the vertebrate embryo the neural crest gives rise to all autonomic, all spinal sensory, and some cranial sensory neurons. Due to a lack of specific markers for sensory neuroblasts, little is known about the earliest stages of sensory and autonomic neuron development. In particular, it is not known if the neural crest contains cells that can generate both sensory and autonomic neurons, or if the two lineages segregate before the onset of neural crest migration. To answer this question, we first established that the stage-specific embryonic antigen 1 (SSEA-1) is a specific marker for embryonic sensory neurons in the quail. Three populations of sensory neuroblasts were observed in primary neural crest explants, 1) a small population consisting of late-migrating, early differentiating cells with a very limited proliferative potential, 2) a larger population of late-migrating, early-differentiating cells with a high proliferative potential, and 3) a large population of early-migrating, late-differentiating cells with a high proliferative potential. In vitro clonal analyses showed that the progeny of individual neural crest cells can indeed give rise to both autonomic (SSEA-1$^-$/dopamine-β-hydroxylase$^+$) and sensory (SSEA-1$^+$/dopamine-β-hydroxylase$^-$) neuroblasts. We observed two types of precursor cells. One type also gave rise to pigment cells in addition to autonomic and sensory neuroblasts, the other one did not generate melanocytes.

Our data are compatible with the view that the neural crest not only contains sensory neurons that segregate early in development, but also at least two types of progenitor cells common to the sensory and autonomic neuronal lineages. (Supported by USPHS grant HD21423)

132.5

CHANGES IN PROTEIN KINASE C ACTIVITIES ARE CORRELATED WITH CHANGES IN THE MORPHOGENETIC AND DIFFERENTIATIVE PROPERTIES OF NEURAL CREST DERIVED CELLS OF EARLY AVIAN EMBRYOS. G. Ciment, L. Hess* and T. Chamberlin*. Dept. Cell Biology & Anatomy and the Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

In previous work, we found that the phorbol ester drug 12-O-tetradecanoyl phorbol acetate (TPA) has major effects on the developmental properties of early neural crest-derived cells. First, TPA-treatment of cultured dorsal root ganglion (DRG) cells increases the extent of migration of these crest-derived cells upon subsequent grafting into host embryos. And second, TPA reverses the developmental restriction of melanogenesis that occurs early in peripheral nervous system development, causing Schwann cell precursors to undergo a metaplastic transformation into melanocytes.

In this study, we examine whether these effects of TPA may be mediated by changes in endogenous levels of protein kinase C (PKC) activities. We report that low levels of PKC activities correlate well both with changes in migratory behavior, as well as with the adventitious pigmentation of DRG cells. This correlation occurs, moreover, both during normal development, as well as with TPA treatment in culture. These results suggest that regulation of endogenous levels of PKC may play a role in controlling both the migration of neural crest cells, as well as their developmental fate.

132.6

IMMORTALIZATION OF CENTRAL DOPAMINERGIC CELLS BY THE FUSION OF ROSTRAL MESENCEPHALIC TEGMENTAL (RMT) CELLS WITH NEUROBLASTOMA (N18TG2) CELLS. H.K. Choi*, A. Heller, P.C. Hoffmann, P.J. Kontur, L.A. Won, D.N. Hammond, B.H. Wainer, and A.P. Fox. Dept. of Pharmacological and Physiological Sciences, The Univ. of Chicago, Chicago, IL 60637.

Clonal hybrid cell lines expressing high levels of dopamine (DA) have been developed from RMT of mice employing somatic cell fusion techniques. RMT cells from embryonic day 14 mice were fused with N18TG2 cells deficient in hypoxanthine phosphoribosyl transferase (HPRT). After the fusion, hybrid cells were selected in a medium containing hypoxanthine/aminopterine/thymidine which selects against HPRT-deficient N18TG2 cells. These hybrid cells retain chromosomes from both RMT and N18TG2 cells. Among the hybrid lines obtained, one expressed a high level of DA (100 ng/mg protein), another a high level of 3,4-dihydroxyphenylalanine (DOPA) (120 ng/mg protein), and a third no detectable level of catecholamines (<0.1 ng/mg protein). The DA and DOPA line also express norepinephrine (30 ng/mg protein). The dopamine line has characteristics typical of central dopamine cells including high endogenous catecholamine levels, catecholamine specific histofluorescence, neurite formation with immunoreactivity to the neurofilament proteins (NFP), large voltage-sensitive sodium currents (2000 pA/cell peak current), and an ability to aggregate in rotation-mediated cell culture. Supported by NIMH MH28942 and GM07151.

132.7

CELLULAR SUBSETS IN NEURAL CREST CELL DIFFERENTIATION
G.D. Maxwell and M.E. Forbes*. Department of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032.

Previous work using cell sorting and cell culture showed that neural crest cells sorted as HNK-1+ give rise to catecholamine-positive (CA+) cells, melanocytes, and unpigmented cells, whereas cells sorted as HNK-1- give rise to melanocytes and unpigmented cells, but few, if any, CA+ cells (Forbes et al. 1987 Soc. for Neurosci. Abstr. 13: 182).

We have extended these results by analyzing the developmental behavior of subpopulations of the HNK-1+ neural crest cells. The differentiation of the brightest third of the HNK-1+ cells was compared to the remaining HNK-1+ cells. Numerous CA+ cells arose from both populations, along with melanocytes and unpigmented cells. In contrast, when the development of the brightest third of the HNK-1+ cells was compared with that of the remaining HNK-1+ cells grown together with the HNK-1- cells, many CA+ cells developed from the brightest third of HNK-1+ cells, but few CA+ cells differentiated from the less bright HNK-1+ cells grown together with the HNK-1- cells. Melanocytes and unpigmented cells were seen in both populations. These results indicate that the capability to become CA+ is not restricted to the brightest HNK-1+ cells, but the presence of HNK-1- cells may alter the ability of some HNK-1+ progenitors to become CA+ cells.

Supported by NIH grant NS 16115.

132.8

REGULATION OF NEUROTRANSMITTER PHENOTYPE IN DEVELOPING PRIMARY SENSORY NEURONS. D.M. Katz. Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

Factors regulating neurotransmitter phenotype in primary sensory neurons, including the role of target tissues, are poorly understood. In the petrosal ganglion (PG) of adult rats, 80-90% of tyrosine hydroxylase (TH)-containing sensory neurons innervate a single target, the carotid body, indicating that catecholaminergic (CA) phenotypic expression and the pattern of peripheral innervation are highly correlated. To define regulation of CA phenotypic development in the PG, immunocytochemistry and Western blots were used to analyze TH expression in vivo and in culture. TH is first detectable at very low levels in carotid body afferents on embryonic day (E) 16.5 in vivo. TH levels rise sharply at birth resulting in a 4-5 fold increase in the number of immunoreactive cells by postnatal day 1. To examine the role of neuron-target interactions in TH expression at E16.5, PG were explanted to culture on E14.5 and grown in the absence of the carotid body. TH-positive neurons were detected after two days in culture, suggesting that target tissues are not required for enzyme expression in vivo . Preliminary data indicate that many developing ganglion cells are capable of expressing TH; therefore, the pattern of target innervation may restrict expression of the CA phenotype to appropriate subpopulations of sensory neurons. Supported by AHA grants 84 983 and PL 653 (Ohio Affiliate).

132.9

NEURAL-CREST ORIGIN OF HEART CONDUCTION TISSUE MYOCYTES IS SUGGESTED BY THE EXPRESSION OF NEUROFILAMENT PROTEINS.
M.Vitadello, S.Schiaffino*and L.Gorza*. C.N.R.-I.F.C.N., Milano and Inst. of General Pathology, Padova, ITALY.

Neurofilaments(NF) are the neuron specific class of intermediate filaments and anti-NF antibodies have been widely utilized as markers of neuronal cells. We present evidence for the presence of the low(NF-L) and middle(NF-M) molecular weight subunits of NF in the rabbit heart conduction system myocytes. 2 anti-NF-M and 1 anti-NF-L mAbs decorate a population of cardiac myocytes exclusively localized in the sino-atrial and atrio-ventricular nodes and His bundle. On immunoblots cardiac proteins recognized by anti-NF mAbs have the same electrophoretic mobility as NF-M and NF-L in the sciatic nerve. Reactivity due to contaminating cardiac nerves was excluded. We performed in addition an immunohistochemical study on hearts of rabbit embryos at various stages of development. It confirms the specificity of anti-NF antibodies for heart conduction system cells. Moreover it reveals that a number of NF-reactive cells in the embryonic hearts are in close contiguity with clusters of labelled cells localized in the 3rd and 4th branchial arches. All of these NF-reactive cells also display immunoreactivity for HNK-1 mAb, a marker of neural-crest derived cells.

132.10

REGULATION OF TYROSINE HYDROXYLASE mRNA IN CATECHOLAMINERGIC CELLS OF THE EMBRYONIC RAT: ANALYSIS BY IN SITU HYBRIDIZATION. G. M. Jonakait, M. Rosenthal* and J. I. Morrell, Dept. of Biol. Sci. and Inst. for Animal Behavior, Rutgers Univ., Newark, N.J. 07102

In situ hybridization was used to examine the appearance of mRNA specific for tyrosine hydroxylase (TH) in the peripheral nervous system of the rat embryo, including sympathetic ganglia (SG) and transiently catecholaminergic cells of the embryonic intestine. mRNA was first detected in SG at E11.5, the age corresponding to the initial immunohistochemical detection of TH protein. By contrast, mRNA for TH was difficult to detect in embryonic intestines at E11.5, but was found instead in cells clustered at the dorsal boundaries of the pharynx and foregut. Cells expressing TH mRNA were infrequently found in embryonic intestines of any age, even when TH protein was apparent. Treatment of pregnant rats with reserpine on E11.5 dramatically increased the number of gut cells at E12.5 with detectable TH mRNA. However, after E13.5 TH mRNA was undetectable even in reserpine-treated guts. These data are consistent with the hypothesis that the microenvironment of the embryonic intestine affects gene expression directly to alter phenotype. Moreover, while reserpine administration briefly increases TH mRNA levels, the effect is short-lived, and does not alter phenotypic conversion. Supported by grants NS23687 and HD22983. GMJ and JIM are Johnson & Johnson Discovery Research Fellows.

132.11

CHANGES IN ULTRASTRUCTURE AND INTERMEDIATE FILAMENT PROTEINS IN RPE AND RETINAL GLIA FROM HUMAN AND RABBIT EPIRETINAL MEMBRANES AND VITREOUS CULTURES. S. A. Vinores, H. A. Sen*, S. F. Hackett* and P. A. Campochiaro*. Dept. Ophthalmology, Univ. of Virginia School of Medicine, Charlottesville, VA 22908.

Cellular epiretinal membranes are a significant cause of visual morbidity. The processes that lead to their development are not well understood. The participation of several cell types, including retinal pigment epithelium (RPE) and retinal glia, has been suggested based upon ultrastructural characteristics and light immunohistochemistry. We used electron immunocytochemistry to study cells in human epiretinal membranes obtained at surgery and compared them to RPE and retinal glia in animal and in vitro models of epiretinal membranes. Our data suggest that RPE cells which are normally keratin positive (K^+) may undergo transformation in epiretinal membranes to undifferentiated K^+ cells with large nuclei and sparse cytoplasm, polarized K^- cells with numerous microvilli, or K^-/fibroblast-like cells deep within the extracellular matrix. Some glial cells (GFA^+) appear to progress to cells which are morphologically indistinguishable from undifferentiated RPE cells. These data suggest that cells in epiretinal membranes may undergo alterations in both morphology and intermediate filament expression due in part to extracellular surroundings, and that neither alone is a reliable marker for cell identification.

132.12

MICROGLIAL AND MACROGLIAL MARKERS ARE SIMULTANEOUSLY EXPRESSED IN AN IMMORTALIZED CEREBELLAR MOUSE CELL CLONE.
B. Pessac and F. Alliot*. Centre de Biologie Cellulaire, C.N.R.S., 94205 Ivry-Sur-Seine Cedex, France.

The glia of the central nervous system (CNS) comprises the macroglia, i.e. astrocytes and oligodendrocytes, and the microglia, the origin of which remains unclear. We have recently shown that very rare single cells, dissociated from various brain regions of the adult and embryonic mice and cocultured on monolayers of an immortalized "velate protoplasmic like" cerebellar astroglial clone (Brain Res., 306:283, 1984), give rise to foci of around 200.000 cells each. The vast majority of these cells exhibit simultaneously a mixed macroglial and microglial phenotype (manuscript in preparation). These data led us to analyze the phenotype of a clonal cell line with an oligodendroglial like morphology that was spontaneously immortalized at the same time as the astroglial clones. All cells of this clone express simultanesouly microglial markers i.e. the Mac-1, F4/80 and Leu M3 epitopes, the Ia antigen and receptors for the acetylated form of the low density lipoprotein as well as macroglial markers, i.e., GFAP, GalC, MBP and PLP. Taken together these data show that the mouse CNS contains quiescent stem cells that can give rise in vitro to cells with a double microglial and macroglial phenotype.

132.13

RESPONSES OF NEONATAL AND ADULT GLIAL PROGENITORS TO NEURONAL CELL LINE-DERIVED MITOGENS. Samuel F. Hunter*, Matthias F. Seidel*, and Jane E. Bottenstein. Univ. of Texas Medical Branch, Dept. of Pharmacology-Toxicology, the Marine Biomedical Institute, and Dept. of Human Biological Chemistry & Genetics, Galveston, TX 77550.

We have reported earlier a trypsin-sensitive, mitogenic activity of conditioned medium (CM) derived from the B104 neuronal cell line (Bottenstein et al., J. Neurosci. Res. 20, in press) on A2B5$^+$ neonatal glial progenitors (GPs) in mixed glial cultures. We now find similar responses in both enriched neonatal GP and 30 day old-rat brain glial cultures. Enriched neonatal GPs were assessed by combined immunostaining and ^{3}H-thymidine autoradiography. After 1 day in tertiary culture, A2B5$^+$ cells had a maximal labelling index (LI) of 37% with 12 μg CM/ml, while control had 5%. At this time, control cultures contained <10% type 1 astrocytes and >75% A2B5$^+$. Continued survival of GPs required addition of 33% astrocyte CM, and after 3 days A2B5$^+$ cells showed a maximal LI of 68% with 16 μg B104 CM/ml, but only 7% with astrocyte CM alone. Enriched GPs respond similarly to those in mixed primary cultures containing 40% astrocytes, so the mitogenic action of CM factors is probably not mediated by the type 1 astrocyte. Mature rat brain glia were plated in 15% calf serum in DME and switched at 3 DIV to O3 medium with or without 8 μg B104 CM/ml. At 7 DIV, CM-treated A2B5$^+$ cells had LIs of 41% versus 14% control, and for galactocerebroside$^+$ (GalC$^+$) cells 3% versus 0.7%. At 10 DIV, A2B5$^+$ and GalC$^+$ cells were increased 8- and 1.7-fold, respectively. (NIH Grant NS20375)

SENSORY SYSTEMS: AUDITORY SYSTEMS I

133.1

PRODUCTION OF ENKEPHALINS BY OLIVOCOCHLEAR NEURONS DEMONSTRATED BY IN SITU mRNA HYBRIDIZATION. A.F. Ryan[1], D.M. Simmons[2,3], A.G. Watts[2], and L.W. Swanson[2,3]. [1]Depts. of Surgery/Otolaryngology and Neurosciences, UCSD School of Medicine and V.A. Medical Center, [2]Neural Systems Laboratory, Salk Institute, and [3]Howard Hughes Medical Institute, La Jolla, CA 92093.

Gerbils were injected in one cochlea with 2% fluorogold and in the other with 2% fast blue. After 24 hrs. their brainstems were prepared for in situ RNA hybridization. Alternate slides were treated with proteinase-K or Triton-X100 to increase hybridization in the fixed tissue. Sections were hybridized with a single stranded RNA complimentary to preproenkephalin mRNA, in which all uridine groups were labeled with ^{35}S. Sections were dipped in liquid emulsion for autoradiographic and fluorescent microscopic analysis.

Fluorogold and fast blue labeled cells were observed in the superior olivary complex and vestibular nuclei (consistent with the location of cochlear and vestibular efferents) of Triton-X-treated sections. Proteinase K digestion reduced fast blue labeling, and had less effect on fluorogold labeling.

Cells positively labeled for preproenkephalin were noted in the ventral nucleus of the trapezoid body, and between the medial superior olivary nucleus and the medial nucleus of the trapezoid body, locations consistent with the medial olivocochlear efferent system. Hybridization was not observed in the lateral superior olivary nucleus, the location of the lateral efferent system in this species. Some retrogradely labeled medial olivocochlear neurons did not display hybridization, indicating that the production of enkephalins is not a universal feature of medial cochlear efferents. Preproenkephalin hybridization colocalized with retrograde labeling in all vestibular efferents examined.

Supported by grant NS14945 from the NINCDS, and the Research Service of the VA. Preproenkephalin template DNA was provided by Dr. S.L. Sabol.

133.2

SYNAPSES FROM OLIVOCOCHLEAR COLLATERALS IN THE MOUSE COCHLEAR NUCLEUS. T.E. Benson[1] and M.C. Brown[2]. Departments of Anatomy and Cellular Biology[1], Cellular and Molecular Physiology[2], and Otolaryngology, Harvard Medical School, Boston, MA 02115; and Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA 02114.

The cochlea receives efferent innervation from cells of the superior olivary complex. Those cells of the medial olivocochlear (OC) group also project axon collaterals to granule-cell regions of the ventral cochlear nucleus (VCN). OC collaterals are thought to form both small boutons and mossy terminals. The latter together with granule and/or Golgi cell dendrites comprise neural glomeruli. In order to study synaptic relationships of OC collaterals in the VCN, we labeled OC axons by HRP injections into the spiral ganglion. Labeled collaterals were characterized by light microscopy (LM) and serial-section electron microscopy (EM).

By LM, OC terminal arbors exhibited extensive branching in and abutting granule-cell regions. Axosomatic contact was rare. Arbors had numerous angular *en passant* swellings and fewer terminal swellings. The morphology of labeled collaterals suggested that much of this specialized arbor was unmyelinated whereas parent axons were myelinated; this was confirmed for several collaterals and parent axons by EM.

The distal portion of the terminal arbor of one labeled collateral was analyzed by extensive EM. This arbor terminated in the posterior division of the VCN on the border of the granule-cell lamina Labeled terminals made asymmetric (thus possibly excitatory) synapses, some having postsynaptic dense bodies. Synapses were found with both tapering and varicose dendrites, suggesting that OC collaterals contact different cell types. Multiple OC synapses were found on a tapering dendrite which issued from a moderately sized (9x20 µm) neuron. The neuron had characteristics of multipolar cells. Although unlabeled mossy terminals were observed, these have not yet been observed coming from the labeled collateral. It is possible that mossy terminals are formed on more proximal portions of the arbor, such as at nodes of Ranvier. Our results indicate that OC collaterals are associated with granule-cell regions, but their postsynaptic targets may be diverse.

[Supported by NIH grants NS13126, NS23508, and NSF grant BNS-20833]

133.3

CENTRAL EFFECTS OF COCHLEAR HAIR CELL LOSS IN DEAFNESS MICE. D.B. Webster. Kresge Hearing Research Laboratory, Department of Otorhinolaryngology, LSU Medical Center, New Orleans, LA 70112.

In deafness mice, neither VIII nerve action potentials nor cochlear microphonics ever develop and hair cells start degenerating at 12 days of age. Using light microscopy, serial sections of the cochleas and brainstems were quantitatively studied in 11 groups of deafness mice ranging from 1 to 460 days of age. Through 12 days of age deafness mice have the same number of spiral ganglion neurons as in hearing CBA/J mice. After this there is a gradual loss of spiral ganglion neurons amounting to a 50% decrease by 460 days of age. The number of ventral cochlear nucleus neurons is normal through 9 days of age but from then through 460 days of age the number remains constant at 20% fewer than in CBA/J mice. Dorsal cochlear nucleus volume growth in deafness mice is indistinguishable from that in normal hearing CBA/J mice. However ventral cochlear nucleus volume stops increasing at 24 days of age and is more than 25% smaller than in CBA/J mice by 90 days of age. Neuronal soma areas in the ventral cochlear nucleus as well as in the superior olivary complex never attain the sizes found in CBA/J mice being on the average 20% smaller, These data are consistent with the hypothesis that central anatomical changes with peripheral deafness are caused by a lack of neural activity, spontaneous and/or evoked.

Supported by NIH grant NS-19238 and Kam's Fund.

133.4

EFFECTS OF AMINO ACID ANTAGONISTS ON AUDITORY NERVE SYNAPTIC POTENTIALS P. A. Starr * and W. F. Sewell Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Department of Otolaryngology, and the Program in Neuroscience, Harvard Medical School, Boston, MA 02114.

The identity of the neurotransmitter released by hair cells of the inner ear is unknown. However, it has been reported that transmission at the hair cell-afferent neuron synapse can be blocked by glutamate antagonists (Annoni *et al., J. Neuroscience* 4:2106, 1984). We have studied the effects of glutamate antagonists on sound-evoked synaptic potentials and on spontaneous miniature synaptic potentials (smsp's) in the goldfish auditory nerve. Because smsp's appear to correspond to the release of a single quantum of transmitter (Furukawa *et al., J. Physiol.* 276:211, 1978), we can infer whether the site of action of the drug is pre- or postsynaptic by comparing the effect of the drug on the amplitude of smsp's to the effect on the amplitude of sound-evoked synaptic potentials.

Synaptic potentials in the auditory (saccular) nerve of ketamine-anesthetized goldfish (10-16 cm long) were monitored with glass micropipettes. The hair cell-afferent nerve synapses were continuously superfused with a balanced salt solution, or with the same solution containing a drug to be tested. The sound stimuli (40 msec tone bursts) were delivered via an earphone into a closed cavity surrounding the fish's body posterior to the gills.

Both a broad-spectrum glutamate antagonist, gamma-DGG (gamma-d-glutamylglycine, 1.0 mM), and a specific NMDA (N-methyl-d-aspartate) receptor antagonist, APV (5-aminophosphonovaleric acid, 2.0 mM), completely and reversibly blocked sound-evoked synaptic potentials. However, the amplitudes of smsp's were only partially blocked at these concentrations. The antagonists had no noticeable effect on the microphonic potential, showing that they did not grossly alter current flow through hair cells. The fact that glutamate antagonists can partially block smsp's suggests that the transmitter receptor on auditory nerve terminals may be pharmacologically related to excitatory amino acid receptors in the CNS. However, the fact that glutamate antagonists can completely block sound-evoked potentials at a concentration that only partially blocks smsp's suggests that they also act presynaptically to reduce the number of quanta released for a given sound stimulus. Thus, the predominant actions of the glutamate antagonists were presynaptic.

133.5

AN ADDITIONAL DIMENSION IN THE TOPOGRAPHIC ORGANIZATION OF THE SPIRAL GANGLION PROJECTION TO THE VENTRAL COCHLEAR NUCLEUS. P. A. LEAKE* and R. L. SNYDER* (SPON: P. T. Ohara). Coleman and Epstein Labs, Dept. of Otolaryngology, Univ. of California, San Francisco, CA 94143-0526.

The morphological organization of inputs from restricted sectors of the cat cochlear spiral ganglion into the cochlear nucleus was studied by making discrete injections (0.05-0.5μL) of horseradish peroxidase (HRP) into the spiral ganglion within Rosenthal's canal. Injections produced Golgi-like labeling of a small cluster of ganglion cells.

Large injections labeled sectors of the spiral ganglion innervating ≥1mm (≈1 critical band distance) of the basilar membrane and resulted in discrete laminae of labeled axons and preterminal fields within each cochlear nucleus subdivision. The positions of these bands were consistent with the "isofrequency" laminae appropriate for the frequencies represented at the injection site. That is, bands of labeled fibers in the posteroventral (PVCN) and anteroventral (AVCN) cochlear nucleus were oriented in a roughly horizontal plane with high frequency laminae situated dorsally and lower frequencies progressively more ventral. These projection laminae extended across the entire lateral to medial dimension of the VCN. Labeled stripes in the dorsal cochlear nucleus (DCN) were oriented at an angle of approximately 60° relative to the laminae in the VCN. In DCN projecting fibers and terminals did not reach the dorsal margin of the nucleus but were excluded from the molecular cell layer.

In contrast to larger injections, very small HRP deposits labeled only a column or partial isofrequency lamina in the VCN. Specifically, injections restricted to the ventral (scala tympani) portion of the spiral ganglion labeled only the lateral portion of VCN isofrequency laminae, while dorsal injections (scala vestibuli aspect of the ganglion) projected to the medial aspect of the isofrequency planes. These data suggest a previously unrecognized topographic organization of the spiral ganglion, orthogonal to the frequency domain. That is, in addition to the spiral dimension (represented by the dorsal to ventral frequency map in the VCN) there is also an orderly and sequential topographic projection of inputs from the ventral to dorsal aspect of the spiral ganglion across the lateral to medial dimension of each lamina.

Supported by NIH Grant NS-16361 and the S. and I. Epstein Fund.

133.6

SEPARATE CODING OF HIGH AND LOW TEMPORAL ENVELOPE FREQUENCIES IN THE AUDITORY FOREBRAIN OF THE MYNAH BIRD. B. Hose and G. Langner. Inst. of Zool., Technical Univ., Schnittspahnstr. 3, 6100 Darmstadt, FRG.

Rhythms and pitch in natural communication sounds correspond to periodic amplitude fluctuations of signal envelopes with low or high envelope frequencies (EFs), respectively. Most auditory forebrain units in field L of mynah birds (Gracula religiosa) are tuned to a certain best envelope frequency (BEF). Field L is three-layered and tonotopically organized, with isofrequency planes cutting across the three layers L_1, L_2 and L_3. L_2 is the input layer. L_1 and L_3 are postsynaptic to L_2. BEFs are mapped within isofrequency planes. A BEF-map across all layers was found in terms of a synchronization criterion, the vectorlength R (Hose, B. et al., *Brain Res*, 422:367, 1987). R = n r is a mixed criterion and takes into account the neuronal discharge rate n as well as the probability r that the discharges are synchronized to the envelope of the stimulus. Further analysis of spike patterns and comparison of R, n and r as a function of EF revealed differences between the layers and between coding of high and low EFs: Besides the topological representation of predominant low BEFs in the postsynaptic layers and predominantly high BEFs in the input layer, a partly translation from a neuronal synchronization code to a rate code from pre- to postsynaptic layers provides evidence that **rhythm** information is **processed** at the forebrain level. In contrast, the percentage of units with temporal tuning mechanisms resembling those in the midbrain (Langner, G., *Exp Brain Res*, 52:333, 1983) suggests that **pitch** information which is already processed at the midbrain level is also **represented** in the input layer of the forebrain level. *Supp. by DFG.*

133.7

TONOTOPIC ORGANIZATION OF THE HUMAN AUDITORY CORTEX UTILIZING A MULTI-CHANNEL SQUID SYSTEM. T. Yamamoto*, W. Hosteller*, S.J. Williamson, and R. Llinás. Dept. of Physiology. & Biophysics, NYU School of Medicine, New York, NY 10016 and Dept. of Physics, New York University, 4 Washington Place. New York, NY 10003.

Progress in multi-channel SQUID (super-conductive quantum interference device) technology and a three-dimensional probe position indicator (PPI) has made it possible to record human brain magnetic fields at 14 sites simultaneously. By recording the surface magnetic fields generated by neuronal activity, the equivalent current dipole location may be estimated within a clinically reasonable time period. This non-invasive, real time (DC to 3KHz) technique has better spatial resolution than EEG or PET, and displays functional information from given brain regions which can be combined with CAT and MRI information. The present system consists of two probes each containing 8 dc SQUIDs and 3 rf SQUIDs, together with PPI and supporting electronics installed in a magnetically shielded room. Typical overall system noise is 20 to 30 fT/ $\sqrt{\text{Hz}}$. Analysis of the iso-magnetic field contour map allows the localization of neuronal activity and the characterization of its source strength. The average error in the dipole location for fields of physiological strength was 2 mm using a spherical head model containing a current dipole in a saline solution. Methods have also been developed for superimposing the characteristic tonotopic organization of the auditory cortex on an MRI. This will be illustrated for randomized auditory stimuli. The work was supported by NIH grant NS13742.

133.8

FISH SPATIAL HEARING REQUIRES BINAURAL ANALYSIS OF SOUND PRESSURE AND PARTICLE MOTION. N.A.M. Schellart and R.J.A. Buwalda (SPON: European Neuroscience Association). Laboratory of Medical Physics, Acad. Med. Center, Univ. of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam.

Without a cochlea, but with a swimbladder as a middle-ear analogon, fish hearing rivals that of higher vertebrates. The gas-filled bladder transforms sound pressure (p) into pulsations with body-fixed orientation that reach the nearby labyrinths, thus displacing the hair cells relative to the massive otoliths. The otolith organs are also stimulated directly by the particle motion vector (v) in line with the source because the fish oscillates with the water. The vectorsum of these "indirect" (p-induced) and "direct" (v) stimulations, which are not in phase, will result in elliptical displacement orbits (Schellart & de Munck, JASA 82:822, 1987). We surmise that these are centrally analyzed, with the sensory maculae mapped out on octaval nuclei, as in gravistatic detection. This provides fish with superior 3-D spatial hearing, without need for the binaural intensity and time difference cues used by man. According to this essentially monaural hypothesis, directional hearing should show strong azimuthal anisotropies.

In search of any such anisotropies we have studied trout in psychophysical heart rate conditioning paradigms, employing superpositioning of standing waves for total control of v direction and of phase and amplitude of v and p. Masked detection thresholds were determined as a function of source direction for two conditions: a 100 Hz probe signal with "normal" p/v phase and amplitude ratios, and a "pure v" signal together with a "pure p" noise masker. Omnidirectional v noise was always present. High or low thresholds were expected near 0° and 180°, since direct and indirect input are then (anti)paralel. However, these expected anisotropies did turn up only if the indirect input was mimicked by a longitudinal v vector of appropriate phase and strength. As in this case the sumvectors for both ears are identical, we conclude that in the normal case the surprising absence of any axis of directional sensitivity must be attributed to the processing of binaural differences introduced by bilaterally symmetrical components of the slightly diverging left and right indirect inputs.

133.9

EXPERIMENTAL VERIFICATION THAT INTERTYMPANIC COUPLING IS THE PREDOMINANT SOURCE OF DIRECTIONAL SOUND SENSITIVITY OF THE TREEFROG'S EAR. R.R. Capranica, Section of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853 and A. Michelsen*, Inst. of Biology, Odense Univ., DK-5230 Odense, Denmark

Behavioral studies have demonstrated that anurans(frogs and toads) can localize a low-frequency sound source with remarkable accuracy even though they lack an external ear (pinna or canal) and have small heads. The basis for this ability presumably resides in a pressure gradient mechanism whereby the anuran's eardrum receives sound at its external surface as well as at its internal surface. By means of laser vibrometry, we have measured the amplitude and phase of vibration of the eardrums of awake, unrestrained green treefrogs (Hyla cinerea) both before and after blocking the movement of the contrlateral eardrum in response to sounds of systematic incidence from 0 to 360°. The eardrum of an untreated adult green treefrog exhibits a change in vibrational amplitude of up to 10 dB or more with changes in azimuth, exhibiting a sharp decrease when the sound impinges directly from the contralateral side compared to the ipsilateral side. This directionality is dependent on frequency and is greatly reduced when the contralateral eardrum is coated with a heavy layer of grease. Coupling through the floor of the mouth, nares, or body via the lungs plays a much smaller role, as shown by selectively blocking each of these routes while measuring the vibrational amplitude and phase of the exposed eardrum

133.10

COMPARATIVE PHYSIOLOGY OF AUDITORY LOCALIZATION IN OWLS. S. F. Volman and M. Konishi. Biol. Div., 216-76, Calif. Inst. of Tech., Pasadena, CA 91125.

Bilateral ear asymmetry evolved in only some lines of owls. Barn owls use asymmetry for two-dimensional sound localization. Azimuth is computed from interaural time differences (ITD), and elevation from interaural intensity differences (IID) created by the vertical asymmetry. In a part of inferior colliculus (ICx), neurons tuned for ITD and IID are arranged into a map of auditory space.

We recorded from ICx in three other owl species: the great horned and burrowing owls with symmetrical ears, and the asymmetrical long-eared owl. In each species we found a map of azimuth entirely dependent on ITD. In the symmetrical owls, ICx neurons were insensitive to elevation; the long-eared owl had weak elevation tuning, probably based on IID. Curiously, in the symmetrical owls, the cells did require binaural stimuli, although they all preferred nearly equal interaural intensity, whereas the barn owl has a range of best IIDs. The frequency ranges of ICx units were: burrowing owl, 2-4 kHz; great horn, 2-5 kHz; long-eared, 2.5-7 kHz; and barn owl, 3-8.5 kHz.

These data suggest three generalizations: 1) All owls use ITD alone to code azimuth; 2) the use of IID cues in asymmetrical owls correlates with higher frequency hearing; and 3) the existence of binaurally exclusive IID tuning in symmetrical owls may have facilitated the evolution of asymmetry.

133.11

COMMISSURAL PROJECTIONS MEDIATE INHIBITION IN A LATERAL LEMNISCAL NUCLEUS OF THE BARN OWL. T.T. Takahashi and M. Konishi, Inst. of Neurosci., Univ. of Oregon, Eugene, OR 97403 and Div. of Biol., Caltech, Pasadena, CA 91125.

Nucleus ventralis lemnisci lateralis pars posterior (VLVp) is the first site in the owl's auditory system at which are found neurons sensitive to interaural level difference, the owl's cue for sound-source elevation. VLVp cells are excited and inhibited by stimulation of contralateral and ipsilateral ears respectively. The excitation arrives via a contralateral input from nucleus angularis, the cochlear nucleus specialized for coding sound level. Present tracer studies revealed commissural projections from one VLVp to the other which can account for the ipsilateral inhibition. Specifically, inhibitory cells in, say, the right VLVp are driven via the left nucleus angularis and project commissurally, thus enabling the left ear to inhibit the ipsilateral (left) VLVp. As a test of this hypothesis, we recorded from neurons in VLVp while locally anesthetizing VLVp of the opposite side (18 neurons, 3 owls). Injection of anesthetic (0.2 ul) rendered the binaural neurons insensitive to ipsilateral stimulation, making them monaural. Recovery took about 20 minutes if injection and recording sites had the same best frequencies. Otherwise recovery was quicker, suggesting that the projection is tonotopically organized. No convincing evidence of other inhibitory sources was observed.

133.12

ORGANIZATION OF INTERAURAL-INTENSITY-DIFFERENCE SENSITIVITY WITHIN FREQUENCY BAND STRIPS OF CAT PRIMARY AUDITORY CORTEX. D. R. F. Irvine, R. Rajan*, and L. M. Aitkin*. Depts. Psychology and Physiology, Monash University, Clayton, Vic. 3168, Australia.

The organization of neuronal sensitivity to interaural intensity differences (IIDs) in the primary auditory cortex (AI) was examined in anesthetized cats. IID-sensitivity functions were obtained for single neurons isolated along the dorso-ventral extent of frequency-band strips in the high-frequency region of AI. Four major classes of IID-sensitivity function were distinguished: those with a clearly defined peak response near zero IID; those with maximum response (either a peak or a plateau) at IIDs involving greater intensity at either the contralateral or ipsilateral ear; those that were relatively flat, indicating no sensitivity to IID. In the dorsal part of AI, it was frequently observed that all the neurons encountered within restricted segments of the frequency-band strip had the same form of IID sensitivity, i.e., there was a spatial segregation of different types of sensitivity. However, in all animals there were some regions, particularly in the ventral part of AI, in which there were rapid transitions in the form of IID sensitivity, even when successive neurons were separated by as little as 20-100 μm across the cortical surface. There appears to be an irregular mosaic of different forms of IID sensitivity across frequency-band strips in AI, and any given IID must therefore result in a complex pattern of activity across such a strip.

PAIN MODULATION: PHARMACOLOGY

134.1

POTENTIATION OF NONOPIOID SWIM ANALGESIA BY THYROTROPIN RELEASING HORMONE ADMINISTRATION INTO THE PERIAQUEDUCTAL GRAY IN RATS. J.A. Robertson, M.L. Cooper*and R.J. Bodnar. Dept. of Psychol. and Neuropsychol. Doctoral Sub-Program, Queens College, CUNY, Flushing, NY 11367.

Intracerebroventricular and intrathecal administration of thyrotropin releasing hormone (TRH) has been shown to modulate analgesic responses with the former injection route potentiating a nonopioid form of swim analgesia in rats. Since TRH is found in serotonergic cells implicated in pain inhibition in the midbrain periaqueductal gray (PAG), the present study evaluated the effects of intracerebral PAG injections of TRH (10 ug) upon nonopioid continuous cold-water swim (CCWS, 2°C, 3.5 min) analgesia on the tail-flick and jump tests in rats. Intracerebral administration of TRH into the PAG significantly potentiated CCWS analgesia on the tail-flick, but not the jump test, and concomitantly reduced CCWS hypothermia. When swim temperatures were increased to 21°C, intracerebral TRH produced similar specific potentiations. In contrast, intracerebral TRH failed to alter basal nociception in the PAG. These results indicate that the PAG is an important site of action for TRH modulation of a nonopioid, neurohormonal form of analgesia.

134.2

SELECTIVE GENDER AND GONADECTOMY EFFECTS UPON CENTRAL MORPHINE ANALGESIA. K.L. Kepler, B. Kest J.M. Kiefel, M.L. Cooper* and R.J. Bodnar. Dept. of Psychol. and Neuropsych. Doctoral Sub-Program Queens College, CUNY, Flushing, NY 11367.

Female rats display significantly less analgesia than age-matched males following either systemic morphine, opioid-mediated or nonopioid-mediated swim analgesia. Gonadectomy reduces these analgesic effects further in both genders. Since pharmacodynamic and pharmacokinetic effects cannot be definitively distinguished for systemic morphine analgesia effects as functions of gender or gonadectomy, the present study evaluated these variables upon morphine analgesia following intracerebroventricular injection. Weight-matched male and female rats received sham or gonadal surgery. One month later, morphine (0.1-40 ug) analgesia was assessed on the tail-flick and jump tests for up to 2 h following microinjection. Male rats displayed significant increases in morphine analgesia on both tests relative to females. Castration, but not ovariectomy also significantly reduced analgesic magnitude as compared to same-sex sham controls. These data strongly suggest both gender and gonadectomy affect morphine analgesia independent of pharmacokinetic effects.

134.3

REDUCTION IN OPIOID AND NONOPIOID FORMS OF SWIM ANALGESIA BY SEROTONIN TYPE 2 RECEPTOR ANTAGONISTS IN RATS. J.M. Kiefel, D. Paul and R.J. Bodnar. Dept. of Psychol., Queens Col., CUNY, Flushing, NY 11367 and Mem. Sloan-Kettering Cancer Ctr., NY, NY 10021.

Although the role of centrifugal serotonin pathways have been established for opiate analgesia, recent work has further implicated the S2 receptor in this response. The present study compared the serotonin receptor antagonist, metysergide with the S2 receptor antagonists, pirenpirone and ketanserin for their ability to alter opioid-mediated intermittent and nonopioid-mediated continuous cold-water swim (ICWS, CCWS, 2° C, 3.5 min) analgesia on the tail-flick and jump tests in male rats. Metysergide (0.1-5.0 mg/kg, IP), pirenpirone (0.04-0.2 mg/kg, IP) and ketanserin (1.0-5.0 mg/kg, IP) each produced dose-dependent decreases (50%) in CCWS analgesia on both tests. In contrast, ICWS analgesia was only marginally decreased by metysergide on the tail-flick, but not the jump test. These data implicate serotonergic, and particularly S2 type receptors in the mediation of nonopioid CCWS analgesia.

134.4

5-HT ANXIOLYTICS, BUSPIRONE AND IPSAPIRONE, POTENTLY BLOCK NON-OPIOID ANALGESIA IN DEFEATED MALE MICE. R.J. Rodgers* and J.K. Shepherd*, (SPON: European Neuroscience Association) Pharmacoethol. Lab., Sch. of Psychol., Bradford University BD7 1DP, England.

In male DBA/2 mice, the experience of social defeat is accompanied by an acute non-opioid form of analgesia. Although initial studies supported the involvement of benzodiazepine recognition sites in the mediation of defeat analgesia, more recent detailed analyses have suggested that this hypothesis is untenable (Rodgers, R.J.& Randall, J.I. Physiol. Behav. 41: 279, 1987). In the present study, the effects of two anxiolytic ligands for 5-HT_{1A} binding sites (buspirone HCl & ipsapirone HCl) have been assessed. Both compounds, administered i.p. 15 minutes prior to testing, were studied over a 2000-fold dose range (0.01-20 mg/kg). Results show that, although largely devoid of intrinsic effects on basal nociception, both drugs potently blocked the analgesic consequences of defeat experience:- minimum effective dose for buspirone=0.5mg/kg, for ipsapirone=0.05mg/kg. These findings provide evidence for the importance of 5-HT_{1A} binding sites in the mediation of a biologically-relevant form of environmental analgesia, and further suggest that this paradigm may be useful in screening for atypical anxiolytics.

We thank Bristol-Myers & Troponwerke for the gifts of drugs used. This work was supported by a YRHA research grant (LE97)

134.5

PARADOXICAL ANALGESIA PRODUCED BY THE OPIATE-ANTAGONIST NALOXONE IS MEDIATED BY INTERACTION AT A SITE WITH CHARACTERSITICS OF THE DELTA-OPIOID RECEPTOR. Y.O. Taiwo*, A.I. Basbaum, F. Perry* & J.D. Levine*, (SPON: S. D. Collins), Div. Neurobiol., UCSF, SF, CA 94143.

The paw-withdrawal and tail-flick tests were used, in 300 g male Sprague-Dawley rats, to measure effects of opioid analgesics on nociceptive thresholds. The analgesic effect of low-dose naloxone (LDN) was abolished in rats that had been pretreated 24 hrs earlier with a large intrathecal dose of naloxone. The latter procedure abolished the analgesic effects of the delta specific ligands DPDPE and DSLET but not that produced by mu ligands DAGO and morphiceptin and the kappa specific ligand U50,488. The analgesic action of LDN (on thermal and mechanical nociceptive threshold tests) also persisted in rats made tolerant to mu-opioid receptor specific ligands, but it was prevented by the delta-receptor specific antagonist, ICI-174,864. We conclude that the analgesic action of low dose naloxone is mediated via a stereospecific binding site with the characteristics of the delta-opioid receptor.

134.6

CENTRAL ALLOXAN REDUCES THE POTENCY OF MORPHINE ANALGESIA. E. Lubin and R.J. Bodnar. Dept. of Psychol. and Neuropsychol. Doctoral Sub-Program, Queens College, CUNY, Flushing, NY 11367.

While peripheral alloxan acts as a pancreatic beta-cell toxin, central administration of far lower doses of alloxan presumably alters central glucoreceptor mechanisms. It is through this latter effect that central alloxan has been thought to reduce opioid-mediated analgesia and hyperphagia induced by 2-deoxy-D-glucose (2DG). The present study extended the role(s) of central alloxan effects by evaluating its actions upon systemic morphine analgesia (1-20 mg/kg, SC) on the tail-flick and jump tests. Nociceptive and analgesic assessments occurred two weeks following intracerebroventricular administration of either vehicle or alloxan (200 ug). Alloxan significantly reduced the potency of analgesia induced by the 2.5 and 5 mg/kg doses of morphine on both nociceptive measures. However, central alloxan failed to shift the dose-response curve of morphine analgesia. In contrast to its central actions, intravenous administration of an identical dose of alloxan failed to affect systemic morphine analgesia. These results suggest that alloxan alters opioid mechanisms necessary for 2DG and morphine analgesia.

134.7

VITAMIN B-INDUCED SUPPRESSION OF SPINAL DORSAL HORN NOCICEPTIVE NEURONS IN THE CAT. Q.-G. Fu*, J. Sandkühler, E. Carstens, B. Stelzer*, M. Zimmermann (SPON: A.F. Haase).
II. Physiologisches Institut der Universität, D-6900 Heidelberg, FRG

Systemic administration of a vitamin-B-compound (Neurobion, E. Merck) produced analgesia in clinical therapy and animal models of pain. The mechanism underlying this analgesic action is not known. We have examined the effects of vitamin B on spinal transmission of nociceptive information.
Single neurons were extracellulary recorded in the spinal dorsal horn of pentobarbital-anaesthetized cats. Radiant heat (50 °C, 10 s) was applied to the glabrous foot pad as a noxious stimulus. Vitamin B compound ($B_1/B_6/B_{12}$, 100 mg/100 mg/1 mg in 3 ml) was injected intravenously (0.3 ml) or applied directly to the spinal cord dorsum at the recording segment by means of a small perspex pool. All neurons selected for this study were multireceptive, i.e. responded to noxious and non-noxious skin stimuli. In 6 of 9 neurons tested heat evoked responses were suppressed by spinal administration of vitamin B compound. The suppression began 3 to 15 min (5 ± 3.6 min, n = 6) after the start of spinal superfusion. The average maximal suppression was to 57.7 ± 11.8 % of control. Spontaneous activity of the neurons was not significantly changed. Intravenous injection also depressed heat evoked responses of the two dorsal horn neurons tested to 74.8 ± 5.2 % of control. This inhibition had a duration of 6 to 9 min.
Our data suggested that vitamin B can directly suppress the responses of spinal neurons to nociceptive stimulation. It is conceivable that this suppression is involved in the analgesia in clinical therapy.
SUPPORTED BY E. MERCK, DARMSTADT, FRG.

134.8

DEPRESSION OF NOCICEPTIVE ACTIVITY EVOKED IN THE RAT THALAMUS BY VITAMIN B COMPLEX AND VITAMIN B_6. I. Jurna* and D. Bonke. Institut für Pharmakologie und Toxikologie der Universität des Saarlandes, D-6650 Homburg/Saar, F.R.G, and E. Merck, D 6100 Darmstadt, F.R.G.

It is frequently proposed that vitamins of the B group alleviate pain. In search of evidence of an analgesic property of these compounds, experiments were carried out on rats under urethane anaesthesia in which activity was elicited in single neurons of the dorsomedial part of the ventral nucleus (VDM) of the thalamus by electrical stimulation of nociceptive afferents in the ipsilateral sural nerve. This activity is highly sensitive to morphine (Carlsson et al., pain (1988) 313). Intraperitoneal injection of 5 ml/kg of a combination of vitamin B_1, B_6 and B_{12} (Neurobion®, containing thiaminchloridehydrochloride 33.4 mg/ml, pyridoxinhydrochloride 33.4 mg/ml and cyanocobalamin 0.34 mg/ml) depressed evoked nociceptive activity by 50 % (number of neurons; N = 9). At a lower dose (Neurobion® 3 ml/kg; N = 6), the depression amounted to 40 %. At both doses the effect manifested itself from 70 min onwards, was at its maximum from 90 to 120 min, and returned to the control level by the end of the experiments at 180 min. Intraperitoneal injection of pyridoxinhydrochloride (167 mg/kg; N = 8) caused depression to an extent and with a time course similar to that observed after 5 ml/kg with the combination of vitamins. Thiaminhydrochloride (167 mg/kg; N = 8) and cyanocobalamin (1.67 mg/kg; N = 10) caused a slight depression from 70 to 120 min. It is concluded that the combination of the vitamins B_1, B_6 and B_{12} may produce analgesia which is mainly due to the action of vitamin B_6.

134.9

ANTINOCICEPTIVE EFFECTS OF VITAMINS B_1, B_6, AND B_{12} IN RATS AND MICE. G. Bartoszyk and A. Wild* E. Merck Pharm. Res., POB4119, 61 Darmstadt/FRG

Combinations of the vitamins B_1, B_6, and B_{12} are clinically used alone or in combination with NSAIDs in some painful conditions (e.g. neuralgia or inflammation, resp.). Animal studies regarding the antinociceptive effects of vitamins have been reported only occasionally with contradictory results (e.g. Eschalier, A., et al., Psychopharmacology 81:228, 1983; Hanck, A., et al., Int. J. Vit. Nutr. Res. 27:189, 1985). Using male Wistar rats (280-360 g) we investigated the clinically used combination of B_1, B_6, and B_{12} in three experimental animal models: benzoquinone writhing test (WR, reduction in number of writhes), heat coil test, and hot plate test (both: increase in latency). Injection of aqueous solutions (content per 1 ml: 33.3 mg B_1, 33.3 mg B_6, 0.3 mg B_{12}) gave dose- and time-dependent response curves. In WR, injection of the single vitamins as well as their combinations, two by two, resulted in less pronounced effects indicating an at least additive effect of the single vitamins. Using female NMRI mice (20-37 g) in the WR, the antinociceptive effects of oral diclofenac and metamizol were enhanced by adding a vitamin combination which was inactive when given alone.

134.10

INHIBITORY ACTIVITY OF ANALOGS OF WIN 48098 IN ISOLATED TISSUE PREPARATIONS IN VITRO IS REFLECTIVE OF A MECHANISM OF ANTINOCICEPTION. Susan J. Ward, M. Miller, D. Luttinger, M.A. Eissenstat and M. Bell. (SPON: D.R. Haubrich). Departments of Pharmacology and Medicinal Chemistry, Sterling-Winthrop Res. Inst., Rensselaer, NY 12144.

In addition to inhibiting prostaglandin (PG) formation, the new analgesic Win 48098 inhibits neuronally-mediated contractions of mouse vas deferens (MVD) preparation apparently by a "novel" presynaptic mechanism (Ward et al., FASEB, 1987). Compounds were designed and synthesized that retain inhibitory activity in the MVD, but which do not inhibit PG formation. Representative examples of the results are summarized below:

	IC50 (μM) in vitro		Antinociceptive ED50 (mg/kg) Mouse		Rat
	MVD	PG	i.v.	p.o.	p.o.
48098	0.56	5.0	5.4	40	12
Analog 1	0.17	>30	9.7	1000	402
Analog 2	0.032	>30	3.8	106	—
Analog 3	0.015	>100	0.5	97	38
Analog 4	0.006	>30	0.12	23	7

Inhibitory potency of compounds in the MVD ranged over 3 orders of magnitude and was correlated with antinociceptive potency. Behavioral depression was observed in doses at and above the antinociceptive ED90 (iv and po) in mice, but was less apparent in the rat. The data suggest that the inhibitory activity of this series of compounds in the MVD may represent a new antinociceptive mechanism; however, this mechanism may also elicit behavioral depression.

134.11

WIN 48,098, A NOVEL AMINOALKYLINDOLE ANALGESIC: INHIBITION OF ADENYLATE CYCLASE IN RAT CEREBELLAR MEMBRANES. M. Pacheco, S.R. Childers and S.J. Ward. Depts. of Neuroscience and Pharmacology, Univ. of Florida Coll. Med., Gainesville, FL 32610 and Sterling-Winthrop Res. Inst., Rensselaer, NY 12144.

Win 48098 is an aminoalkylindole (AAI) analgesic with a dual mechanism of action: inhibition of cyclooxygenase (CO) and a "novel" mechanism with characteristics of a neurotransmitter receptor, reflected by inhibition of neurally mediated contractions in the mouse vas deferens (MVD) preparation. In the present study, Win 48098 and structurally related analogs devoid of CO activity were evaluated for effects on adenylate cyclase (AC) in brain. Crude membranes were prepared from various regions of rat brain, and AC activity was assayed in the presence and absence of 10 μM Win 48,098. AC was assayed with 100 mM NaCl and 50 μM GTP, in the presence and absence of 0.1-1.0 μM forskolin, using [3H]ATP as substrate in an HPLC procedure.

AAI's inhibited both basal and forskolin-stimulated AC activity by 20-35% in membranes from cerebellum. However, little or no inhibition was detected in other regions of rat brain, including striatum, brainstem, hypothalamus, thalamus, hippocampus, amygdala or cortex. The potencies of several AAI analogs to inhibit AC paralleled their potencies in the MVD:

Compound	IC_{50} vs. AC (μM)	IC_{50} in MVD (μM)
Win 48098	5	0.5
Analog 1	2	0.1
Analog 2	0.9	0.015
Analog 3	0.5	0.006

The effect of these analogs on AC was magnesium-dependent, with lower concentrations of magnesium (<5 mM) providing maximal inhibition levels. Inhibition of AC by these compounds was GTP-dependent, since removal of guanine nucleotides totally eliminated the inhibitory activity. Like other G-protein coupled receptor systems in brain, inhibitory activity was supported by GDP and GMP, but not by non-hydrolyzable GTP analogs like Gpp(NH)p and GTP-γ-S. These data suggest that these aminoalkylindole analgesics act through specific, G-protein-linked receptors in the CNS to inhibit AC.

134.12

THE ACTION OF ANESTHETICS AND DIPHENYLHYDANTOIN ON REPETITIVE RESPONSES OF CORNEAL A-DELTA MECHANORECEPTORS. Hilary W. Thompson,* Brett Dupuy,* and Roger W. Beuerman. LSU Eye Center, New Orleans, LA 70112.

A subpopulation of A-delta corneal mechanoreceptors in the rabbit responds with 1-3 action potentials to each cycle of a mechanical stimulus presented at low frequencies (1 Hz) for several hours. Recording were made from the long ciliary nerve. Mechanoreceptors were stimulated with 10-60 μm displacements of a 0.5 mm dia steel ball attached to a piezoelectric actuator. Drugs were injected into a constant flow of isotonic saline directed at the point of mechanical stimulation. Drug effect was defined as a decline in response probability below 90%. Drug concentrations that did not completely inhibit response produced shifts in response latency. Drug action, in terms of time to onset of effect (TOE) and duration (D), was defined for various topical anesthetics (benoxinate, proparacaine, and tetracaine): benoxinate: TOE = 6±1.5 (mean±SEM) sec, D = 580±376 sec at 10^{-2} M; proparacaine: TOE = 11±6.3 sec, D = 88±34 sec at 10^{-2}. Diphenylhydantoin, an anticonvulsant, acted at concentrations less than $5x10^{-4}$ M: TOE = 30±12 sec, D = 50±20 sec. The mechanism of action of diphenylhydantoin on the corneal innervation may be due to its effects on calcium channels. This technique permits assessment of pharmacological agents on peripheral free nerve endings, and represents a model for study of the ionic mechanisms of mechanoreceptive transduction in the cornea.

NEUROTOXICITY I

135.1

LACK OF TOLERANCE DEVELOPMENT TO SUBCHRONIC TOXICITY OF SOMAN AND TABUN. R. C. Gupta and W-D. Dettbarn. Dept. of Pharmacology, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232

Male Sprague-Dawley rats were exposed daily to sublethal doses of soman (40 ug/Kg,sc) and tabun (50 ug/Kg,sc) for 4 weeks. With both the nerve agents 40 to 50% animals showed anticholinesterase toxicity signs after receiving four or five consecutive doses and died when given three to five more daily doses. Remaining 50 to 60% animals did not exhibit any signs of toxicity at any time up to 4 weeks of treatment. During an early episode of soman toxicity (day 5), when toxic signs were evident, a marked reduction ($P < 0.01$) in the activities of esterases, such as AChE, BuChE, and CarbE, was seen in the discrete brain regions and skeletal muscles of different characterstics. AChE activity in brain and skeletal muscles and CarbE activity in brain remained significantly ($P < 0.01$) depressed throughout the experiment; whereas BuChE activity in all the tissues (especially muscles), and CarbE activity in the liver (80%) and in the plasma (50%) indicated substantial recovery when examined after 4 weeks. Similar findings were also observed with tabun. The lack of tolerance development to the toxicity of soman or tabun following their subchronic exposure, unlike reported for DFP (Gupta, et al., Toxicol. Appl. Pharmacol. 84: 541, 1986) could be due to either non-recovering inactivation of AChE activity or insensitivity of in vivo protein synthesis.

135.2

MECHANISMS OF CHEMICAL INJURY IN RAT PERIPHERAL NERVE. M.W. Kalichman, O.A. Holovda*, H.C. Powell*, and R.R. Myers*. Depts. of Anesthesiology and Pathology, Univ. of California, San Diego, CA 92093.

Sprague-Dawley rats were used to study mechanisms of injury to saphenous or sciatic nerve following extraneural injection of various concentrations of local anesthetics including 2-chloroprocaine, procaine, etidocaine, and lidocaine, as well as neurolytics such as glycerol and phenol, in volumes of 0.2-1ml. Local anesthetics *are not normally used for their ability to produce neurolysis*; however, *in sufficient* concentration, all of the drugs tested demonstrated a concentration-dependent increase in long-lasting nerve injury, as indicated by the accumulation of endoneurial edema, cytoplasmic lipid droplets, and axonal degeneration and demyelination, at 48 hrs after administration. The following possible mechanisms of injury were considered: (1) *Physicochemical properties of the solutions tested.* Control studies using solutions characterized by a wide range of pH, tonicity, and lipid solubility, as well as various additives and vehicles, revealed no correlation with injury other than that predicted by neurolytic concentration. (2) *Ischemia.* Extraneural application of test solutions might compromise transperineurial vessels either by direct vasoconstriction or indirectly as a result of increased endoneurial fluid pressure associated with edema formation. In either case, injury should be restricted to the subperineurial region rather than centrally, where the axial circulation persists in spite of a loss of the transperineurial blood supply. Chemical neurolysis is not restricted to the subperineurial region; therefore, ischemia is not likely the principal mechanism of nerve injury. (3) *Specific chemical interactions between neurolytic agents and the affected cells.* Electron microscopic studies have demonstrated a highly selective neurotoxic property of local anesthetics: Schwann cells of unmyelinated fibers readily undergo lytic changes; whereas, myelinated fiber Schwann cells are much more likely to accumulate cytoplasmic lipid inclusions. These selective changes argue against a non-specific mechanism of injury such as might be expected with ischemia or general alterations of the endoneurial microenvironment. Additionally, in electrophysiological studies, diverse local anesthetics were found to inhibit nerve conduction acutely with the same relative potency demonstrated for the neurotoxic effect of endoneurial edema accumulation at 48hrs. These histopathologic and electrophysiologic data are consistent with the hypothesis that some chemical neurolytics produce injury to peripheral nerve by cell-specific effects at an appropriate receptor or ion channel.

135.3

MPTP LETHALITY IN MICE: EFFECT OF MAO-B INHIBITION AND AMINE UPTAKE INHIBITION. R. W. Fuller, S. K. Hemrick-Luecke* and K. W. Perry*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is neurotoxic to nigrostriatal dopamine neurons, and its neurotoxicity is prevented by inhibition of monoamine oxidase type B (MAO-B) or by inhibition of the dopamine uptake carrier. We now report that the acute lethality of MPTP in Charles River CFW mice is also prevented by selective inhibition of MAO-B with deprenyl. Deprenyl (0.01-10 mg/kg i.p.) pretreatment prevented lethality and the observed behavioral effects at 1-3 hrs after high doses of MPTP (30-90 mg/kg s.c.). 1-Methyl-4-phenylpyridinium (MPP+) was more potent in causing lethality than was MPTP, and deprenyl did not affect its lethality. MPTP lethality was not antagonized by EXP 561, an uptake inhibitor that prevented the neurotoxic effects of a lower dose of MPTP on striatal dopamine and cortical norepinephrine neurons. The protective effect of deprenyl against MPTP-induced lethality paralleled the inhibition of MAO-B in brain but not in liver and persisted out to 14 days. Deprenyl pretreatment markedly reduced MPP+ concentrations in brain after MPTP injection, without altering MPTP concentrations in brain. Centrally formed metabolites of MPTP, probably MPP+, seem to account for the lethality of MPTP at higher doses as well as its neurotoxic effects toward nigrostriatal dopamine neurons at lower doses.

135.4

LONG-TERM COGNITIVE AND MOTOR DEFICITS IN MPTP-TREATED MONKEYS. J.R. Taylor, R.H. Roth, J.R. Sladek, Jr., D.E. Redmond, Jr. Yale Univ. Sch. of Med., New Haven, CT 06510, and Univ. of Rochester Sch. of Med., Rochester NY 14641.

To assess the stability of neural deficits produced by MPTP we studied performance on an object retrieval task. The task requires retrieval of a banana slice from a transparent box open on one side. The orientation of the open side, location of the box, and position of the banana in the box were manipulated in order to vary the cognitive and motor difficulty of the trials. African green monkeys (N=6) were treated with MPTP (2-3 mg/kg). A control group was sham-treated (N=5). The subjects examined were without any neurological or behavioral deficits but probably had dopamine depletions. When acquiring this task 8-12 months after MPTP treatment they had motor and cognitive deficits. During 3 months of testing, MPTP-treated subjects had motor problems and cognitive deficits, such as responding at the transparent side of the box. They were less successful at retrieving the reward on the first reach than controls. The impairments were stable throughout testing. On select trials when an identical, but opaque, box was used MPTP-treated subjects decreased reaches at the transparent side of the box, while motor problems were increased. The task is sensitive to cognitive and motoric deficits associated with this model of Parkinson's disease and confirms that these deficits are stable for many months. NINCDS #PO1 NS24032; Axion/St. Kitts Biomed., Res. Found.

135.5

ASYMPTOMATIC AND SYMPTOMATIC MPTP-TREATED PRIMATES: REGIONAL HETEROGENEITY IN MESOSTRIATAL TOXICITY. J.D. Elsworth, A.Y. Deutch, D.E. Redmond, Jr., J.R. Sladek, Jr. and R.H. Roth. Depts. Pharmacol. & Psychiat., Yale Univ. Sch. Med., New Haven, CT 06510; Rochester Sch. Med., NY.

Primates treated with the same dose of MPTP exhibit different degrees of parkinsonism. Regional biochemical differences in the mesostriatal dopamine (DA) system were examined in asymptomatic (Asymp) and severely symptomatic (Symp) vervet monkeys 1-2 months after receiving MPTP. Large differences in DA depletions existed between Asymp and Symp groups in cell body areas. DA losses occurred at an earlier stage of MPTP toxicity in medial substantia nigra than in lateral retrorubral field, central substantia nigra and dorsal ventral tegmental area. Severe DA depletions were observed in striatum in both Asymp and Symp groups, suggesting that MPTP toxicity is initially exerted at the terminals. Unlike idiopathic, but similar to postencephalitic Parkinson's disease, DA losses in putamen were not greater than in caudate nucleus. Heterogeneity in MPTP susceptibility was apparent within striatum; regional DA losses in Asymp ranged from 75-99% and in Symp from 95-99%. The regional differences between Asymp and Symp groups in caudate nucleus and putamen suggest possible sites at which transplants may be most favorably placed for reversing parkinsonism and suggest that low but sustained DA production by grafts may be sufficient to ameliorate parkinsonian motor abnormalities. NINCDS PO1 NS24032.

135.6

LONG-TERM EFFECTS OF BRIEF EXPOSURE TO 1-METHYL-4-PHENYLPYRIDINE (MPP^+) IN ORGANOTYPIC CULTURES OF CANINE SUBSTANTIA NIGRA (SN). B. Christie-Pope* and W.O. Whetsell, Jr. (SPON: M.D. Johnson). Neuropathology, Vanderbilt Sch. of Med., Nashville, TN 37232.

Earlier investigations have shown that organotypic cultures of canine SN develop swelling of neuronal mitochondria followed by generalized disruption of all cellular components (neurons and glia) when continuously incubated in MPP^+ for up to 15 hours (Christie-Pope *et al.*, Neurosci. Abstr. 13:788, 1987). The present study examined ultrastructural changes in canine SN cultures and caudate (CA) cultures exposed to 0.1nM MPP^+ for 1.5 hours or 3 hours then washed and maintained on usual feeding medium for different periods of time. At 30 days after 1.5 hours exposure to MPP^+, SN cultures exhibited increased glial processes with evidence of neuronal degeneration. Prominent mitochondrial changes were observed in neurons including occurrence of large, swollen mitochondria in some nerve cells and increased numbers of normal-size mitochondria in other nerve cells; mitochondrial changes were not evident in glial cells. SN cultures exposed to MPP^+ for 3 hours then studied 30 days later showed similar ultrastructural changes. Neither sibling SN cultures maintained on usual feeding medium and studied at the same times as the MPP^+-exposed cultures nor other SN cultures maintained normally for up to 100 days showed such ultrastructural changes. CA cultures treated with MPP^+ for 1.5 or 3 hours and studied at 30 days or later showed no ultrastructural alterations compared to control CA cultures. These observations indicate that while prolonged exposure to MPP^+, even at very low concentrations, produces a non-specific toxicity in canine SN or CA cultures (*ibid.*), brief exposure to MPP^+ shows a remote and specific toxicity for SN neurons but not CA neurons in this *in vitro* model. [Supported by a grant from the United Parkinson's Foundation and a Pharmaceutical Manufacturers' Association Foundation Fellowship Award (BCP).]

135.7

ROLE OF GLYCINE IN THE NEUROTOXIC EFFECTS OF GLUTAMATE IN HIPPOCAMPAL CELL CULTURE. I. SHALABY AND M. PROCHNIAK*. Pfizer Central Research, Groton, CT 06340.

We examined the role of glycine in glutamate-induced neurodegeneration using 3 week old cultures of fetal rat hippocampal cells. Glutamate was added for 15 min to the cultures, washed, and cells were allowed to remain 24 hr after which time the cell media was analyzed for lactate dehydrogenase (LDH) activity as an index of cell toxicity. Glutamate induced a concentration-dependent increase in LDH at an ED50 of 200uM with a maximal elevation of 3 fold above controls. NMDA receptor blockers AP-7, PCP, and MK-801 antagonized the effects of glutamate, and were able to save neurons from ongoing degeneration. Glycine at concentrations up to 10uM did not potentiate the neurotoxic effects of glutamate. These results suggested that glutamate application might release sufficient endogenous glycine to potentiate its neurotoxic actions. To examine this possibility we incubated hippocampal cultures with [3H]glycine, thereafter repeatedly incubated cells in buffer from which 5 min fractions were taken to assess [3H]glycine release. 70mM potassium depolarization and 100uM glutamate induced a 2-fold and 1.0mM glutamate a 3-4 fold increase in [3H]glycine release compared to non-stimulated controls. In conclusion, glutamate induced a concentration-dependent release of glycine from hippocampal cell cultures. The glutamate-induced release of glycine may be sufficient to potentiate the neurodegenerative effects of glutamate, thereby rendering additional exogenous glycine application ineffective.

135.8

CSF PGE2 ELEVATION AFTER WATER CONTRAST MYELOGRAPHY. L. Maloney*, R. Evans*, D. Coombs*, L. Cromwell*, R. Colburn*. (SPON: S.J. Velez) Anesth. Research Lab., Dartmouth-Hitchcock Medical Center, Hanover, NH 03756.

Prostaglandin E2 (PGE2) is released with inflammation. We investigated whether myelography with water soluble iopamidol results in acute inflammation/arachnoiditis. METHODS: 59 patients undergoing lumbar myelography (disc disease/stenosis) gave informed consent (IRB approved). A 22 gauge spinal needle was placed fluoroscopically. Three ml of CSF were removed; 1.5-2ml was transferred to polypropylene tubes containing 15 mcg indomethacin to arrest arachadonic acid metabolism. The CSF was centrifuged and frozen at -70 C till analysis. Ten to 15 ml of iopamidol (E.R. Squibb, Princeton, NJ) was then injected. Eleven of the 59 came to surgery (herniated nucleus pulposis or nerve root entrapment). After dural exposure, CSF was withdrawn (25 gauge spinal needle). All blood contaminated samples were discarded. PGE2, cell count, and protein were evaluated in the CSF. RESULTS: Mean age all patients premyelogram vs surgical subgroup were comparable (49+15, 47+14 resp., p=NS). Protein and PGE2 were not different premyelogram in surgical and nonsurgical. Table 1 shows mean + S.E. CSF PGE2 and protein (surgical subgroup only). PGE2 and protein were significantly increased at surgery compared to premyelogram. There was no statistical relationship between cell count and PGE2. DISCUSSION: Injection of contrast dye likely generates inflammation marked by increased CSF PGE2 and protein. Origin of this PGE2 is unknown. PGE2 may influence healing/fibrosis and subsequent surgical recovery (under study).

Table 1	* Premyelogram	Surgical
PGE_2 (picogm/ml)	0.70 ± 0.34	7.88± 9.37
Protein (mg/dl)+	56.0 ± 26.9	74.5± 33.6

*P<0.02(premyel. v. surg. PGE_2), +P<0.01 (premyel. v. surg. protein)

135.9

Cummulative and Residual Effects of Low Level Lead Exposure on Development and Behavioral Activity in Three Generations of Mice. D.A. Rasile*, P.J. Donovick, and R.G. Burright*. Environmental Neuropsychology Lab, SUNY Binghamton, Binghamton, N.Y. 13901

In the past two decades, numerous experimental studies have established the teratogenic effects of low-level lead exposure on development and behavioral activity in laboratory animals. However, few attempts have been made to systematically study the cummulative and residual consequences of lead exposure across multiple generations. The present study addresses these issues and attempts to determine the cross-generational effects of lead toxicity on developmental measures such as body weight, age of eye opening and latency of home nest return--and on behavioral activity measures such as activity wheel, open field, and swimming, in Binghamton Heterogenous Stock mice.

135.10

A SENSITIVE HPLC METHOD FOR THE DETERMINATION OF β-N-METHYLAMINO-L-ALANINE (BMAA) IN CYCAD SEED AND ANIMAL TISSUES. G.E. Kisby, D.N. Roy *, and P.S. Spencer. Institute of Neurotoxicology, Albert Einstein Coll. Med., Bronx, NY 10461.

We have developed a method for detecting subpicomolar concentrations of β-N-methylamino-L-alanine (BMAA), a neurotoxin found in the seed of cycad plants (*Cycas* spp.) which has been linked to western Pacific amyotrophic lateral sclerosis and parkinsonism-dementia complex. BMAA and other amino acids were derivatized with 9-fluorenylmethyl chloroformate (FMOC) prior to HPLC separation and fluorescence detection (excitation λ 254 nm and emisson λ 315 nm). All amino acids, including BMAA, eluted from the column within 22 min. BMAA (t_r= 18.02 ±0.07 min) was detected in *Cycas circinalis* L. seed and in serum, cerebrospinal fluid and brain tissue from BMAA-treated monkeys and rats. The purity of synthetic FMOC-BMAA was established by a single peak after HPLC analysis (t_r=18.17 min) and by mass spectrometry (m/e 341.2). Physiological amino acids could also be detected since they were highly resolved from BMAA. Amino acids and BMAA were linear over the concentration range of 0.15 μM-7.5 μM with a relative standard deviation ranging from 2.1-6.7%. This method should prove useful in determining the biological fate and metabolism of BMAA in plant and animal tissues.

This work was supported by a grant from the NIH (NS 19611).

135.11

REGIONAL BRAIN QUINOLINATE INCREASES DURING AND AFTER HYPOGLYCEMIA. M.P. Heyes*, R.N. Auer, M. Papagapiou and S.P. Markey* (SPON: Q. Pittman) Lab. of Clinical Science, N.I.M.H., Bethesda, MD 20892 and Dept. of Pathology, University of Calgary, Alberta T2N 4N1, Canada.

Hypoglycemia (HG) is associated with neurodegeneration that is attenuated by NMDA receptor antagonists. HG increases brain L-tryptophan concentrations (Agardh: Diabetes, 28: 804, 1979) and could increase the synthesis of quinolinic acid (During: This Meeting), a neurotoxin, convulsant and NMDA receptor agonist. We investigated 1 h of HG and 1 h recovery (R) in rat on quinolinic acid (QUIN), L-TRP, 5-HT and 5-HIAA concentrations in cortex, striatum, thalamus and hippocampus; and striatal DA, DOPAC and HVA concentrations. In all regions, QUIN increased 2-3 fold during HG and 2-3 fold further during R; 5-HT decreased > 50% during H and R whereas 5-HIAA increased 2 fold during H and R. L-TRP increased > 50% during R only. Striatal DA decreased 74% during H only, DOPAC and HVA increased > 200% during H and R. We conclude that H induces increased synthesis of QUIN and release of brain 5-HT and striatal DA. QUIN may have etiologic importance in the neurodegeneration and convulsions of H.

135.12

COMPARATIVE NEUROTOXICITY OF FENFLURAMINE AND 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA). J.A. Wagner and S.J. Peroutka. Department of Neurology, Stanford University Medical Center, Stanford, CA 94305.

Fenfluramine is a phenethylamine used clinically as an oral anoretic and structurally related to MDMA. Biochemical and pathologic evidence suggest that MDMA produces a selective reduction of serotonin (5-HT) nerve terminals in rat and monkey. [^{3}H]Paroxetine was used as biochemical marker in order to assess the extent of 5-HT nerve terminal damage after administration of a single dose (0, 1, 3, 10, or 30 mg/kg s.c.) of fenfluramine. Rats were sacrificed two weeks later. B_{max} levels of [^{3}H]paroxetine were, respectively, 42.1 $\pm$ 3.4, 38.2 $\pm$ 3.4, 34.3 $\pm$ 2.5, 21.8 $\pm$ 2.2, and 9.9 $\pm$ 2.1 fmoles/mg wet weight. No change was seen in K_D. By contrast, only 30 mg/kg MDMA caused a significant reduction in [^{3}H]paroxetine binding. No [^{3}H]paroxetine binding alterations were observed rats sacrificed within 24 hours of fenfluramine or MDMA administration. These data suggest that fenfluramine and MDMA produce similar patterns of 5-HT neurotoxicity. The implications to the human users of these drugs will be discussed.

135.13

ASSESSMENT OF COMPLEX BEHAVIOR IN THE RHESUS MONKEY: EFFECTS OF CHRONIC MARIJUANA SMOKE EXPOSURE. W. Slikker, Jr.*, M.G. Paule, D.E. McMillan*, J.R. Bailey*, A.C. Scallet and S. F. Ali. Div. of Reprod. & Develop. Toxicol., NCTR, Jefferson, AR 72079 and Dept. of Pharmacol., UAMS, Little Rock, AR 72205.

A large scale study involving 30 periadolescent male rhesus monkeys was designed to assess the behavioral effects of exposure to marijuana smoke for 1 yr. All monkeys were trained (n=7-8/group) under 5 complex operant schedules, matched for performance levels and body weights, and exposed to either the smoke of 1 marijuana cigarette (mask) once a day, 7 days/wk, or 2 days/wk. Two control groups, one receiving smoke from an extracted cigarette devoid of cannabinoids (placebo) and 1 sham exposed (no smoke), were also exposed 7 days/wk. Behavioral assessments occurred 23 hr after exposures. Chronic marijuana smoke decreased task completion in conditioned-position response tasks and decreased rate and accuracy in incremental repeated acquisition tasks. Additionally, when compared to either placebo or sham groups, progressive-ratio response rates and breakpoints were significantly decreased in both the low and the high dose marijuana smoke groups. These data indicate that complex behaviors can be routinely assessed in a large number of monkeys and that long-term toxicity studies using inhaled compounds are feasible. The data also suggest that chronic marijuana exposure produces deficits in complex behavior. (Supported in part by NIDA-IAG #224-83-0005).

CARDIOVASCULAR REGULATION III

136.1

SOME PROPERTIES OF THE SYMPATHO-INHIBITION FROM THE CAUDAL VENTROLATERAL MEDULLA OBLONGATA (CVLM) IN THE CAT. K.Dembowsky*, J.Czachurski* and H.Seller*(SPON:D.W.Richter). I.Physiol.Inst., Universität Heidelberg, Heidelberg, F.R.G.

The CVLM contains a group of neurones which upon their activation by glutamate injections cause a complete inhibition of sympathetic activity. Recordings of the activity in several sympathetic nerves revealed that this inhibition is more pronounced in the renal than the cardiac nerve or white ramus T3. This inhibition was accompanied by a strong tonic excitation of phrenic nerve activity. Since this inhibition could still be evoked after a contralateral hemisection and a section of the ipsilateral ventral quadrant of the spinal cord, it is concluded that its spinal pathway is confined to the ipsilateral dorsolateral funiculus. This inhibition also persisted after blockade of alpha2-receptors with rauwolscine, making an involvement of noradrenaline as transmitter in this system unlikely. The question if this inhibition is due to disfacilitation or post-synaptic inhibition of sympathetic preganglionic neurones (SPN) was addressed with intracellular recordings of SPNs. Glutamate injections into CVLM resulted in a reduction of the on-going synaptic activity in SPNs and/or a membrane hyperpolarization. In 2 SPNs this was associated with no change in input resistance (R_N), whereas in 3 other SPNs a transient reduction of R_N was observed. Further evidence for post-synaptic inhibition of SPNs from CVLM was obtained in experiments in which IPSPs were evoked in SPNs in response to electrical stimulation of this area.

136.2

LATERAL TEGMENTAL FIELD SYMPATHOINHIBITORY (LTF-SI) NEURONS PROJECT TO MEDULLARY RAPHE NUCLEI. S.M. Barman and G.L. Gebber. Dept. Pharmacol., Mich. St. Univ., E. Lansing, MI 48824.

Both the LTF and raphe nuclei of the cat medulla contain neurons that are in SI pathways. Their activity is synchronized to sympathetic nerve discharge (SND), and their firing rate is increased during the inhibition of SND produced by baroreceptor reflex activation. LTF-SI neurons fire before raphe-SI neurons during the cardiac-related burst of SND. Whereas raphe-SI neurons innervate the intermediolateral nucleus, the axons of LTF-SI neurons do not project to the spinal cord. The current study was designed to test the hypothesis that LTF-SI neurons project to the raphe nuclei. We antidromically activated 18 LTF-SI neurons by microstimulation of the region of the medullary raphe (2-3 mm rostral to the obex) that contains raphespinal-SI neurons. The onset latency of antidromic activation was 19±3 ms when threshold current (133±24 μA) was used. This value was close to the mean difference (17 ms) between the spontaneous firing times of LTF-SI and R-SI neurons. LTF-SI neurons could not be antidromically activated by high intensity (1 mA) current applied either 2 mm lateral to the midline, contralateral to the recording site or in the raphe nuclei 1 mm caudal to the obex. The onset latency of antidromic activation of these neurons was shortened when threshold current was applied 1.5 mm lateral to the midline, ipsilateral to the recording site. Taken together, these data are consistent with the hypothesis that LTF-SI neurons innervate and excite raphespinal neurons which then inhibit SND. (Supported by NIH grant HL13187.)

136.3

IDENTIFICATION OF SEROTONERGIC AND SYMPATHETIC NEURONS IN MEDULLARY RAPHE NUCLEI. RB McCall and ME Clement. The Upjohn Company, Kalamazoo, MI 49001.

The purpose of this study was to identify midline medullary serotonin (5-HT) neurons and to determine if these neurons were distinct from raphe sympathoinhibitory and sympathoexcitatory neurons. Identification of medullary 5-HT neurons was based on electrophysiological and pharmacological similarities to dorsal raphe 5-HT neurons. Sympathoinhibitory and sympathoexcitatory neurons were characterized by an irregular discharge pattern which was temporally related to inferior cardiac sympathetic nerve discharge (SND) and to the cardiac cycle. Sympathoinhibitory neurons increased their discharge rate and the discharge of sympathoexcitatory neurons decreased during baroreceptor reflex activation. A third type of neuron fired in an extremely regular fashion. The discharges of regularly firing neurons were not temporally related to SND and were not affected during baroreceptor reflex activation. Regularly firing neurons had a spontaneous discharge rate of 1.1 spikes/s. Regularly firing neurons and sympathoinhibitory neurons could be antidromically activated by electrical stimulation of the intermediolateral cell column of the spinal cord. Axonal conduction velocities in the medullospinal pathway of regularly firing neurons was 1.3 m\s and 2.4 m\s for sympathoinhibitory neurons. Regularly firing neurons were completly inhibited by iontophoretic 5-HT or by i.v. or iontophoretic application of the 5-HT$_{1A}$ agonist 8-OH DPAT while much higher doses of 8-OH DPAT failed to affect the discharges of sympathoinhibitory and sympathoexcitatory neurons. Based on the striking similarities between regularly firing medullary neurons and dorsal raphe 5-HT neurons it is concluded that the regularly firing neurons are 5-HT containing neurons. Medullary 5-HT neurons are distinct from sympathoinhibitory and sympathoexcitatory neurons. Studies in our laboratory indicate that 5-HT neurons excite sympathetic preganglionic neurons.

136.4

RESPIRATORY MODULATION OF SYMPATHETIC PREGANGLIONIC NEURONS IN THE MID-THORACIC SPINAL CORD OF THE RAT. *S.F. Morrison, and D.J. Reis.* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

The nucleus reticularis rostroventrolateralis (RVL) contains neurons that provide a major source of tonic sympathoexcitation as well as neurons that may be involved in the generation of the basal respiratory rhythm. The responses of sympathetic preganglionic neurons (SPNs) to stimulation of the RVL indicate that SPNs are driven by (1) a rapidly conducting RVL-spinal pathway, (2) RVL-spinal neurons with slowly conducting axons, or (3) both systems. In the present study we compared the respiratory modulation of SPNs with their responses to RVL stimulation to determine if the respiratory modulation of their discharge could be attributed to either the rapidly or the slowly conducting RVL-spinal pathway. SPNs were antidromically identified in the T6-T8 spinal segments of urethane-anesthetized,vagotomized, paralyzed, ventilated rats. The spontaneous activity of 80% of the SPNs was increased during the period of increased phrenic nerve discharge, while the activity of the remaining 20% was lower during the phrenic burst than during the phrenic silence. Of the SPNs in the former group, 36% were excited at a short latency (< 30 msec), 46% at a long latency (> 100 msec), and 18% had a bimodal response to RVL stimulation. Those with decreased firing in inspiration were exclusively responsive to the rapidly conducting pathway. The finding that respiratory modulation of SPNs was not correlated with the temporal characteristics of their responses to RVL stimulation suggests that respiratory modulation of SPNs derives either (1) from the respiratory modulation of the discharge of both classes (rapidly and slowly conducting) of RVL-spinal sympathoexcitatory neurons or (2) from the respiratory modulation of inputs to SPNs from a source other than the RVL. (Supported by NIH grants HL18974 and NS22721.)

136.5

PHENYLETHANOLAMINE N-METHYLTRANSFERASE-CONTAINING TERMINALS SYNAPSE DIRECTLY ON SYMPATHETIC PREGANGLIONIC NEURONS IN THE RAT. *T.A. Milner, S.F. Morrison, C. Abate and D.J. Reis* Div. of Neurobiology and Molecular Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

The electron microscopic localization of a polyclonal antiserum directed against the adrenaline synthesizing enzyme, phenylethanolamine N-methyltransferase (PNMT) was examined in the intermediolateral cell column (IML) of the rat thoracic spinal cord utilizing the peroxidase-antiperoxidase labeling method. Terminals containing PNMT-immunoreactivity (I) constituted 76% (117 out of 154) of the labeled profiles. These terminals (0.5-1.4 µm in diameter) contained a few mitochondria and a large population of small clear and from 1-6 large dense-core vesicles. Of 117 terminals, 93 formed associations with unlabeled perikarya and dendrites. The synaptic junctions on perikarya were rare (n = 4) and exclusively symmetric; those on proximal (large) dendrites were more numerous (31% of 89) and were primarily symmetric membrane specializations. The vast majority (69% of 89) of the terminals with PNMT-I formed asymmetric synaptic junctions on distal (small) dendrites and dendritic spines. Moreover, many of the terminals with PNMT-I as well as their postsynaptic targets were closely invested with, or apposed to fibrous astrocytic processes.

In a subsequent set of experiments, immunoautoradiographic labeling for PNMT was combined with horseradish peroxidase (HRP) retrograde identification of sympathetic preganglionic neurons (SPNs). In these sections, terminals with PNMT-I directly synapsed on the HRP-containing (i.e., retrogradely labeled SPNs) perikarya and dendrites. The axosomatic synapses observed between PNMT-labeled terminals and SPN perikarya were exclusively symmetric; whereas the type and distribution of axodendritic association varied such that the majority were asymmetric on small dendrites and dendritic spines. The findings provide ultrastructural evidence that in the rat IML, adrenergic (i.e., PNMT-containing) terminals (a) may be either excitatory (asymmetric) or inhibitory (symmetric) depending on their site of termination and (b) can influence sympathetic nerve discharge through a direct effect on the SPN cell membrane. (Supported by NIH HL 18974.)

136.6

QUANTITATIVE-TOPOGRAPHIC ANALYSIS OF ADRENERGIC AND NON-ADRENERGIC SPINAL PROJECTIONS OF CARDIOVASCULAR AREA OF RVL. *S.L. Cravo*, D.A. Ruggiero, M.Anwar*, D.J. Reis.* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

The cardiovascular area (CAA) of RVL was defined in urethane-anesthetized rats by microinjections of the neuroexcitatory agent L-Glutamate (L-Glu, 1 nmol, 15 nL, pH 7.4). An area where the L-Glu provoked pressor responses larger than 10 mmHg (maximal response 30 mmHg) was defined along a longitudinal strip of 400 µm comprising the rostral third of the C1 adrenergic neurons group. Spinal projections from CAA were studied using the retrograde tracer WGA-HRP and immunocytochemistry for the presence of tyrosine hydroxylase (TH) and phenylethanolamine-N-methyltransferase (PNMT). After multiple injections of WGA-HRP in all segments of the spinal cord: a) 209±1 neurons (n=3) were retrogradely labeled with HRP in both CAA; b) 224±8 neurons were immunocytochemically stained for TH or PNMT; c) 137±14 (65±7% of the total number of cells labeled with HRP) neurons contained both retrograde and immuno-labels; d) the same number of neurons contained TH and PNMT; hence all are adrenergic. After injections restricted to thoracic spinal segments (n=3), 190±12 neurons were labeled with HRP, 259±6 with TH or PNMT, and 148±9 (78±1% of total number of HRP cells) contained both labels. Non-adrenergic cervical spinal projecting neurons were preferentially distributed dorsally to the adrenergic-spinal projecting cells but also intermixed with them. Adrenergic and non-adrenergic neurons projecting to the lumbar-sacral cord were localized medially to CAA. We conclude that in the rat the CAA of RVL is restricted, that in this area over 75% of thoracic projections are from adrenergic neurons, and that the reticulospinal projections from the entire RVL to the cord are topographically organized.

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136.7

EFFECTS OF KYNURENATE (KYN) ON CAT SYMPATHETIC NERVE DISCHARGE (SND). G.L. Gebber, S.M. Barman and K.J. Varner. Dept. Pharmacol., Mich. State Univ., E. Lansing, MI 48824.

We investigated the role of excitatory amino acids in the genesis and control of SND in Dial-urethane anesthetized cats. Inferior cardiac SND was recorded before and after intracisternal (ic) or local medullary injection of the glutamate-receptor antagonist, KYN. Power density spectral analysis demonstrated that ic KYN (25-50 µmol) reduced SND (≥50%) and eliminated its cardiac-related rhythmic component. The former effect was reproduced by microinjection of KYN (50 nl of 250 mM solution) into the rostral ventrolateral medulla (RVLM) while the latter effect was observed when KYN was injected into the nucleus of the solitary tract. The primary component (>90% of total activity) in SND after ic KYN was a 2- to 6-Hz rhythm, not unlike that observed in baroreceptor-denervated cats. This rhythm is generated by brain stem circuits that provide driving input to RVLM sympathoexcitatory neurons innervating the spinal intermediolateral nucleus (Barman and Gebber, J. Neurophysiol. 57: 1410-1424, 1987). In baroreceptor-denervated cats, KYN (ic or RVLM microinjection) reduced SND without changing the relative proportion of neural activity in the 2- to 6-Hz band. Microinjection of KYN into the medullary lateral tegmental field (a potential source of the 2- to 6-Hz rhythm) failed to affect the power or frequency components in SND. These results indicate that the reduction of SND produced by KYN does not involve perturbation of 2- to 6-Hz rhythm generation. (Supported by NIH grant HL13187.)

136.8

EFFECTS OF KYNURENATE (KYN) ON RAT SYMPATHETIC NERVE DISCHARGE (SND). M. Zviman*, G.L. Gebber and S.M. Barman. (SPON: J.L. Bennett). Depts. of Pharmacol. and Physiol. Mich. State Univ., E. Lansing, MI 48824.

Sun et al. (Brain Res. 438: 23-40, 1988) have located neurons with pacemaker-like activity (beating frequency ≈20 Hz) in the rat rostral ventrolateral medulla (RVLM) after intracisternal (ic) injection of the glutamate-receptor antagonist, KYN. They proposed that these neurons are responsible for SND. The fact that RVLM pacemaker neurons discharge independently of each other after ic KYN blocks their synaptic inputs leads to the prediction that SND will be asynchronous. The results of the current study show that this is not the case. Splanchnic SND was recorded in urethane-anesthetized rats before and after ic KYN (5-25 µmol). As reported by Sun et al., KYN initially increased SND, eliminated its cardiac-related rhythmic component and prevented its inhibition upon baroreceptor-reflex activation. However, SND remained synchronized after ic KYN. Power density spectra of SND contained peaks between 2- and 8-Hz that accounted for >80% of total activity both before and after ic KYN. These results raise two possibilities. First, the limited-band white noise output of independently discharging RVLM pacemaker neurons is transformed in the spinal cord to a 2- to 8-Hz rhythm. Second, RVLM pacemaker neurons are not the primary factor involved in generating SND. These possibilities are currently under investigation. (Supported by NIH grant HL13187.)

136.9

BLOCKADE OF EXCITATORY AMINO ACID RECEPTORS IN RABBIT CAUDAL VENTROLATERAL MEDULLA DOES NOT ABOLISH THE BARORECEPTOR-VASOMOTOR REFLEX. W.W. Blessing, Centre for Neuroscience, Flinders University of South Australia, Bedford Park, SA

There is controversy as to whether or not inhibitory vasomotor neurons in the caudal ventrolateral medulla (CVLM) constitute the inhibitory link in the central baroreceptor-vasomotor pathway. Recent studies in favour of this hypothesis have suggested that blockade of excitatory amino acid (EAA) receptors in the rat CVLM interrupts the baroreceptor-vasomotor reflex (Gordon, F.J. Am. J. Physiol. 252: R628, 1987, Guyenet, P.G. et al, Brain Res. 407: 272, 1987). The present study investigates whether or not this is the case in the rabbit. N. Z. White rabbits (2.5-3 kg), previously prepared with vascular cuff occluders, were anesthetized with urethan (1.4 g/kg i.v.), paralysed and mechanically ventilated. Sympathetic nerve activity (rSNA) was recorded from the left renal nerve and arterial pressure (AP) was recorded from a catheter in the central ear artery. The reflex change in rSNA in response to electrical stimulation of the left aortic depressor nerve (ADN) and to increase or decrease in AP using occluders was recorded before and after bilateral CVLM injection (5 nmol in 100 nl) of either kynurenic acid (KA) or 2-amino-5-phosphonovaleric acid (APV). The rSNA response to ADN stimulation was abolished with EAA receptor blockade (see Table). However response to AP increase or decrease was preserved, consistent with Blessing and Willoughby (Am. J. Physiol. 253: H777, 1987).

		Percent change in rSNA		
		ADN	Increase AP	Decrease AP
KA	Pre-inj	-66±9 (6)	-76±6 (4)	+37±7 (4)
	Post-inj	-14±10**	-64±1	+61±8
APV	Pre-inj	-72±5 (6)	-69±6 (6)	+41±8 (6)
	Post-inj	+1±4 **	-46±6**	+43±1 7

136.10

BLOCKADE OF PARABRACHIAL PRESSOR RESPONSES BY SPINAL ADMINISTRATION OF AN N-METHYL-D-ASPARTIC ACID (NMDA) RECEPTOR ANTAGONIST. M.M. Kalis* and F.J. Gordon (SPON: B.L. Brown). Dept. Pharm., Emory Univ., Atlanta, GA 30322.

These studies investigated a potential role for spinal excitatory amino acid (EAA) receptors in the mediation of pressor responses evoked by electrical stimulation (50 uA, 0.5 msec pulses) of the lateral parabrachial complex. Rats were anesthetized with urethane, vagotomized, paralyzed and respirated. Stimulation of the parabrachial complex at 10, 20 and 40 Hz increased mean arterial pressure (MAP) by 15 ±4, 36 ±5 and 57 ±4 mmHg, respectively. Thirty min after intrathecal (i.t.) administration of the NMDA receptor antagonist D-2-amino-7-phosphono-heptanoic acid (D-AP7; 0.2 umol/10 ul) MAP had fallen from 97 ±4 to 62 ±2 mmHg and parabrachial pressor responses were reduced to -2 ±2, 1 ±2 and 6 ±2 mmHg. Neither i.v. D-AP7 nor i.t. infusion of vehicle had any effect on parabrachial pressor responses. I.t. infusion (10 ul) of NMDA (0.5 umol) or kainic acid (3 nmol) increased MAP by 43 ±7 and 38 ±6 mmHg respectively. D-AP7 eliminated the pressor action of NMDA without affecting that of kainic acid. These results indicate that the increase in MAP produced by electrical stimulation of the lateral parabrachial complex may be dependent upon synaptic activation of spinal NMDA receptors, and suggest that EAA neurotransmitters may play a role in mediating cardiovascular responses evoked by activation of descending sympathoexcitatory pathways. (NIH HL-36907)

136.11

BLOCKADE OF SPINAL PATHWAYS CAUSES NON-UNIFORM DECREASES IN PRE- AND POSTGANGLIONIC DISCHARGE. L.C.WEAVER AND L. QU* (SPON: B.Timney). The John P. Robarts Research Institute and Dept. of Physiol., Univ. of Western Ontario, London, Ontario, and Neurobiol. Dept., Northeastern Ohio Univ. Coll. of Med., Rootstown, Ohio.

Although discharge of many sympathetic nerves decreases substantially upon transection of the spinal cord in cats, firing of splenic and mesenteric nerves continues unabated (Brain Res. 338:123, 1985; J.Physiol. 396:155, 1988). Experiments were done in anesthetized cats to determine if ongoing sympathetic outflow to the splanchnic circulation depends less upon supraspinal excitatory drive than that directed to other vascular beds. Transection of the cervical spinal cord (CIX) significantly decreased firing of gastric, hepatic, adrenal and lumbar chain sympathetic nerves. Discharge of gastric and hepatic nerves decreased (-49% and -75%, respectively) as much as that of adrenal and lumbar chain nerves (-39% and -60%, respectively). Thus, sustained discharge after CIX is not necessarily characteristic of innervation of the splanchnic circulation. In contrast, discharge of preganglionic greater splanchnic nerves was not decreased after CIX, although the firing pattern changed from rhythmic to asynchronous. This suggests that preganglionic rhythmicity may be important for effective synaptic transmission to some but not all postganglionic neurons. Support: Can. Ht. Fdn. and NIH grant HL21436.

136.12

RENAL AND GREATER SPLANCHNIC NERVE RESPONSES TO BLOCKADE OF THE ROSTRAL VENTROLATERAL MEDULLA IN RATS. C.P. YARDLEY*, K. HAYES*, and L.C. WEAVER. The John P. Robarts Research Institute and Department of Physiology, University of Western Ontario, London, Ontario, Canada.

In cats, transection of the spinal cord decreases discharge of renal nerves, but does not decrease firing of preganglionic greater splanchnic nerves (Neurosci. Abst: 1988), suggesting that actions on postganglionic nerves do not necessarily reflect changes produced in preganglionic sympathetic nerves. Experiments were done to compare effects of blockade of excitatory drive from rostral ventrolateral medulla (RVLM) on renal and pre- and postganglionic splanchnic nerves in rats. Glycine (1M) was microinjected (150 nl) unilaterally into the RVLM of urethane anesthetized rats while recording renal (RNA) or greater splanchnic nerve activity (GSPNA) and blood pressure (BP). Eleven injections decreased RNA by 45% and BP by 36%. After seven other injections, mean GSPNA decreased by only 18% and BP fell by 30%. Moreover, the preganglionic component of SPNA (after hexamethonium) was reduced by only 13%, whereas RNA (sample from sites causing similar decreases in BP) was reduced by 33%. These results show that, in the rat, tonic discharge of RVLM neurons is more important for maintaining renal than pre-and postganglionic components of greater splanchnic nerve discharge. Supported by the Canadian Heart Foundation and NIH grant HL21436.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: VESTIBULAR SYSTEM II

137.1

VESTIBULAR AMPULLARY EPITHELIA IN A TURTLE, P.SCRIPTA: STRUCTURE AND INNERVATION. A.M. Brichta & E.H. Peterson. Department of Zoological & Biomedical Sciences & College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701.

As part of a larger study aimed at understanding vestibular control of head movement in *P.scripta* we are characterizing the functional architecture of vestibular primary afferents. We have begun by assessing: 1) organization of the ampullary neuroepithelia using light and scanning electron microscopy (SEM) and 2) morphology and spatial distribution of vestibular peripheral processes using horseradish peroxidase (HRP).

SEM analysis of ampullary neuroepithelia revealed statistically significant regional differences in hair cell density, luminal surface profiles, and cilia complexes. Peripheral hair cell density is high (39±6 hair cells/1500um^2); mean profile areas are small (11.5 ± 3.7um^2) and tend to be elliptical. Stereocilia are generally smaller in diameter, length, and number than those found centrally. In contrast central hair cell density is low (26±1 hair cells/1500um^2), while outline areas are large (17.4 ± 5.3um^2) and more nearly circular. To relate hair cell morphologies to their innervation, we labelled peripheral endings of vestibular primary afferents using an *in vitro* HRP technique (Brichta and Peterson, '87, Soc. Neurosci. Abs.13:634). All afferents were traced back to their cell somas in the vestibular ganglia. There are three morphological types of vestibular primaries - calyceal, dimorphic, and bouton - that differ significantly in fiber diameter (Brichta and Peterson, '87). Reconstruction of individual primaries reveals that these types also differ in collecting territories, branching density and terminal number. Calyceal fibers terminate only in central regions of the epithelium and never branch before entering the crista. In contrast, bouton endings are found preferentially at the epithelial periphery, and dimorphic endings have a relatively homogeneous distribution. Both types sometimes branch before entering the crista; daughter branches may innervate widely separated regions of the epithelium, but always form the same terminal type. Terminal sprays of all three types are oriented normal to the long axis of the crista; the linear collecting distances of calyces are shorter (X=25.5±11 um) than those of dimorphic (X=54±20um) and bouton types (X=109±73um). Calyceal sprays had fewer terminal processes (X=4) than dimorphic or bouton types (>8). Taken together, these data suggest that there may be multiple information channels in *Pseudemys* vestibular nerve that differ in morphology, hair cell contacts, and spatial organization within the sensory epithelium.
(Supported by NIH grant RO1 NS23498)

137.2

HORIZONTAL CANAL AFFERENTS TERMINATE IN THE LATERAL VESTIBULAR NUCLEUS. G.A. Kevetter and A.A. Perachio. Dept. Otolaryng., U. TX. Med. Branch, Galveston, TX 77550.

The central distribution of afferents that innervate the horizontal semicircular canal was studied in order to continue the identification of areas that receive segregated and integrated input in response to head accelerations in both the vertical and horizontal planes. The lateral vestibular nucleus (LVN) was the focus of this study since it has been considered to receive primarily an otolith input. Horseradish peroxidase was ionophoretically applied to either the crista ampullaris of the lateral canal or intra-axonally into physiologically identified vestibular afferents in the gerbil. Labeled fibers and terminals were located in all four major vestibular nuclei and portions of the cerebellum. As the fibers entered the brainstem, they divided into an ascending and a descending branch. In the ventral LVN, medially directed collaterals were present. Swellings, resembling boutons en passant, were observed on these collaterals as they traversed the LVN and continued to the medial nucleus. Labeled terminals were abundant in the lateral portion of LVN. Afferents that were injected intracellularly had a distribution pattern similar to that seen after extracellular injections. Most injected afferents had an irregular spontaneous discharge rate. Additional features of the termination patterns in the LVN could be determined. Collaterals of the descending branch were thick and directed medially. Many small, thin branches emerged from these collaterals and ramified throughout the rostral caudal extent of LVN with many terminal boutons in the LVN. Many fewer fibers and terminals were located in the dorsal LVN. (Supported by NSF BNS 84/18559, NIH NS24391, and NASA NAG2-26).

137.3

VESTIBULAR PROJECTIONS TO MOTOR CORTEX IN MACACA NEMESTRINA. O.B. White and J.C. Brinkman*, Experimental Neurology, John Curtin School, A.N.U., Canberra, A.C.T. 2601, AUSTRALIA.

There are direct projections from the vestibular nuclei to nucleus VPLo in the monkey thalamus. Previous studies have identified vestibular responses only in areas 2 and 3a of the monkey cerebral cortex (Lang et al, Brain Res. 177:3, 1979), despite the fact that VPLo projects to area 4 (Friedman, D. and Jones, E., J. Neurophysiol. 45:59, 1981). In this study single unit recordings were made from the motor cortex of conscious monkeys during active performance of a stereotyped task, passive manipulation of the contralateral limbs and, during depolarization of the contralateral round window. Recording sites were confirmed histologically to be in area 4. Thirty-nine of 158 units tested responded to vestibular stimulation, 8 at latencies < 8 msecs. Unit responses were either phasic or sustained throughout the stimulus period. Twenty of 39 units also responded to passive manipulation of the contralateral limbs or axial musculature. An additional 9 units responded only to a combination of reaching out and labyrinthine depolarization. Thus, we have identified a vestibular field in that region of motor cortex which receives kinaesthetic afferent input from axial and proximal limb structures.

Supported by an NH&MRC grant to OBW.

137.4

PASSIVE MEMBRANE PROPERTIES AND SPONTANEOUS SYNAPTIC ACTIVITY OF BULLFROG UTRICULAR AFFERENTS. R.A. Baird. Neurological Sciences Institute, Portland, OR 97209.

To study, in vitro, synaptic transmission and impulse initiation in the bullfrog utricular macula, microelectrodes were inserted into the apical surface of the sensory epithelium. Stable intracellular recordings were obtained from both supporting hair cells (SHCs) and unmyelinated afferent nerve terminals (ANTs). The two cells are readily distinguished by their responses to intracellular current. SHCs have large (-70 to -90 mV) resting potentials, low (10-30 MΩ) input resistances, and are electrically passive. ANTs, on the other hand, display smaller (-30 to -65 mV) resting potentials, larger (40-175 MΩ) input resistances, and respond with single spikes to small (0.1-1.0 nA) positive currents.

Most ANTs have spontaneous spiking and synaptic activity. The spike discharges of striolar ANTs are low (1-10 sp/s) and irregular. Extrastriolar ANTs have higher (10-20 sp/s), more regular, discharges with large postspike afterpolarizations. Spikes in all ANTs are notched at 20% of their amplitude and triggered off summated synaptic events. The smallest events, assumed to arise from single synaptic sites, are monophasic potentials of 0.2-0.6 mv with durations of 2-10 ms. Larger (1-2mV) events with no inflections on their rising or falling phase are seen, particularly in striolar ANTs, and may represent the synaptic input of hair cells multiply innervated by the afferent terminal. [Supported by NIH NS23584].

137.5

INPUTS FROM REGULARLY AND IRREGULARLY DISCHARGING VESTIBULAR NERVE AFFERENTS TO VESTIBULOSPINAL NEURONS OF THE SQUIRREL MONKEY. R. Boyle*, S.M. Highstein, and J.M. Goldberg (SPON: M. Meikle). Oregon Health Sciences Univ., Portland, OR 97201, Washington Univ., St. Louis, MO 63110, and Univ. of Chicago, Chicago, IL 60637.

In a previous study (J. Neurophysiol., 58:700, 1987) an electrophysiological paradigm, based on the differential excitability of irregular (I) and regular (R) afferents, was used to characterize the monosynaptic inputs from the ipsilateral vestibular nerve (V_i) to second-order vestibular nuclei neurons projecting to the oculomotor nucleus, flocculus, and spinal cord. Here we extend the analysis and record the monosynaptic EPSPs elicited by V_i stimulation of antidromically identified vestibulospinal neurons to different spinal segments in anesthetized squirrel monkeys (Saimiri Sciureus). Stimulating electrodes were placed on V_i, in the rostral MLF, and at spinal levels C_1, C_6, and T_{12}. Fifty-three neurons were identified as lateral vestibulospinal (LVST) neurons. The majority of cervical-projecting LVST neurons (22/25, 88%) receive a predominant mixed (M) to I afferent input. Of the 28 lumbar-projecting LVST neurons, 15 receive an I, 3 an M and 10 an R input; no correlation was observed between the neuron's afferent input and axonal conduction velocity. Nineteen neurons were identified as medial vestibulospinal (MVST) neurons. MVST neurons to the cervical plexus (n=4) receive an I afferent input, whereas those to the brachial plexus (n=4) receive an R input. Eleven MVST neurons projected both rostrally and caudally in the MLF; these bifurcating MVST neurons receive a predominant R to M afferent input (9/11, 82%). The results suggest that LVST and MVST neurons projecting to different segments of the spinal cord differ in their profile of V_i inputs.

137.6

BRAINSTEM AFFERENTS TO THE MONKEY'S VENTROLATERAL VESTIBULAR NUCLEUS,TVP NEURONS. T.P. Langer* and C.A. Scudder. (SPON: M.C. Chubb) Dept. Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195

Tonic-Vestibular-Pause (TVP) neurons, a group of neurons in the ventrolateral vestibular nucleus that discharge at a rate proportional to eye position and head velocity, and pause during saccades, participate in the vestibulo-ocular reflex. We injected HRP among recorded TVP neurons, labeling a small part of the ventrolateral vestibular nucleus. Labeled neurons were demonstrated with the TMB method.

Labeled neurons densely filled the ipsilateral ventrolateral vestibular nucleus, but did not extend into adjacent vestibular nuclei. Scattered neurons labeled in the medial and inferior vestibular nuclei, caudal to the injection, but many more cells labeled in the contralateral medial and inferior vestibular nuclei, particularly the rostral third of the medial nucleus. The supravestibular nuclei were lightly labeled, bilaterally. Neurons were labeled in the reticular formation that contains inhibitory burst neurons, about twice as often ipsilaterally. Many cells labeled in the ipsilateral lateral cuneate nucleus, the lateral reticular nucleus, bilaterally, and the contralateral dorsal accessory olive. (Supported by RO1 EY03212)

137.7

RESPONSES OF MEDIAL VESTIBULAR NUCLEI (MVN) NEURONS DURING HARMONIC LINEAR ACCELERATION IN DECEREBRATE RATS. G.A. Bush and A.A. Perachio, Dept. Otolaryngol., Univ. TX Med. Branch, Galveston TX 77550.

Spontaneously active MVN neurons were studied for their responses to both angular and linear acceleration. The responses were qualitatively characterized by rotating the animal's head around an earth vertical axis in the horizontal plane. Each cell's response to linear horizontal translational motion (0.6 Hz; peak acceleration of ±0.145g) along the sagittal (x) and interaural (y) head axes as well as vectors oriented ±45° to the midsagittal plane was tested. Type I (n=46) neurons had significantly ($p<0.01$) higher resting rates, mean ±SD 35.0±21.8 spikes/sec compared to 20.4±12.0 for type II cells (n=43). Both type I (69%) and type II (67%) neurons responded to dynamic linear acceleration. Seventy percent of the type I cells' responses were phase related to acceleration for each axis tested compared to 50% of the type II cells. Within this group, 83% of the type I and 75% of the type II cells showed greater sensitivity to y- than x-head axis translational motion. With respect to the vector producing the greatest response, 40% of the type I cells had a mean ±SD phase lead of 27.0° ±6.0, whereas the remaining type I cells had a mean ±SD phase lag of 20.9° ±10.4. With one exception, type II neurons exhibited a response phase lead, mean ±SD=13.3° ±3.2. Type I cells, which all responded throughout the stimulus period, showed a greater mean ±SD gain, 35.6±7.0 than non-rectifying type II neurons, 28.0±9.5 spikes/sec/g. Half of the type II cells analyzed exhibited some degree of rectification (silencing) during the stimulus. These type II neurons had lower average spontaneous firing rates, 18.0±10.5 spikes/sec, and a greater mean ±SD gain, 43.4±7.1 spikes/sec/g, when compared to either type I or non-rectifying type II cells. The average peak response ±SD had a phase lead of 49.4° ±31.8 re head acceleration. Approximately half of the rectifying type II cells responded in phase with head velocity, accounting for the large phase lead. (Supported by NASA grants NAG2-26, NTG 44-008-801; NIH grant NS24391)

137.8

EFFECTS OF SINUSOIDAL NECK ROTATION ON LOCUS COERULEUS-COMPLEX NEURONS. O.Pompeiano,C.D.Barnes,D.Manzoni*,G.Stampacchia* and P.d'Ascanio*. Dept. Physiol.& Biochem.,Pisa Univ.,56100 Pisa,Italy.

In precollicular decerebrate cats the activity of 99 locus coeruleus (LC)-complex neurons, 11 of which activated antidromically by stimulation of the spinal cord at T_{12}-L_1, was recorded during sinusoidal neck rotation. Among the noradrenergic LC-complex neurons (which had a slow and regular resting discharge and a typical response to a noxious stimulus characterized by a burst of activity followed by a silent period), 73 (73.7%) responded to neck rotation at 0.15 Hz, ±10°. In particular, 40 of 73 units (54.8%) were excited during side-down and depressed during side-up neck rotation, while 18 of 73 units (24.7%) showed the opposite pattern (average phase lead of +34.2° re to position). The observation that the predominant response pattern of LC-complex neurons to neck rotation was opposite to that of vestibulospinal (VS) neurons projecting to the same segments of the spinal cord should be related to the fact that while the VS neurons exert a direct excitatory influence on ipsilateral limb extensor motoneurons, the coeruleospinal (CS) neurons exert an inhibitory influence on the related Renshaw (R)-cells (Fung S.J. et al. Brain Res.402: 351-354,1987). It appears, therefore, that during side-up neck rotation ipsilateral limb extensor motoneurons are excited by an increased discharge of VS neurons, while the related R-cells are disinhibited by a reduced discharge of CS neurons. This would enhance the functional coupling of motoneurons with the corresponding R-cells, thus limiting the response gain of limb extensors to neck stimulation.

137.9

SPATIAL PROPERTIES OF SECOND ORDER VESTIBULOOCULAR RELAY NEURONS IN THE ALERT CAT. S. I. Perlmutter, K. Fukushima, B.W. Peterson, J.F. Baker, Northwestern Univ. Med. School, Chicago, IL 60611

Last year we reported that part of the spatial transformation in the VOR occurs as early as the second-order vestibuloocular relay neuron (2VON) in decerebrate cats. We have now analyzed the properties of 35 2VONs in the alert cat, identified by their monosynaptic response to electrical stimulation of labyrinth and antidromic response to stimulation of midbrain MLF. Responses were recorded to 0.5 Hz, 10° rotations in 3-10 vertical and horizontal planes. Neurons were classified according to the canal from which they received their strongest input. Of 20 2VONs that received their primary input from the posterior canal (PC: mean gain 2.2+1.0 spikes/s/deg/s), 16 responded maximally to rotation in a vertical plane within 10° of the PC plane while 4 received sufficient convergent input from the orthogonal vertical canal to shift their plane of maximum response >10° from the PC plane towards the roll plane and close to the plane of the oblique eye muscle. Of 15 neurons that received primarily anterior canal (AC) input (mean gain 3.2+0.9 spikes/s/deg/s), 13 were non-convergent, while 2 had maximum response planes which were >10° from the AC plane in the direction of the pitch plane. Thus **spatial transformation occurs at the 2VON level in alert cats.** 2VONs also received convergent horizontal canal (HC) input (mean gain for all neurons 0.4+0.3 spikes/s/deg/s). All 7 AC neurons whose maximum response plane was tilted >10° from the vertical by HC input exhibited Type II horizontal responses. Of 15 PC cells with tilts >10°, 11 exhibited Type I, 4 Type II responses.

None of 12PC and 6AC neurons examined showed a clear pause during saccades; 8/12 PC and 6/6 AC showed a significant correlation (sensitivity 0.5-2.4 spikes/s/deg) with vertical eye position (up for AC, down for PC). Responses were mostly tonic.

Pairing horizontal rotation with vertical optokinetic stimulus leads to development of vertical vestibuloocular responses to horizontal rotations in the dark (Brain Res. 371(1986)162). Two of 4 2VONs tested showed a large change in maximum response vector after such paired stimuli, producing more horizontal component. Thus **2VONs appear to participate in the adaptive change of the VOR.**

Supported by grants EY 06485, EY 05289, EY 07342, NS 17489, NS 22999.

137.10

VESTIBULAR COMPENSATION: AN IN VIVO AND IN VITRO STUDY OF SECOND ORDER VESTIBULAR NEURONS. **C.de Waele*, M.Serafin*, M.Mühlethaler and P.P.Vidal. Lab. de physio. Neurosens.,CNRS, Paris, France and Dept de Physiol. CMU Geneve Switzerland**

Following the surgical removal of one labyrinth, guinea pigs exhibit a postural and oculomotor syndrome, due to a decrease of the resting discharge of second order vestibular neurons(dVII) in the deafferented side and an increase in the intact side. The recovery of a near balanced discharge between both sides is considered to be an essential mechanism for vestibular compensation. We have therefore recorded in situ the resting discharge of dVII before, immediately after and one week following surgery. In the acute stage neurons were found to be silent whereas already 4 days later they recovered a normal basal discharge. Preliminary studies have shown in brain slices and in the isolated whole brain in vitro the presence of at least three neuronal cell types in the vestibular nuclei. The exploration of their membrane properties after hemilabyrintectomy should provide an insight into the mechanisms of restauration of the resting discharge. (supported in part by grant no 3.288-0.85 from swiss NSF).

137.11

RECOVERY OF ACTIVITY OF TYPE II NEURONS IN THE MEDIAL VESTIBULAR NUCLEUS (MVN) FOLLOWING UNILATERAL LABYRINTHECTOMY IN THE DECEREBRATE GERBIL. S.D. Newlands* and A.A. Perachio. Depts. Otolaryng., Physiology & Biophysics, U. Texas Med. Branch, Galveston, TX 77550.

Recordings were made bilaterally from the MVN of gerbils in three types of preparations; labyrinth intact controls, acutely right labyrinthectomized (2 to 10 hours), and chronically right labyrinthectomized (4 to 6 weeks). Dynamic responses to horizontal rotation at 1.3 and .13 Hz (peak velocity 52°/sec) were recorded. An average cycle histogram was computed, curve fitted with the first and second harmonic, and the gain (in spikes/sec/deg/sec) and phase re velocity (in degrees) of the peak response were calculated. In normal gerbils, the gain and phase (±S.E.M.) of type II neurons at 1.3 Hz (n=22) were .61±.03 and -3.0±.9, and at .13 Hz (n=17) were .49±.03 and 23.6±2.9. Acute right labyrinthectomy resulted in a significant reduction in gain at both frequencies and a phase advance at .13 Hz bilaterally and an increased phase lag at 1.3 Hz on the left. The gain at 1.3 Hz was asymmetric and significantly higher on the right. In the right MVN at 1.3 Hz (n=35), gain = .30±.01 and phase = -1.6±.8; and at .13 Hz (n=27), gain = .19±.01 and phase = 48.1±0.4. On the left at 1.3 Hz (n=13), gain = .18±.01 and phase = -17.6±1.7; and at .13 Hz (n=9), gain = .13±.01 and phase = 58.7±2.4. Type II neurons were difficult to find in the left nucleus. In the compensated animals, the gain at 1.3 Hz improved significantly on the left, remained low relative to controls at .13 Hz bilaterally, and became equivalent bilaterally at both frequencies. The phase advanced at 1.3 Hz bilaterally. In the right nucleus at 1.3 Hz (n=22), gain = .37±.02 and phase = 14.8±.6; and at .13 Hz (n=19), gain = .19±.01 and phase = 43.5±.9. In the left MVN at 1.3 Hz (n=10), the gain = .35±.03 and phase = 9.1±1.5; and at .13 Hz (n=9), gain = .20±.01 and phase = 44.1±1.9. These data indicate that compensation of type II dynamic responses is incomplete and frequency dependent. (Supported by NASA grant NAG 2-26 and NIH grant NS24391)

137.12

NALOXONE INDUCES AN EXCITATORY EFFECT ON THE VESTIBULAR SYSTEM PRIMARY AFFERENTS. E. Soto, M. E. Pérez* and R. Vega.* Universidad Autónoma de Puebla, Depto de Ciencias Fisiologicas - ICUAP, Apartado Postal 406, Puebla, Pue. México.

It has been reported that in the cochlea and vestibular system there exist immunorreactivity to some neuropeptides, particularly enkephalines. With the aim to contribute to the understanding of peptides role in synaptic transmission on the peripherial vestibular organ, we studied the effect of naloxone on the activity of vestibular system primary afferents of the axolotl (Ambystoma mexicanum).

Multiunit recordings from afferent fibers were done by a suction electrode. Naloxone 10^{-4} and 10^{-3} M was applied by microperfusion in volumes of 20 µl. We found that naloxone 10^{-4} M induces an increase of about 40% of the basal resting discharge, its effect lasting about 2 min. Naloxone did not modify the response of afferent fibers to mechanical stimulation nor the effect induced by glutamate 10^{-3} M or kainate 10^{-4} M.

These results suggest that enkephalines participate in synaptic transmission in the inner ear of amphibians, exerting an inhibitory influence upon its synaptic target.

137.13

THE EFFECTS OF CHOLINERGIC AGONISTS ON THE PASSIVE MEMBRANE PROPERTIES OF RAT MEDIAL VESTIBULAR NEURONS IN VITRO. K.D. Phelan and J.P. Gallagher. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

We have previously demonstrated that primary afferent transmission to the medial vestibular nucleus (MVN) is not mediated by acetylcholine (ACh), but rather an excitatory amino acid (Soc. Neurosci. Abstr. 11:104). The purpose of the present study was to determine the effects of cholinergic agonists on rat MVN neurons using intracellular recording methods in an in vitro transverse slice preparation.

Superfusion of ACh (10 µM) produced membrane depolarization (<10 mV), increased spontaneous firing, and decreased input resistance (<20%). Muscarine (10 µM), a selective muscarinic agonist, predominantly caused membrane depolarization (<10 mV) and increased spontaneous firing through an apparent decrease (<20%) of a K^+ conductance. In some instances, muscarine produced membrane hyperpolarization and decreased resistance. Pretreatment with atropine (1 µM) reversibly blocked the effects of muscarine. The selective nicotinic agonists, nicotine (20 µM) and dimethylphenylpiperazinium (DMPP) produced membrane depolarization (<10 mV), increased spontaneous firing, and decreased input resistance (<40%). Pretreatment with mecamylamine (10 µM) reversibly suppressed these effects. The effects of each cholinergic agonist persisted in the presence of tetrodotoxin or a low Ca^{2+}/high Mg^{2+} solution.

These findings indicate that MVN neurons possess muscarinic as well as nicotinic cholinergic receptors. Our results suggest cholinergic mechanisms may play a prominent role in the modulation of the activity of MVN neurons.

(Supported by NASA Grant NAG2-260)

137.14

EFFECTS OF ACUTE HEMILABYRINTHECTOMY ON THE RESPONSES OF IPSILATERAL VESTIBULAR NUCLEAR NEURONS TO BIDIRECTIONAL OFF-VERTICAL AXIS ROTATIONS (OVAR). Y.S. Chan, Y.M. Cheung and J.C. Hwang. Dept. of Physiology, University of Hong Kong, Sassoon Road, Hong Kong.

This study aims to explore how coding of spatial and directional information about the animal's horizontal plane is processed in vestibular nuclear neurons on the lesioned side by subjecting the decerebrate hemilabyrinthectomized (HL) cats to constant-velocity OVAR in the clockwise (CW) and counterclockwise (CCW) directions. At 4-18 hr after HL, the responses of central vestibular units could be classified into 2 groups based on their responses to bidirectional 10° OVAR (1.75°/s). The first group of units exhibited response patterns similar to those obtained in cats with intact labyrinths (Chan et al., Brain Res., 406, 1987). These units responded to either CW or CCW OVAR with position-dependent discharge modulation (with comparable gains in either direction). About 2/3 of the units exhibited phase lead response, while the remaining 1/3 showed phase lag response. The mean response gain of these units, however, was only 1/3 of that observed in labyrinth intact animals. The other group of units showed discharge modulation only to 10° OVAR in either the CW or CCW direction but not to both. Some units of the latter group became responsive to bidirectional OVAR with increase in the amplitude of head tilt or the velocity of rotation, although the gain ratio of CW and CCW were not necessarily equal to 1.

For each bidirectionally responsive unit, the characterized directional axis which represents the direction given by the averaged position of the CW/CCW discharge maxima was determined. Such directional axis of each unit remained fairly stable at different head tilts (5-25°) or velocities of rotation (<15°/s), a phenomenon similar to that observed in intact preparations. In both the intact and HL preparations, the directional axes of the units were distributed fairly uniformly along the pitch and roll axes and the subtended angles. There was an apparent change in the relative percentage of type 1 to type 2 units, and that of type α to type β units in HL cats as compared to those of the intact cats. Such observed difference in the population of cells sampled could be due to the restricted sensory inputs from the contralateral intact otoliths. (Supported by research grants from C.R.C.G., Sun Yat Sen Fund and Leung K. K. Fund.)

138.1

LINEAR VESTIBULO-OCULAR REFLEX (LVOR) OF SQUIRREL MONKEY: I. BASIC CHARACTERISTICS. D.L.Tomko and G.D.Paige. NASA Ames Research Center, Moffett Field, CA 94035.

Horizontal (H-LVOR), vertical (V-LVOR) and torsional (T-LVOR) eye movements were recorded with dual search coils in 6 squirrel monkeys during linear accelerations (0.5-5.0 Hz, 0.36g Pk) delivered by an air bearing linear sled. Linear stimuli were generated along the animals' naso-occipital (NO), inter-aural (IA) and rostro-caudal (RC) axes. For each stimulus axis, 4 of the following six animal positions were tested: upright, inverted, nose up or down, and left or right ear down. Gains (deg/s/g) and phases (eye velocity re head velocity) were calculated.

Two LVORs, H-LVOR during IA and V-LVOR during RC oscillation were compensatory, with eye velocity opposing head velocity (phase near 180 deg), aiding maintenance of gaze fixation. Gains for both reflexes rose from 15 to 30 deg/sec/g as frequency increased from 0.5 to 5.0 Hz. A noncompensatory T-LVOR during IA and V-LVOR during NO oscillation were also recorded whose gains were smaller (8-15 deg/sec/g) than the compensatory reflexes. For all axes of motion, no systematic modification in gain or phase was observed as a function of head orientation. Thus, the LVORs from 0.5 to 5.0 Hz behave as a set of specific vectorially-defined reflexes analogous to the angular VORs.

Supported by NASA Space Medicine Tasks 199-22-92-02 and -03 and NASA grant NCA2-222.

138.2

LINEAR VESTIBULO-OCULAR REFLEX (LVOR) OF SQUIRREL MONKEY II: VISUAL-VESTIBULAR INTERACTIONS. G.D. Paige and D.L. Tomko, NASA Ames Research Center, Moffett Field, CA 94035.

Horizontal (H-LVOR) and vertical (V-LVOR) eye movements were recorded (coil technique) in monkeys secured in a 3 axis positioning device riding on air bearings. Linear oscillations (0.5-5.0 Hz, 0.36g pk.) were generated along the naso-occipital (NO), inter-aural (IA) and rostro-caudal (RC) axes, in darkness and in light while monkeys looked at the container wall (visual suppression; LVSVOR).

The H-LVOR during IA and V-LVOR during RC oscillation were compensatory: eye velocity opposed head velocity. Compared to LVOR gain (o/s/g) in darkness , LVSVOR gain for both reflexes dropped to near 0 at 0.5 Hz. However, gain rose as frequency increased, to nearly double that in darkness at 5 Hz. Since visual influences on eye movements are nearly gone at 5 Hz, we surmised that a measure of target distance (TD), likely vergence-dependent, biased the LVSVOR for fixation on the container wall (TD=20 cm). Darkness presumably allowed distant "fixation" and smaller gains. When vergence was measured during oscillations (binocular coils), vergence angle and gain were closely linked. A simple geometric model explains the findings.

During NO motion above 0.5 Hz, responses depended on gaze re axis of motion (e.g. rightward during right gaze, upward during up gaze, etc.). This unique switching in the LVOR is consistent with a compensatory, TD- and gaze-dependent geometric model.

Support: NASA Space Med. Tasks 199-22-92-02 & 3; NCA2-222.

138.3

RELATIVE CONTRIBUTIONS OF COMPENSATORY HEAD AND EYE MOVEMENTS TO VISUAL STABILIZATION DURING CIRCULAR LOCOMOTION IN LIGHT. David Solomon and Bernard Cohen, Depts. of Neurology and Physiology, Mt. Sinai School of Medicine, City University of New York, 10029

We studied characteristics of gaze during running in light to determine the effects of properties of the visual surround. The dimensions of the running platform and experimental chamber were such that the monkey's axis of rotation (.3 m) was comparable to surround distances, necessitating a gain change to stabilize the visual surround. Eye velocity re head ($\dot{E}h$), head velocity re body ($\dot{H}b$), body velocity in space ($\dot{B}s$) and angular position relative to the surround were recorded as the animal circled in light. The curtain to the chamber was either open or closed, changing the surround distance in the direction of gaze during part of each revolution. An open curtain allowed for the possibility of an investigator appearing with a fruit reward. $\dot{E}h$ and $\dot{H}b$ were scaled and summed to yield gaze velocity re body ($\dot{G}b$). There was continuous compensatory nystagmus of the eyes and head during running. Ocular quick phases were larger and of shorter duration when the head was free than when it was fixed during passive rotation, probably due to the VOR acting toward the end of the movement.

Slow phase gain was calculated by dividing $\dot{G}b$ by $\dot{B}s$ and averaging according to angular postition. Gains were as low as .7, indicating some tolerance for retinal slip. When passing the presumed area of interest, gain varied from 1.0 to 1.3, depending on the animal's distance from the visual surround. $\dot{E}h$ gain was typically around .6 (range .3-.9) and $\dot{H}b$ gain around .3 (.2-.7). $\dot{H}b$ gain built to a maximum with a corresponding drop in relative $\dot{E}h$ contribution when $\dot{G}b$ gain was highest while running past the sector of interest. Thus, eye-head coordination strategies are used differentially when prolonged gaze stabilization of a particular field is desired. Supported by NS00294 & EY01867.

138.4

PITCH AND YAW VESTIBULO-OCULAR REFLEXES RESPOND DIFFERENTLY TO GRAVITY SENSE. S.A. Rude, K.D. Powell*, J.F. Baker. Dept. of Physiology, Northwestern Univ. Sch. of Med., Chicago, IL 60611.

Last year we presented a study of the horizontal vestibulo-ocular reflex (VOR) in the cat showing that during low frequency yaw rotations the horizontal VOR is significantly improved with the addition of a changing gravity stimulus. This improvement was observed when the animal was positioned on either its left or right side, despite reversed saccular otolith versus canal stimulus phases in the two positions. Does the vertical VOR show an analogous improvement in its low-frequency response during pitch rotations in prone and supine positions as compared to pitch on the side, where there is no changing gravity stimulus?

We made electro-oculographic recordings of the pitch vertical VOR during small amplitude oscillations from 0.01 Hz to 1.0 Hz in the prone, supine (rolled 180° from prone), left and right side (rolled ±90° from prone), and nose down (pitched 90° nose down) positions. Although the prone position vertical VOR was accurate at all frequencies, on side pitch produced phase advances below 0.1 Hz with corresponding gain decreases. On side vertical VOR was asymmetric, with stronger upward than downward slow phase. This confirms Tomko *et al.* (*Exp. Brain Res.*, 69:307, 1988) who used much larger amplitude rotations. The supine vertical VOR below 0.1 Hz showed average phase advances greater than on side VOR, and generally lower gains. However, supine VOR was difficult to interpret at 0.01 Hz and showed marked asymmetry of the upward vs. downward response. Barmack obtained similar results in rabbits (*J. Physiol.*, 314:547, 1981). Nose down orientations produced accurate VVOR phase responses though gain was somewhat lower than while prone.

Thus the vertical VOR was improved in the prone position, which involved a changing gravity stimulus. However, when the phase of the utricular stimulus was reversed with respect to the canal stimulus by placing the cat in the supine position the low frequency vertical VOR was less accurate than without a changing gravity stimulus. Support: EY05289, EY06485, EY07342.

138.5

ADAPTATION IMPROVES THE LOW FREQUENCY PITCH VESTIBULO-OCULAR REFLEX (PVOR) IN SUPINE CATS. K.D. Powell*, S.A. Rude, J.M. Banovetz*, S.I. Perlmutter, B.W. Peterson, J.F. Baker. Interdepartmental Graduate Program in Neuroscience and Dept. Physiology, Northwestern Univ., Chicago, IL 60611. Support: EY05289, EY06485, EY07342.

In the preceding abstract we show that the pitch vestibulo-ocular reflex (PVOR) of cats in the prone position is accurate for whole body rotations at frequencies as low as 0.01Hz, but that the PVOR of cats in the supine position has advanced phase and lower gain below 0.1 Hz. This poor performance may be due to reversal of the utricular stimulus in the supine position. Is the deficiency of the supine PVOR a result of "hard wiring" of the PVOR neural network, or is it an adaptive response to greater experience in the prone versus supine position? If it is the latter, then an adaptation paradigm should produce improvement in the supine PVOR.

We attempted to adapt the supine VOR in 3 cats in 6 experiments. The PVOR was electrooculographically recorded during whole body pitch rotations in darkness at several frequencies in the prone, supine, and 90° rolled positions, before and after adaptation. During 2 hr adaptation, a supine cat was pitched at 0.05 Hz and viewed an optokinetic pattern which rotated at 0.05 Hz, but 180° out of phase with the head and thus in phase with desired VOR. In all 6 experiments low frequency PVOR gain in the supine position was significantly increased by adaptation. In 5 experiments low frequency PVOR phase in the supine position was significantly less advanced after adaptation. The PVOR of the cat in prone position was not made less accurate by the adaptation. We conclude that inaccuracy of the PVOR of the cat in the supine position is in part caused by inexperience. While supine PVOR improved, changes were smaller than we have observed in 0.05 Hz VOR direction adaptation experiments. The small effect could be due to habituation, asymmetry of up versus down eye movements, or less VOR plasticity in the supine position.

138.6

MACULO-OCULAR REFLEX OF THE RAT ELICITED BY LINEAR ACCELERATION AND OFF-VERTICAL AXIS ROTATION. B.J.M. Hess[1]* and N. Dieringer[2]*. Neurol. Clinic[1] Zürich (CH); Physiol. Institut[2], Munich (FRG).

The maculo-ocular reflex (MOR) was studied in brown rats during static tilt, sinusoidal linear acceleration on a sled and during constant-velocity rotation on a table tilted up to 30° re earth vertical (OVAR). Eye movements were recorded with a magnetic field search coil system which allowed to measure linearly horizontal, vertical and torsional components with a double search coil. Static tilt or sinusoidal linear horizontal accelerations (0.2-1.0 Hz, up to 30 cm) elicited predominantly vertical and torsional MOR responses with a gain up to 0.25, depending on the orientation of the head re direction of acceleration. OVAR elicited vertical and torsional eye movements with 2-3 times higher gains than corresponding MOR responses. An additional horizontal component, consisting of a bias velocity and a superimposed sinusoidal modulation showed a clear directional asymmetry for the two eyes: Responses of the eye ipsilateral to the direction of a table rotation had a smaller gain. The phase values of vertical and torsional OVAR responses reflected the animal's best orientation re acceleration for optimal MOR responses on the sled. (SPON: ENA)

138.7

EYE AND NECK MOTOR COUPLING IS DEPENDENT ON THE PRESENCE OF ORIENTING STIMULI. J. M. Banovetz*, K. Fukushima, S. A. Rude, J. F. Baker, and B. W. Peterson. Depts. of Physiol., Northwestern Univ., Chgo, IL and Hokkido Univ., Sapporo, Japan. Support: EY05289, EY06485, EY07342

Recent evidence suggests that neck reflex responses are the product of collateral excitation by oculomotor systems (Vidal, et. al.,1982). We propose that eye-neck coupling is dependent on visual or other sensory targets to which the animal trys to orient. Orienting responses may lead to neck EMG, torque, and position responses that are well correlated with eye movements. In the absence of such targets, the neck motor system is uncoupled and functions other than orienting can be effectively studied.

We have studied the extent of eye-neck coupling by measuring EOGs, Biventer, Complexus, Splenius, Occipitoscapularis, Rectus Major, and Obliquus EMGs, and torque production about the C1-C2 and C1-skull joints during whole body rotations in 3 dimensions. In the absence of target stimuli, the EMG's and torques are closely correlated with the vestibular stimulus, and are unrelated to saccadic eye movements. When the animal is rotated in the light, however, with consequent visual stimulation, the neck torques show a clear correlation with eye position. The important difference is the presence of target stimuli. Furthermore, when the vestibuloocular reflex (VOR) is adapted for two hours by rotating the animal in the dark with an optokinetic stimulus rotating synchronously in an orthogonal direction, the VOR develops an adaptive component in the optokinetic direction, but the neck torques demonstrate little or no adaptation. This evidence suggests that, although there may be overlap in the vestibular neuron populations that drive the ocular and neck reflexes, the neck motor output is not driven by or closely related to eye position, and the vestibular control loops are largely seperate. Coordinated eye and neck movements may be controlled by higher centers, such as the Superior Colliculus, perhaps activating a specific coupling pathway such as the tecto-reticulospinal pathway of Grantyn and Berthoz (1983, 1985).

138.8

GAZE-ADJUSTING SACCADIC COMPONENT OF GOAL-DIRECTED VESTIBULO-OCULAR FUNCTION. Bernard N. Segal & Joseph Bloom*. Dept. Otolaryngol., Sir MB Davis-Jewish General Hosp. & McGill Univ., Montréal, Qué., Canada H3T1E2

If a subject is briefly turned during short darkness intervals while trying to "look" at a point on the wall, the eyes tend to be kept on the unseen point by both slow-phase and rapid eye movements (Exp Brain Res 70: 26-32 (1988)). In a given turn, perhaps several rapid movements may occur, whose cumulative effect tends to correct inaccurate slow-phase movements. We now describe properties of individual rapid movements causing this effect.

Gaze (direction of regard in space) was reconstructed by adding computer-sampled head (re: space) and eye (re: head) position signals, the latter being re-calibrated during each turn. Parameters of rapid movements (initial and final gaze error (re: target); amplitude and peak velocity relationship (i.e., "main sequence"); etc.) were calculated.

Preliminary results indicated that the proportion of error-correcting rapid movements was greatest in subjects exhibiting the most slow-phase error. For example, a subject having ≈2/3 of rapid eye movements that reduced gaze error exhibited average slow-phase error, over all turns, that was ≈1/10 of head movement. Moreover, main sequence relationships computed with respect to space (i.e., gaze) were less variable than those computed with respect to the head (i.e., eye). These results suggested that excessive slow-phase error evoked rapid movements which responded to gaze error, and which combined linearly with slow-phase movements. Thus, such rapid movements appeared to be gaze-adjusting saccades which corrected variability of the slow-phase component of goal-directed vestibulo-ocular function.

Supported by Canadian MRC, Sir MBD-Jewish General Hosp. & McGill Univ.

138.9

EFFECT OF COMBINED LINEAR AND ANGULAR ACCELERATION ON THE VESTIBULO-OCULAR REFLEX (VOR). E.W. Sargent* and G.D. Paige (SPON: C. Scudder), Dept. of Otolaryngology, Washington Univ. School of Med., St. Louis, MO 63110.

The VOR during linear motion (LVOR) was studied indirectly from responses to angular rotation with the head placed eccentrically from the axis of rotation. Eye movements (dual search coil technique) were recorded from squirrel monkeys during angular oscillations generated with the subjects secured in a 3-D positioning device mounted on a radial rail fixed on a rate table. Head position was varied (0-40 cm), as was frequency (0.01-4.0 Hz) and velocity (25-200°/s pk). Low velocity, high frequency trials induced high tangential acceleration (T; .44g pk), whereas high velocities and low frequencies favored centripetal acceleration. With animals upright gain (pk eye/head velocities) of the horizontal VOR increased with T acceleration in the interaural (IA) axis when the monkey faced away from the rotation axis. When facing the axis, VOR gain dropped. The mean gain of the upright IA LVOR was around 70°/s/g. IA accelerations were also assessed with the monkey supine with the head eccentric (generating a torsional but not horizontal VOR). Mean gain of the supine IA LVOR was 30° /s/g. The difference may result from canal-otolith interactions generated by coplanar (vs. orthogonal) accelerations. Responses seen in other planes (e.g., vertical) during combined accelerations will also be discussed.
(Support: NASA NCA2-222; NIH NS07278)

138.10

A WIDE RANGE OF OPTOKINETIC VELOCITIES ELICITS ADAPTATION OF VESTIBULO-OCULAR REFLEX (VOR) DIRECTION IN CATS. C.R. Mason*, S.A. Rude, J.F. Baker (Spon: W. Todd Rainey). Interdepartmental Graduate Program in Neuroscience and Dept. Physiology, Northwestern University Chicago, IL 60611.

Errors in the vestibulo-ocular reflex (VOR) produce retinal slip which, in turn, produces adaptation of the VOR and decreased retinal slip. Different experimental paradigms using lenses or optokinetic projectors produce varying velocities of retinal slip. We are studying the relative effectiveness of different optokinetic velocities in producing VOR direction adaptation.

Three cats were subjected to 0.25 Hz sinusoidal whole body pitch rotations while viewing a 0.25 Hz optokinetic stimulus oscillating horizontally in synchrony with the body rotation. Each cat was subjected to 2 hour training sessions at peak velocities of 6, 12, 24, 48, and 80°/s. Horizontal and vertical electro-oculographic recordings at a variety of pitch rotation frequencies (0.02, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.5 Hz) were made in the dark before and after the training session. Saccades were removed from the records and adaptation was measured as the vectorial difference between pre- and post- adaptation gain of horizontal VOR during pitch.

Maximal VOR direction adaptation occurred at an optokinetic stimulus peak velocity of 24°/s, with decreased adaptation at extremes of velocity.

Two of the 3 animals received aspiration lesions of cerebellar nodulus and uvula, which may be involved in optokinetic following and VOR adaptation. VOR direction adaptation gain was significantly lower after the lesions; however, both animals still exhibited clear VOR adaptation.

We conclude that a wide range of peak velocities of optokinetic stimuli can be paired with head rotation to produce VOR adaptation, and that nodulo-uvular lesions reduce but do not abolish VOR direction adaptation.
Support: EY05289, EY06485, EY07342

138.11

AGE AND LESION DEPENDENCE OF SIZE OF DEITERS GABA IMMUNOPOSITIVE TERMINAL STRUCTURES IN WEAVER MUTANT MICE. J. Bäurle*, U. Grüsser-Cornehls* and B. G. Grover*. (SPON: European Neuroscience Association). Dept. of Physiology, Freie Univ. Berlin, Arnimallee 22, 1000 Berlin 33 (FRG.

The postnatal degeneration of cerebellar granule cells in Weaver mutant mice leads to a reorganization of neuronal circuitry and plastic changes in subjacent cerebellar structures (Grüsser-Cornehls U. and Grover B. G. Neurosci., 13:1224,1987). The goal of the present investigation was to determine whether aging or cerebellar lesions result in size changes of terminals abutting vestibular nuclear cells, from which at least some portion must represent Purkinje cell terminals. Control and Weaver mutant mice ranging in age from 7 days to 9 months and adult cerbellectomized mice were examined.

Camera lucida drawings of terminal structures were made using plan-apo-oil-phase-contrast optics (Zeiss) from material immunoreacted for GABA. The cross-sectional terminal area was evaluated with a Kontron Mini-Mop after Xerox enlargement.

In control mice terminal sizes were smallest in 7-day-old mice (range: 0.2 to 1.6 um^2), increased slowly thereafter up to 5 to 7 months (range: 0.2 to 2-3 um^2), then decreased again. The same was found for mutant mice with the significant difference that terminal sizes encompassed a larger range (7 days: 0.2 to 2.2 um^2, 5 to 7 months: 0.2 to 6 um^2). In cerebellectomized mutant mice terminal sizes were smaller than in unoperated and even smaller than those in age-matched unoperated normals. The increased number of large terminal structures seems to be specific to weaver mutants and is probably one of the functional and plastic changes in cascade caused by the degeneration of granule cells. (Supp. by a DFG grant, Gr 276/19-5)

138.12

HISTOCHEMICAL CORRELATES OF VESTIBULAR COMPENSATION FOLLOWING HEMILABYRINTHECTOMY IN RATS, R.W. Sikes, Dept of Physical Therapy, Northeastern Univ, Boston, MA 02115.

Unilateral damage to the vestibular labyrinth produces profound disturbances of posture and eye movement that are rapidly compensated in most species. In rats, posture returns to nearly normal within hours, and abnormal nystagmus dissipates within days. This study examined changes in relative levels of cytochrome oxidase (CO) in the rat during vestibular compensation.

The membranous labyrinth was surgically removed on the left side in anesthetized Long-Evans rats (200-400g). After a period of 10 hours to one week, the rats were deeply anesthetized and perfused with fixative. Frozen sections were processed histochemically to reveal the levels of CO in the brainstem and spinal cord. The optical density of the reaction product in nuclei ipsi- and contralateral to the lesion was determined and expressed as percent difference.

Differences in CO levels between sides were observed in several nuclei. Density in the ventral horn of the contralateral lower cervical spinal cord was about 16% greater than the ipsilateral side, and densely stained motor neurons were more frequently seen contralaterally. Both the rostral medial and superior vestibular nuclei had greater CO levels contralaterally in the acute animals. The CO levels in the lateral cuneate nucleus were greater ipsilaterally.

138.13

L-BACLOFEN AND THE VOR BEFORE AND AFTER NODULO-UVULECTOMY; GABA RECEPTOR HYPERSENSITIVITY IN THE VESTIBULAR NUCLEI? D. Helwig* and B.Cohen.(SPON: H. Cohen). Depts. of Neurology and Physiology. Mt. Sinai School of Medicine CUNY, New York, N.Y. 10029

We have previously shown that a racemic mixture of baclofen, when injected intramuscularly in doses of 1-5 mg/kg, acts centrally to produce a dose dependent shortening of the dominant time constant (Tc) of the VOR and of OKAN (Cohen et al. 1987). The reduction is at steady state for 1-3 hours after injection and disappears within 12-18 hours. The Tc, measured in the steady state, varied linearly with dose in 8 animals, falling 11%/mg/kg; r = 0.92). Baclofen also causes a reduction in the gain of OKN and the nystagmus produced by off-vertical axis rotation (OVAR) . These responses are all functions of the velocity storage mechanism in the vestibular system (Raphan et al. 1977, 1979), suggesting that inhibitory control of velocity storage is established by GABA, acting on GABAb receptors. In the present study we compared effects of d- and l-baclofen on the VOR and OKAN time constant with the racemic mixture in rhesus monkeys before and after nodulo-uvulectomy. L-baclofen was about twice as effective as the racemic mixture in shortening the VOR and OKAN Tc's, shifting the dose-response curve to the left. D-baclofen was ineffective in changing the Tc's at dosages up to 5 mg/kg. After nodulo-uvulectomy both the racemic mixture and the l- form were more effective in producing a decrease in the Tc than before operation and the dose response curve was shifted to the left. The data indicate that l-baclofen is the active enantiomer that produces inhibition of velocity storage. Postoperative findings are consistent with the hypothesis that a loss of the output of nodular and uvular Purkinje cells to the vestibular nuclei produces GABAb receptor hypersensitivity. Supported by NS00294, EY01867 and a grant from the Young Men's Philanthropic League.

138.14

SIMULATIONS OF LIZARD HEAD MOVEMENTS DUE TO VISUAL AND VESTIBULAR STIMULATION. H.B.Wang* and J.H.Anderson. Depts. of Otolaryngology and Physiology, Univ. of Minn., Minneapolis, MN 55455.

Experimental recordings in the lizard have shown that the head movements due to body rotation in the dark and due to the rotation of the visual surround (optokinetic stimulation, OKS) are dependent on the amplitude of the stimulus. The gain of the response first increases and then decreases as the stimulus amplitude increases. This may be due to a threshold and a saturation which is dependent on the neck angle. When body rotation is combined with the OKS in a synergistic manner, there is a linear summation of the expected responses to each stimulus. However, in the conflict situation (no movement of the body relative to the visual surround) there is not a linear interaction.

In order to characterize these non-linearities further and to define the relative roles which different reflexes play under closed-loop conditions, a model has been developed. Simulations indicate that the effective elasticity of the head/neck system is dependent on the neck angle. Adaptation which is characteristic of the vestibular and proprioceptive inputs is required to give appropriate head velocity profiles. A decrease in the gain of the vestibular input may be present during sensory conflict, but in the synergistic case it can cause oscillations. (Supported by NS-12125 and NS-16567.)

138.15

THE EFFECTS OF GRAVITOINERTIAL FORCE LEVEL ON SUPPRESSION OF VESTIBULAR NYSTAGMUS BY HEAD TILTS. P. DiZio and J.R. Lackner, Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

The time constant of post-rotary nystagmus decay (T) is shorter in free fall (0G) than in normal (1G) or increased (1.8G) gravitoinertial force (gif) levels, and is further shortened by 40° pitch head movements in 1G and 1.8G but not 0G.* Here, we studied 90° post-rotary head tilts. In 0G, 1G and 1.8G four blindfolded subjects were rotated at 60°/s with their heads 45° ventriflexed; they were then stopped suddenly and instructed either to keep their head still or to tilt it back 90°. As previously, we recorded the horizontal electrooculogram and used a log-linear fit to compute T (below, in seconds).

Post-rotary head position	0G	1G	1.8G
No tilt, 45° ventriflexed	10.1	16.8	15.5
90° tilt back from 45° ventriflexed	10.0	8.6	8.7
40° tilt back from 20° ventriflexed*	10.4	11.2	11.4

The data confirm that without head movements T is shorter in 0G than in 1G and 1.8G. In 1G and 1.8G, post-rotary head tilts shorten T by an amount proportional to head tilt amplitude. Head tilts in 1G and 1.8G shorten T significantly below the 0G, no head tilt value, yet in 0G head tilts do not further shorten T. This means sensory-motor signals related to head tilt relative to gif, not tilt relative to the trunk or head movements per se, trigger shortening of post-rotary nystagmus.

*Lackner & DiZio, Soc. for Neurosci. Abst., 12(1), 71.3, 1986. Supported by NASA contract NAS9-15147.

138.16

POSTURAL SYNDROM FOLLOWING GLOBAL AND SELECTIVE LESION OF THE VESTIBULAR APPARATUS IN GUINEA PIG. VIDAL P.P., de WAELE C., GRAF W. (SPON: J.J. Dreyfuss)

The present study was undertaken in order to determine to what extent biomechanical constraints might influence the postural syndrom observed after hemilabyrinthectomy. In the first part of this work, X-ray photographies from above and profile were performed in hemilabyratinthecomized guinea pigs and the different parameters of the postural syndrom analyzed. We found that the head rotation in the horizontal plane, towards the side of the lesion, was mainly due to a rotation of the C1 C2 joint whereas the ipsilateral tilt of the head in the frontal plane was caused by a rotation of the thoracic vertebrae which provocked an en bloc bascule of the cervical column. In the second part, selective lesions of the different branches of the vestibular nerve were realized in order to study the resulting postural syndroms. The section of the horizontal ampullar nerve modify the head neck geometry exclusively in the hozontal plane whereas the destruction of the utricle caused a large head tilt only in the frontal plane. The results lead us to suggest that skeletal geometry influences not only postural control but also its pathology. Furthermore, it appears that to the functionnal segmentation of the cervical column corresponds a functionnal partition of the vestibular afferences.

138.17

MOTION SICKNESS IN SUNCUS MURINUS: A NEW EXPERIMENTAL ANIMAL. N.Matsuki*, S.Ueno*, T.Kaji* and H.Saito*(SPON: M.Kimura). Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., Univ. of Tokyo, Tokyo 113, Japan.

Our previous report have shown that Suncus murinus (house musk shrew), a species of Insectivora, vomits in response to various emetic agents and several antiemetic drugs prevented it (S.Ueno et al., Life Sci.41,513,1987). In the present study, we found that the Suncus murinus vomited when they received reciprocal motion stimulus. Healthy adult Suncus murinus of either sex weighing 40 to 70g were used for the experiments. The animal was placed in a transparent cage on a shaker and received mild reciprocal shaking on a horizontal axis (amplitude: 10-40mm; frequency: 0.5-3Hz) for 5min. The motion stimulus induced the vomiting which was dependent on both amplitude and frequency. When the motion stimulus was repeated, the animals acquired adaptation to the motion sickness. The motion sickness was prevented by the pretreatment of antihistamines. Scopolamine was less effective. Dopaminergic(D_1) antagonists strongly inhibited the motion-induced emesis, and autoradiographic study showed the existence of D_1-receptor in the area postrema, suggesting the involvement dopaminergic nervous system. These results indicate that Suncus murinus is a good animal model not only for the research on motion sickness and adaptation but also for the development of anti-motion sickness drugs.

138.18

POSTURAL DISTURBANCES IN PAROXYSMAL POSITIONAL VERTIGO. A.Katsarkas and R.Kearney. Dept. of Otolaryngology and Biomedical Engineering Unit, McGill University, Montreal, Quebec, H3A 1A1, Canada.

Postural sway was measured in fourteen patients suffering from paroxysmal positional vertigo in which the nystagmus elicited during attacks was the short rotatory-oblique type (Katsarkas, A: Ann. Otol. Rhinol. Laryngol.; 96: 305-308, 1987). Patients stood on a force plate for 20 s while the antero-posterior and medial-lateral projections of the center of force were recorded and standard deviations computed. Recordings were made before and after placing the patient's head in the provocative position. Vertigo was elicited and the nystagmus was observed in 10/14 patients; neither vertigo nor nystagmus was induced in the other four, during these recordings.

A paired t test of the sway data ($p<0.01$) showed the medial-lateral sway unchanged in all patients. The antero-posterior sway increased following the provocative maneuver in patients who experienced vertigo and developed nystagmus, while it did not change in those who did not.

It is concluded that paroxysmal positional vertigo, with nystagmus of the rotatory-oblique type of short duration, increases the antero-posterior sway immediately after the vertigo attack, while the medial-lateral sway remains unchanged. These observations have medico-legal implications.

138.19

MODELLING THE THREE DIMENSIONAL STRUCTURE OF THE VELOCITY STORAGE INTEGRATOR. D. Sturm and T. Raphan. Dept. of CIS, Brooklyn College of CUNY, Brooklyn, N.Y. 11210

Velocity storage is present in vertical and roll components of compensatory eye movements if the head is oriented appropriately with regard to gravity (Raphan & Cohen, 1983; Matsuo & Cohen, 1984; Raphan & Cohen, 1987). To explain this, it has been postulated that gravity orients the principal axes of the three dimensional representation of velocity storage towards the space vertical (Raphan & Cohen, 1987; Sturm and Raphan, 1988). Velocity storage which is expressed by OKAN (Raphan et al, 1979), has been represented as a dynamical system, $\dot{x} = Hx$, where x is a three dimensional vector representing the state of the system and H is the matrix containing the parameters that govern its dynamic behavior. The parameters of H were calculated such that the eignevectors associated with the body vertical in the upright position tended to align with gravity. The model simulates the absence of vertical and roll OKAN in the upright position and the increases in vertical and roll velocity storage as a function of head position. It also predicts and simulates the cross-coupling from the horizontal to vertical and roll planes when subjects are on their sides or supine, respectively (Raphan & Cohen, 1988). The model indicates that velocity storage has a three dimensional structure and that the eigenvalues and eigenvectors of its system matrix are closely linked to the gravitational field.
Support: NASA #NAS 9-17720 and NIH EY04148,NS00294

138.20

VISUAL-VESTIBULAR INTERACTION IN PSYCHOSIS: EFFECTS OF BACKGROUND ILLUMINATION R.T. Pivik, P.M. Cooper*. Univ. of Ottawa & Ottawa General Hosp., Ottawa, Canada

The failure of fixation suppression, impaired smooth pursuit tracking under light conditions and significant improvement during dark conditions in psychotic patients (Pivik, et al, Prog. Neuro-Psychopharm. Biol. Psychiat., 11:259, 1987) suggest dysfunction of cerebellar and brainstem structures in these patients. The present study examined the effects of variations in background illumination on the slow phase velocity (SPV) during fixation suppression in psychiatric patients. Psychotic patients [23 actively-ill (A) 23 remitted (R)] and 23 normal controls (C) were studied. Caloric nystagmus was recorded electrooculographically with assessment of visual fixation and arousal. Relative to controls, patient groups showed decreased fixation suppression during both light and dark conditions ($p < .02$). Group light-dark suppression ratios were: C--1.6; R--1.2; and A—.97, i.e., C data were similar to those reported for normal subjects, and, like cerebellar patients (Hood and Waniewski, J. Neurol. Sci., 63:27, 1984) group A data were virtually unaffected by background illumination. R group performance was intermediate. These similarities between cerebellar and psychotic patients provide further evidence suggestive of vestibulo-cerebellar dysfunction in psychotic patients.
(Assisted by: Ontario Mental Health Foundation)

SPINAL CORD AND BRAINSTEM: IMMUNOCYTOCHEMISTRY

139.1

IMMUNOCYTOCHEMICAL LOCALIZATION OF GABA IN NEURONS PROJECTING TO THE DRG. C.A. Livingston and A.J. Berger. Physiol. & Biophys., Univ. of Wash. Sch. Med., Seattle, WA 98195.

Monosynaptic inhibition of dorsal respiratory group (DRG) inspiratory neurons by Botzinger Complex (BOT) expiratory neurons located near the retrofacial nucleus (RFN) may be mediated by γ-aminobutyric acid (GABA). Using a double-labeling technique, we localized GABA-like immunoreactivity (GABA-LI) in neurons projecting to the DRG. In anesthetized cats, we located the DRG by recording inspiratory related activity in the ventrolateral nucleus of the solitary tract using glass microelectrodes containing 10-25% horseradish peroxidase (HRP). HRP was iontophoresed unilaterally into the DRG. The anesthetized cats were maintained for ∿24 h, then perfused with physiological saline followed by 1% paraformaldehyde/2% glutaraldehyde in 0.1 M phosphate buffer (pH=7.4). Serial transverse sections of the medulla were processed to visualize the retrograde tracer, HRP, and GABA-LI. Reticular neurons retrogradely labeled from the DRG (punctate black staining) were scattered bilaterally in the lateral tegmental field and lateral reticular nucleus, but were most densely distributed in the rostral ventrolateral medulla. 20-30% of all cells retrogradely labeled from the DRG were also positive for GABA-LI (punctate black staining/homogeneous brown background). The exclusive distribution of these double-labeled cells in the rostral medulla near the RFN suggests that they are BOT neurons. Presumptive GABA-ergic terminals were noted in the DRG. These observations indicate that GABA is a transmitter utilized by BOT expiratory neurons. (NS 14857 & HHS NS 07097)

139.2

COLOCALIZATION OF 5-HT FIBERS AND MOTONEURON POOLS IN THE CHICK SPINAL CORD DETERMINED WITH CHOLERATOXIN HRP AND IMMUNOHISTOCHEMISTRY. S. Homma*, K. Kohno* and N. Okado. Dept. Anat., Inst. Basic Med. Sci., Univ. Tsukuba, Ibaraki 305, Japan.

50-100 µl of 0.1 % HRP conjugated with choleratoxin subunit B (List lab) was injected in the muscle, and chicks were perfused with Zamboni's fixative. The alternative sections of the spinal cord were processed with TMB and with an antibody against 5-HT. In the lumbar spinal cord (segments XXIV-XXXI), the distribution pattern of 5-HT immunoreactive fibers in the lateral motor column (LMC) varied for different MNPs. In segments XXV and XXVI, a high density of 5-HT fibers was located in the dorsolateral and dorsomedial parts of the LMC, which corresponds to the MNPs of the iliotibial and ischioflexorius muscles, respectively. In segment XXVII, there were two regions with high density of 5-HT fibers in the MNPs of the iliofibular and dorsal shank muscles (lateral), and of the accessory muscles (mediolateral). In the caudal part of the lumbo-sacral spinal cord (segments XXIX - XXXI), a high density of immunoreactive fibers was found in the dorsolateral LMC, as in the MNP of the caudiliotibial muscle. The present study has demonstrated that the pattern of distribution of 5-HT fibers varies considerably between different MNPs suggesting that MNPs may be differentially affected by ongoing changes of 5-HT synaptic transmission.

139.3

IDENTIFICATION OF CATECHOLAMINE FIBERS AND SYNAPSES IN LAMINA IX OF THE RAT LUMBAR SPINAL CORD. E.W. Johnson* and H.O. Nornes. Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO. (Supported by NIH #NS21309-02)

A double labeling technique was used to identify catecholamine (CA) fibers and terminals simultaneously with retrogradely labeled motor neurons in the lumbar cord. Both techniques used a peroxidase label and a DAB reaction product for identification. Motor neurons were retrogradely labeled by injecting the gastrocnemius muscle with WGA-HRP. The immunocytochemical procedure employed primary antibodies directed against tyrosine hydroxylase (TH) - the rate limiting enzyme in CA synthesis.

At the light microscopic level, the TH-immunoreactive fibers were distinguished from the labeled motor neurons and could be found surrounding them. For ultrastructural analyses, labeled motor neurons and surrounding fibers were isolated for electron microscopy.

Distinctive differences between the two labels were confirmed at the ultrastructural level. The HRP in the motor neurons was in electron dense vesicles distributed within the cytoplasm of somata and proximal dendrites. TH-immunoreactive fibers were filled with an amorphous electron dense reaction product which also surrounded the mitochondria.

CA fibers were observed to form synapses on processes within lamina IX of the rat lumbar spinal cord. Contacts between CA fibers and retrogradely labeled motor neurons have also been observed.

This double labeling protocol is a reliable method for identifying catecholamine fibers simultaneously with motor neurons at the LM and EM levels. The presence of CA synaptic terminals in lamina IX of the lumbar spinal cord is consistent with the implicated role of this system in modulation of reflexes and locomotion.

139.4

CATECHOLAMINE NEURONS IN THE BRAINSTEM OF THE BABOON. I. Nadelhaft, R. Segal*, S.K. Wolfson, E.E. Cook* and P.J. Jannetta. VA Med. Ctr., Univ. of Pittsburgh, Depts. of Neurosurgery and Pharmacology, and Montefiore Hosp., Pittsburgh, PA.

As a part of our study of neurons in the ventrolateral medulla (VLM) and their role in the control of blood pressure we have determined the locations of catecholaminergic neurons in the baboon medulla. Antisera to dopamine beta hydroxylase and phenylethanolamine-N-methyltransferase were used to identify noradrenergic (NA) and adrenergic (A) neurons respectively. In a typical animal (8.7 kg) NA neurons were found from 1 to 10 mm rostral to obex and had a broad peak at about 4 mm whereas A neurons were located between 2.5 and 8 mm from obex with a peak at about 6 mm. There were about 10,000 NA neurons and about 8,000 A neurons (uncorrected). These were equally divided between sides. The cells had a fusiform shape (45 x 15 microns) and exhibited apical processes. A neurons were restricted to the VLM whereas NA neurons were found in the VLM and also in a narrow band extending dorsally to the floor of the fourth ventricle. In conclusion, the distribution and morphology of these neurons in the primate is similar to that found in the lower animals.

139.5

CATECHOLAMINE INNERVATION OF DEVELOPING DENDRITE BUNDLES IN THE LUMBOSACRAL SPINAL CORD OF THE RAT. **P.E. Kunkler* and W.J. Anderson. Indiana University School of Medicine, Terre Haute Center for Medical Education, Terre Haute, IN**

In previous investigations from our laboratory, the morphological description of dendrite bundles (DB) in the lumbar spinal cord of the rat have been described. The motoneurons (MN) related to these DB's have been identified as innervating the levator and bulbocavernosus muscles. Since these MN groups are dimorphic in nature, our interest was in determining the noradrenergic innervation. Catecholamine (CA) histofluorescence in 30 day old rats showed the CA fibers enter the DB perpendicular to the horizontally-oriented dendrites and then turns rostrally or caudally to course adjacent to the dendrites or form pericellular rings around DB related MN's. At day 1 and 5, CA fibers could be seen in concentrated amongst the MN's. At day 7 this increased slightly, and by day 10 reached it's maximum intensity. At day 15, the intensity decreases until day 20 where it is similar to 30 and 45 day old rats. This pattern correlates exactly with the longitudinal dendrite growth in these MN's.

139.6

ORIGIN OF CATECHOLAMINERGIC PROJECTIONS TO THE SPINAL CORD IN THE DEVELOPING OPOSSUM. R.R. Pindzola, R.H. Ho and G.F. Martin. Department of Anatomy and Neuroscience Program, The Ohio State University College of Medicine, Columbus, Ohio, 43210.

Tyrosine hydroxylase-like immunoreactive (TH-IR) axons are present in the marginal zone of the spinal cord in newborn opossums and they innervate the gray matter over an extended period of time. The brainstem origin of such axons, presumably catecholaminergic, to the lumbar spinal cord was studied in developing opossums using a combination of the retrograde transport of fluorescent dyes and immunofluorescence. By at least postnatal day 20, TH-IR neurons that project to the lumbar cord could be identified within all the nuclei which contain them in adult animals (Pindzola et al. Neurosci. Abst., 12(2), 1547, 1986). Retrogradely labeled neurons containing TH-IR were also found in areas not containing them in adult animals. For example, they were present in the dorsal part of the locus coeruleus, a region which does not project to the spinal cord in adult animals, but does so in young opossums. It is possible that these neurons provide transient catecholaminergic projections to the spinal cord. (Funded by NS25095).

SPINAL CORD AND BRAINSTEM: ANATOMY

140.1

THE FINE STRUCTURE OF RUBROSPINAL PROJECTIONS IN THE NONHUMAN PRIMATE DEMONSTRATED BY ANTEROGRADE AXONAL TRANSPORT OF WGA-HRP. D.D. Ralston, A.M. Milroy* and G. Holstege. The Department of Anatomy, University of California, San Francisco, California 94143.

The spinal cord of the primate receives descending motor projections for processing motor output to axial and distal musculature from a variety of sources: corticospinal, vestibulospinal, reticulospinal and rubrospinal. This latter system in the primate, a phylogenetically older extrapyramidal system, has long been considered subordinate to the corticospinal tract, utilizing the interneuronal population of the intermediate spinal grey matter to affect, polysynaptically, the motoneurons lying in the lateral motoneuron pools of the ventral horn that subserve distal musculature of the upper and lower extremities. This study demonstrates the nature of the ultrastructural projections from the red nucleus to the spinal intermediate and ventral grey matter.

Macaca fascicularis were used to determine the nature of these projections. The daily care, housing, anaesthesia and pre- and postoperative care were provided according to N.I.H. and U.C.S.F. Animal Care Committee guidelines. Injections of 5% WGA-HRP were placed into the red nucleus allowing survival times of 2-3 days at which time the animals were perfused intracardially with aldehyde solutions. The tissue was reacted for TMB-EM (Olucha, '85) and prepared for electron microscopy following a slow osmication method (Henry, '85) and then viewed with a JOEL 100 CX electron microscope.

The results indicate that the red nucleus projects primarily to intermediate grey matter but also to the motoneuronal pools. Labeled round vesicle terminals making asymmetric contacts predominate, synapsing upon small, medium and large dendrites and occasional cell somata of both regions. Pleomorphic and flat vesicle terminals have also been observed to contain label. Some vesicles within the terminals are coated and occasionally subsynaptic densities demonstrate Taxi bodies. The large neurons in the ventral horn receiving labeled synapses have not been identified as α- or γ-motoneurons.

We conclude from this study that the rubrospinal tract projects primarily to the intermediate grey matter to affect the motoneurons subserving distal musculature via a polysynaptic pathway. However, there is a direct monosynaptic rubrospinal projection to motoneurons in the ventral horn inferring greater influence of the red nucleus on motor output then previously thought. This study provides additional evidence for the mechanism of processing descending rubral projections. (Supported by NS-23347 from N.I.H.)

140.2

ANATOMICAL EVIDENCE FOR DIRECT RUBROSPINAL PROJECTIONS TO MOTONEURONS IN THE MONKEY. A LIGHT MICROSCOPICAL WGA-HRP STUDY. B. F. M. Blok*, G. Holstege and D. Daly Ralston (SPON: W. R. MEHLER) Dept. Anatomy University California San Francisco CA 94143

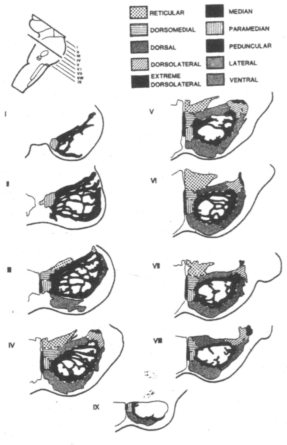
Recently, direct rubrospinal projections to motoneurons have been demonstrated in the cat (Holstege 1987; McCurdy et al. 1987). This raised the question whether this was also the case in monkey. Therefore, in 3 cases relatively large unilateral WGA-HRP injections were made in the mesencephalic and rostral pontine tegmentum of the rhesus monkey. In 2 cases the injection-sites involved the red nucleus. After 4 days survival, the animals were deeply anaesthetized and perfused with an aldehyde solution. Serial vibratome sections were made of the brainstem and spinal cord. They were processed using TMB as a chromogen.

In all 3 cases the ipsilateral interstitiospinal tract was labeled, distributing fibers to the medial part of the intermediate zone, throughout the length of the spinal cord. In the two cases, in which the magnocellular red nucleus was injected, labeled fibers were also present in the contralateral rubrospinal fiber bundle, terminating in Rexed's laminae V, VI and VII, but not in lamina VIII. In one case these projections were found throughout the length of the spinal cord, but in the other case, in which the injection was smaller and more medial, labeled fibers were sparse at lumbosacral levels. In both cases labeled fibers were also distributed to the motoneuronal cell groups, innervating muscles of hand and peripheral parts of the arm and in the case with the large injection they were also found in the motoneuronal cell groups innervating the muscles of the feet and peripheral parts of the leg. No projections were found to motoneurons innervating more proximal muscles of the body. These results indicate that in the monkey, the rubrospinal tract terminates in the same parts of the spinal gray as the contralateral corticospinal pathway. Supported by NASA Grant 2-484903-26308

140.3

CYTOARCHITECTURE OF BASIS PONTIS IN RHESUS MONKEY. J.D.Schmahmann & D.N.Pandya. Boston University & Bedford VA Hospital.

Early workers defined distinct nuclei within the mammalian basis pontis, however these findings have been only loosely utilized in recent studies. Using cresyl violet, thionin, and acetylcholinesterase stained transverse sections, the pontine nuclei of ten rhesus monkeys were studied in terms of the size, shape, degree of Nissl staining, packing density, and orientation of their neurons. The boundaries and characteristics of the nuclei were thereby established.



140.4

COURSE OF SPINOCEREBELLAR AXONS IN THE VENTRAL AND LATERAL FUNICULI OF THE CAT SPINAL CORD. G. Grant and Q. Xu*. Dept. of Anatomy, Karolinska Institutet, S 104 01 Stockholm, Sweden.

The aim of this study was to produce selective labeling of spinocerebellar fibers in the spinal white matter by retrograde axonal transport and to locate such retrogradely labeled spinocerebellar fibers originating from different spinal levels. Following unilateral transections of the ventral and lateral funiculi at different spinal levels or lesions of the restiform body, HRP or WGA-HRP was injected bilaterally into the cerebellum. This resulted in different labeling patterns related to the level and extent of the lesion. Based on this finding, the fiber course of the spinocerebellar fibers in the spinal white matter could be established. It was found that the fibers of the dorsal and ventral spinocerebellar tracts (DSCT and VSCT) ascending in the dorsolateral and ventrolateral fasciculi undergo a shift during their rostral course. Interestingly, the dorsalmost part of the VSCT, originating from the sacrococcygeal region, shifts into the dorsolateral fasciculus to join the DSCT. Both the DSCT and the VSCT are topically arranged. In addition to the course of the fibers of the DSCT and the VSCT, the course in the white matter for spinocerebellar fibers originating from the cervical enlargement and from the central cervical nucleus could also be established.

140.5

MOTONEURONS INNERVATING THE RAT AXIAL MUSCLE LONGISSIMUS CERVICIS ARE NOT CONFINED TO THE MEDIAL MOTOR COLUMN OF THE CERVICAL SPINAL CORD.
M. Kramer and T. W. Deacon. Biological Anthropology, Harvard University, Cambridge, MA 02138.

It is generally accepted that the neurons of the medial motor column provide the sole innervation of axial musculature, while the neurons of the lateral motor column innervate the remainder of body musculature. As a continuation of our previous work which characterized the motoneuronal organization of axial muscles in the rat and macaque, the present study focuses on the organization of motoneurons innervating the cervical epaxial muscle, the longissimus cervicis, of the rat. In this study 1-3 microliters of WGA-HRP were injected into the longissimus cervicis of the rat (N=4). Care was taken to prevent spread of the tracer to the overlying shoulder and neck muscles. In addition, adjacent epaxial muscles were cut and reacted to verify the injection site.

The motoneurons in the cervical spinal cord of the rat can be cytoarchitectonically divided into 3 nuclei: a medial motor column nucleus, M, and two lateral motor column nuclei, VL and DL. Following injections into the longissimus cervicis labelled motoneurons are observed in the medial motor column nucleus, M. In addition, labelled motoneurons are observed in the VL nucleus of the lateral motor column. These data demonstrate that the longissimus cervicis of the rat is innervated by motoneurons located in both the medial and the lateral motor columns. This finding suggests that the traditional view that the motoneurons innervating axial musculature are confined solely to the medial motor column does not adequately describe the innnervation of the epaxial muscle, the longissimus cervicis, of the rat.

140.6

CORTICOSPINAL AXONS FROM HAMSTER SENSORY AND MOTOR CORTEX PROJECT DIFFERENTIALLY TO DORSAL AND VENTRAL HORN SPINAL CORD NEURONS.
R.Z. Kuang* and K. Kalil. (SPON: W. Welker). Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

The rodent sensorimotor cortex has been divided by physiological mapping studies into a broad sensory area in which the body surface is topographically mapped and a narrower motor area from which movements of different regions of the body can be elicited. Regions of sensorimotor overlap have also been reported. However, no detailed anatomical studies have been carried out in rodents to determine whether, as in primates, efferent corticospinal projections arising from layer 5 also reflect these functional distinctions.

Iontophoretic injections of the plant lectin *phaseolus vulgaris* Leucoagglutinin (PHA-L) were used to label small regions of either the sensory or motor cortex in adult hamsters. Survival times of 2 weeks were used to ensure complete filling of corticospinal axon arbors and their terminal boutons. The overall pattern of projections showed that, as in primates, the sensory cortex innervates only the dorsal horn with the heaviest labeling in the medial region of laminae 3, 4 and 5, whereas the motor cortex projects almost exclusively to laminae 5-9 of the ventral horn, with the densest innervation in lamina 7. Moreover, although individual fibers ramify widely, they project exclusively to either the dorsal or the ventral horn. Single axon arbors were reconstructed through serial sections to demonstrate that, as in primates, single corticospinal fibers may project directly to motor neurons occupying several motoneuronal cell groups in lamina 9. These results suggest that projections of the rodent corticospinal pathway reflect their dual sensory and motor cortical origin and may thus have a dual function in modulating afferent sensory inputs to the dorsal horn as well as exerting a direct effect on motoneurons and their associated interneurons in the ventral horn. (Supported by NIH Grant NS-14428).

140.7

A NEW CERVICAL MEDULLARY MOTOR NEURON DENDRITE BUNDLE: FUNCTIONAL IMPLICATIONS OF BRAINSTEM SPINAL CORD INTERACTION
J.B. Taylor and W.J. Anderson. Indiana University School of Medicine, Terre Haute Center for Medical Education, Terre Haute, Indiana.

Previous investigations in our laboratory have morphologically described compact dendrite bundles in the lumbar and cervical spinal cord of the rat. The cervical dendrite bundle group is composed of three motoneuron dendrite columns including the medial dendrite bundle (MDB), the central dendrite bundle (CDB) and the lateral dendrite bundle (LDB), C2-C6. This study describes a new dendritic bundling found in the medulla at the level of the obex which is continuous with the previously characterized cervical bundles. The columns of motoneurons are composed of a MDB and a LDB which converge at C1 to become central and then diverge into the MDB and the LDB once again. There appears to be a direct interaction with the hypoglossal nucleus and possibly the raphe obscurus in the medulla. This motoneuron continuity of dendritic interactions between the medulla and cervical region raises many interesting concepts of spinal cord function.

140.8

DO NUCLEUS AMBIGUUS (NA) CELLS WHICH PROJECT TO THE CEREBELLUM ALSO PROJECT THROUGH THE VAGUS? A FLUORESCENT TRACER STUDY.
J. Lovell and S.L. Stuesse. Neurobiology Department, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

Scattered reports have indicated that various cranial nerve nuclei, including NA, project to the cerebellum. Most previous studies utilized the less sensitive retrograde tracer horseradish peroxidase (HRP). We verified the presence of cerebellar afferents in NA and determined if these cells also sent axons through the vagus by the use of a double labeling technique involving the fluorescent tracers True Blue (TB) and Fluorogold (FG). Rats received approximately 35nl TB in the cervical vagus sheath and 2-3 injections of the same amount of FG at various sites in the cerebellum. 2 days post-injection, 40um serial transverse brainstem sections were obtained and observed. FG labeling was found throughout the length of NA, with the most cells (usu. 10-12/section) occuring near the obex, 4-5 cells caudal to the obex and 2-3 rostral to it. Cells closest to the obex also contained the most FG. A topography of cell types existed such that FG cells were always on the interior of NA surrounded by TB cells. Thus NA projects throughout its length to the cerebellum, with the most projections arising near the obex. NA cells which project to cerebellum are sepa te from those projecting through the vagus.

140.9

NUCLEAR ORIGINS OF PROJECTIONS FROM THE BRAINSTEM RETICULAR FORMATION TO THE CEREBELLUM IN THE RAT.
D.B. Newman and C.Y. Ginsberg*, Department of Anatomy, USUHS, Bethesda, MD 20814-4799

Projections from the brainstem reticular formation (RF) to the cerebellum were examined in hooded rats using retrograde transport of 1% HRP-WGA or 10% HRP. Tissue was processed using TMB histochemistry. Retrogradely-labeled neurons were most numerous in classic precerebellar RF nuclei such as the medullary lateral reticular and paramedian reticular nuclei, as well as the pontine reticulotegmental nucleus. However, many isodendritic, diffuse RF nuclei contained significant numbers of labeled neurons, including medullary RF nuclei such as reticularis dorsalis, ventralis pars alpha, and magnocellularis, pontine nuclei such as pontis caudalis alpha and pontis oralis medialis; and the pedunculopontine nucleus at isthmic levels.

Vermal injections tended to produce more retrogradely labeled RF neurons than hemispheric injections, and injections involving the deep nuclei produced more RF labeling than those confined to the cerebellar cortex.

Within individual RF nuclei, the morphology of labeled neurons was identical to that reported previously by this laboratory subsequent to spinal or cortical HRP injections.

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140.10

CAT MOTONEURONS INNERVATING THE URETHRAL AND ANAL SPHINCTERS HAVE DIFFERENT DENDRITIC ARBORS.
Q. Li* M. G. Leedy, M.S. Beattie, and J.C. Bresnahan. Depts. of Anatomy and Surgery, Ohio State Univ., Columbus, OH. 43210.

Cholera toxin conjugated to HRP was injected into the anal (AS) or urethral (US) striated sphincters of anesthetized female cats to compare locations and dendritic arborization patterns of motoneurons within Onuf's nucleus. TMB processing showed that AS motoneurons are localized dorsomedially in Onuf's nucleus (caudal S1 and rostral S2 segments), while US motoneurons are located ventrolaterally. Dendritic arbors are distinctly different: **AS** dendrites extend dorsally to the **dorsal and interband regions** of the sacral parasympathetic nucleus (SPN); they also extend ventro- and dorsolaterally into the **lateral funiculus. US** dendrites project laterally, then ventrally into mainly the **lateral band** of the SPN; other dendritic arbors invaded the **ventral funiculus.** Both AS and US dendrites project to lamina X. Thus, dendritic arbors of AS and US motoneurons are associated with regions of the SPN subserving defecation and micturition, respectively, and may receive different patterns of descending input. Supported by NS-10165.

140.11

FINE STRUCTURE AND SYNAPTOLOGY OF ESOPHAGEAL MOTONEURONS IN THE RAT. M. R. Moyles* and D. A. Hopkins. Department of Anatomy, Dalhousie University, Halifax, NS, B3H 4H7.

The nucleus ambiguus (AMB) has three major subdivisions which project to the upper alimentary tract in the rat (Bieger and Hopkins, '87). The esophagomotor component, located in the compact formation of the AMB (AMBcom), receives afferent projections from several levels of the neuraxis but very little is known regarding its ultrastructure and synaptology. Therefore, an electron microscopic study of the AMBcom was undertaken.

Transverse or sagittal sections of the medulla oblongata containing the AMBcom of unoperated rats and rats which received lesions of the vagus nerve or injections of WGA-HRP into the esophageal wall or nucleus of the tractus solitarius (NTS) were processed for electron microscopy.

Esophageal motoneurons in the AMBcom were densely packed and had longitudinally oriented dendrites. Somata were characterized by a round nucleus, centrally located nucleolus and abundant short segments of rough endoplasmic reticulum which were not organized into typical Nissl bodies. Perikarya and dendrites were frequently closely apposed without intervening glial processes. The neuropil contained many small-diameter axons adjacent to the perikarya and dendrites of esophageal motoneurons. Axosomatic synapses were very infrequent but axodendritic synapses were numerous and most axon terminals contained round clear vesicles with a few dense-cored vesicles and formed asymmetric junctions with one or more dendrites. After injections of WGA-HRP in the NTS, labeled axon terminals in the AMBcom were characterized by round vesicles and asymmetric synaptic junctions.

In conclusion, the AMBcom contains atypical motoneurons and appears to receive excitatory inputs from the central subnucleus of the NTS.

Supported by MRC of Canada.

140.12

MOTOR NEURONS FOR ACCESSORY RESPIRATORY MUSCLES: COMPARISON WITH PHRENIC AND NON-RESPIRATORY NEURONS. C. G. Charlton, B. Crowell* and R. Benson*. Neuroscience Unit, Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

Motor neurons (MN) for the pectoralis (PE), trapezius (TR), external oblique (EO) and rectus abdominis (RA) were retrogradely labeled to determine if there are distinctions that are related to their role in supportive skeletal and in respiratory functions. These MN were compared with the triceps (T) and phrenic MN.

The accessory respiratory muscles (AMR) MN were mainly confined to the ipsilateral ventrolateral tip of the ventral grey of C6-7. Within this locale the majority of MN for the PE were medial to ventromedial, those for the TR were lateral and those for the EO ventrolateral. The T MN were dorsal to the AMR MN. No MN were observed for the RA in C2 to T2. A second MN column were labeled for the TR in the medioventral ventral grey of C4-6.

The MN for the AMR were oval, of medium size and surrounded by labeled fascicles that may represent mono-synaptic spinal afferent terminals. Like the P MN the AMR MN superimposed a high density of substance P receptors. The T MN were large, elongated with distinct labeled processes and an absence of labeled fascicles. The MN within each column occurred in clumps. The columns are seperated, probably, for achieving discriminatory influences.

Supported by NIMH Grant No. RR03032.

140.13

THE MORPHOLOGY OF JAW-MUSCLE SPINDLE AFFERENTS. D. Dessem* and A. Taylor. Sherrington School of Physiology, St. Thomas's Hospital Medical School, London, England, SE1 7EH.

Intra-axonal penetrations of jaw-elevator muscle spindle afferents in rats were characterized by their increased firing to jaw-opening (muscle stretch), gentle probing of the jaw muscles, failure to be activated by stimulation of the inferior alveolar and maxillary nerves and for some penetrations, activation from the masseter nerve. Horseradish peroxidase was injected for 35-245nA min. into stable penetrations with membrane potentials more negative than -40mV and the tissue was processed histochemically to allow visualization of the axons. All stained axons were located in the motor root of the trigeminal nerve and could be followed dorsomedially before bifurcating into an ascending branch in the tract of the mesencephalic nucleus and a descending branch in the tract of Probst. The largest concentration of axon collaterals and synaptic boutons was in the region of the trigeminal motor nucleus with synaptic boutons most numerous dorsal to the motor nucleus. It is not clear however, how many of these contacts are onto cells in this region and how many are onto distal dendrites of motorneurons. Boutons within the motor nucleus were always restricted to a small portion of the nucleus with some closely associated with stained motorneurons presumably representing somatic contacts. Axon collaterals and boutons were also found in the parvicellular reticular formation caudal to the trigeminal motor nucleus, the reticular formation at the level of the facial nucleus, and overlying somata of caudal mesencephalic trigeminal cells.

140.14

TOPOGRAPHICAL ARRANGEMENT AND DENDRITIC ARCHITECTURE OF HYPOGLOSSAL MOTONEURONS IN THE RAT. X. Bao, S.M. Altschuler* and R.R. Miselis. Depts. of Animal Biology and Pediatrics and Inst. of Neurol. Sci., Univ. of Pennsylvania, Philadelphia, PA 19104.

Cholera toxin-horseradish peroxidase conjugate prepared in our laboratory was injected in small quanities into the geniohyoid, genioglossus, styloglossus and hyoglossus muscles to determine the topographic arrangement and dendritic arborizations of the motoneurons of the hypoglossal nucleus (HN) . Motoneurons innervating the geniohyoid and genioglossus muscles were located just outside and ventrolateral to HN caudally and in the ventral HN rostrally. The styloglossus and hyoglossus motoneurons were in the lateral (caudally) and dorsal portion of the HN (rostrally). A few dendrites extended to the nucleus of solitary tract, medial vestibulat nucleus and raphe obscurus. Many dendrites were in the reticular formation and others formed a plexus under the fourth ventricle and around the central canal. Another delicate dendritic plexus formed a light encapsulation of the dorsal HN motoneurons. The upper boundary of the latter was sharpley defined along the ventral border of the dorsal motor nucleus. Therefore, there is a wide ranging target for central afferents to the HN and an interesting interlocking of dendrites and cell bodies of the two motoneuronal groups of the HN. Supported by GM27739 and the MacArthur Foundation.

140.15

THE INFERIOR OLIVARY COMPLEX (IOC) OF THE MANATEE, TRICHECHUS MANATUS: UNEQUIVOCAL EVIDENCE FOR SUBNUCLEUS C AND NUCLEUS BETA AS SEPARATE ENTITIES. M-C. Holst, J.I. Johnson, R. L. Reep, W.I. Welker. Anat., Mich. State U., E. Lansing, MI 48824; Neurosci., U. Fla., Gainesville, FL 32610; Neurophysiol., U. Wis., Madison WI 53706.

The caudorostral elaboration of the medial olive (MO) and associated minor nuclei is particularly evident in the large, well differentiated manatee IOC. Frontal, horizontal and sagittal, Nissl and myelin stained serial sections reveal the caudal pole (subnucleus b) connects medially to subnucleus c, and laterally to a ventral subnucleus a, resulting in a "c-shape". More rostrally, the lateral part of this "c" (subnucleus b) disappears. Concomitant dorsoventral enlargement of subnuclei a and c, and appearance of nucleus beta results in an "s-shape".

The manatee MO emphasizes a general phenomenon of the mammalian MO: the restriction of subnucleus b to caudalmost levels. We see it, for example, in marsupial possums and placental raccoons. Mid IOC levels include subnucleus c (rather than b) and a separate nucleus beta. Supported by NSF Grant BSR 8503687.

140.16

THE GRACILE NUCLEUS IN SPONTANEOUSLY DIABETIC BB-RATS. S.S.W. Tay, W.C. Wong and A.A.J. Kamal.* Anatomy Dept., Natl. Univ. of S'pore, Kent Ridge, Singapore 0511.

Systematic and progressive changes occur in the gracile tract of spontaneously diabetic BB-rats (Sima & Yagihashi, Diabetes Res. Clin. Pract., 1 : 289, 1986). The present study examines the gracile nucleus in the same species.

Prediabetic BB-rats (Health and Welfare Canada, Ottawa, Ontario) were carefully monitored. After detection of diabetes, insulin was administered daily. Diabetic rats were anesthetized and killed by perfusion with a mixed aldehyde solution at 3 and 7 days, 1, 3 and 6 months. Age-matched Wistar rats served as controls. Tissues containing the gracile nucleus were processed for electron microscopy. At 3-7 days post-diabetes, axons, axon terminals and dendrites showed electron dense and electron lucent degeneration. Degenerating axons were characterized by swollen mitochondria, vacuolation, accumulation of glycogen granules, tubulovesicular elements, neurofilaments and dense lamellar bodies. Degenerating axon terminals made synaptic contacts with cell somata, axons and dendrites. Degenerating dendrites were postsynaptic to normal and degenerating axon terminals. At 1-3 months, electron dense and electron lucent degenerating axons, axon terminals and dendrites were present. Several neuronal cell bodies showed dissolution of their cytoplasm and nucleus. Unidentifiable debris were engulfed by macrophages. At 6 months, degenerating elements were similar to those at earlier intervals. Terminal degenerating axons appeared amorphous and vacuolated. Early degenerating axon terminals and dendrites were still prevalent. It is concluded that neuronal degeneration occurs in the gracile nucleus of diabetic BB-rats.

140.17

RETROGRADE, TRANSNEURONAL TRANSPORT OF PSEUDORABIES VIRUS IN RAT HYPOGLOSSAL AND VAGAL MOTONEURONS: LIGHT AND ELECTRON MICROSCOPIC ANALYSIS. L. Rinaman, M.E. Whealy*, J.S. Schwaber, A. Robbins*, J.P. Card, and L. Enquist*, Inst. of Neurological Sciences, Univ. of Pennsylvania, Philadelphia, Pa., and E.I. DuPont Co., Wilmington, Del. 19898.

In a recent light microscopic immunohistochemical investigation, Ugolini and coworkers (Brain Res., 422: 242, '87) demonstrated retrograde transneuronal transfer of herpes simplex 1 virus following injection of the virus into either the tongue or hypoglossal nerve. In the present study we have utilized light and electron microscopy to define the central neuronal distribution of pseudorabies virus following injection into either the tongue or stomach. Light microscopic immunohistochemistry revealed discrete labeling of motor neurons consistent with the known innervation of the respective organs; ie. virus-containing neurons in the hypoglossal nucleus were only observed ipsilateral to the injection, while injection of the ventral stomach musculature selectively labeled motoneurons in the left vagal nucleus. The number of labeled neurons and the intensity of labeling increased with longer survival times and both injection paradigms resulted in trans-neuronal labeling of second order neurons. Electron microscopic analysis of the motor nuclei revealed virus particles in motor neuron perikarya and dendrites as well as within afferents synapsing upon these profiles. In addition, the ultrastructural analysis demonstrated that glial profiles rarely contained virus, even when located immediately adjacent to severely afflicted neurons.

CLINICAL CNS NEUROPHYSIOLOGY I

141.1

REDUCED NA (VISUAL ERP) INDEXES MISALLOCATION OF ATTENTION IN SCHIZOPHRENIC CHILDREN. R.J. Strandburg, J.T. Marsh, W.S. Brown*, R.F. Asarnow* and D. Guthrie*. Dept. of Psychiatry, UCLA, LA, CA. 90024.

ERPs were recorded from 17 normal and 13 schizophrenic children during performance a reaction time task (RT) and a visual discrimination, the Span of Apprehension Task (Span), which is sensitive to vulnerability factors in schizophrenia. Identical stimuli (12 letter arrays) were presented in both conditions. Subjects responded to the visual arrays in the RT condition and differentially to the presence of either a T or F in the Span condition.

EEG was recorded at 19 scalp sites, and ERPs included activity 1 second before to 1 second after the Span arrays. Difference potentials (Span - RT) were computed to isolate endogenous activity (NA) associated with the Span discrimination. Topographic fields were computed at the peak of N1 and NA.

Schizophrenics were found to produce significantly less NA activity than normals. This endogenous negativity differed in its topography and time course from the exogenous components (P1, N1 and P2). NA is associated with attentional effort during pattern recognition (Ritter et al. 1983). Our results support the hypothesis that schizophrenics are impaired in their ability to allocate adequate attentional resources for Span processing. This deficit is apparent quite early in discriminative processing.

141.2

EYE MOVEMENTS IN SCHIZOPHRENIA: CLINICAL AND NEUROBIOLOGICAL CORRELATES. G.Thaker, R.Buchanan*, B.Kirkpatrick*, C.Tamminga; Maryland Psychiatric Research Center, P.O. 21247, Baltimore, MD 21228.

Although abnormality of smooth pursuit eye movement (SPEM) has been extensively described in schizophrenic patients, its underlying mechanism and clinical correlates remain unclear. To further explore these issues we carried out eye movement examination in saccade, anti-saccade, fixation, and smooth pursuit paradigms in schizophrenic patients. The presentation of each paradigm was randomized. 54 schizophrenic patients (DSM III) and 19 normal controls have participated in this ongoing study. Distractibility score obtained from anti-saccade paradigm, qualitative SPEM score, number of saccades during fixation tasks, and latency for volitional saccades differentiated the patients from the normal controls. However, saccadic distractibility did not correlate with SPEM score but was significantly associated with tardive dyskinesia (TD; r=0.59). In contrast, SPEM score and latency for volitional saccades did not correlate with TD; the latter significantly differentiated schizophrenic patients with deficit state (mean volitional saccade latency, 356 $\pm$ 66 msecs) from non-deficit patients (299 $\pm$ 60, $p<0.01$). Significance of using such oculomotor paradigms to study heterogenous illness like schizophrenia and its neuroanatomic implications will be discussed.

141.3

MIDLATENCY AUDITORY EVOKED RESPONSES IN SCHIZOPHRENICS R. Erwin, M. Mawhinney-Hee*, and R.E. Gur*, Dept. of Psychiatry, Univ. of Penn., Philadelphia, PA 19104.

Studies using a paired stimulus protocol have found that the long-latency auditory evoked component P50 has an abnormal recovery cycle in schizophrenics (Freedman et al., Biol. Psych., 18:537, 1983). Recent studies (Erwin and Buchwald, EEG suppl., 40:461, 1987) examing the effects of stimulus rate on the midlatency response (MLR) component P1 (50-65 ms latency) found P1 recovery cycles in normals similar to P50 recovery cycles in normals. Evidence from both the human and cat model suggests that the P1 is generated by a component of the ascending reticular activating system. The present study attempted to replicate the findings in schizophrenics using a rate protocol and MLR recording procedures.

MLRs were recorded from a group of 11 schizophrenics and 24 normal adults in response to clicks (.1ms, 50 db HL) presented at 3 rates: .9/sec, 5.1/sec and 9.9/sec. Evoked potentials were recorded from the vertex (Cz) and referenced to linked mandibles.

Analyses indicated that schizophrenic P1 amplitudes did not decrease as much as normal P1 amplitudes at faster rates of stimulation (rate x group ($F(2,68)=3.67$, $p<.05$)). These findings suggest that the P50 and P1 component reflect similar brain mechanisms and that abnormal recovery cycles found in schizophrenics may reflect dysfunction in the ascending reticular activating system.

(Supported by NIMH Grant MH43880).

141.4

EEG AND MEG (MAGNETOENCEPHALOGRAPHY) MAPPING OF THE AUDITORY 40HZ RESPONSE: A STUDY OF DEPRESSION. U.Ribary, H.Weinberg*, B.Johnson*, R.J.Ancill* and S.Holliday*. Brain Behavior Lab., Simon Fraser Univ., Vancouver, B.C., V5A 1S6, Canada.

Two relatively new technologies, the mapping and the analysis of digital distributions of electric (EEG) and magnetic (MEG) fields, were combined, in order to study midlatency responses and source estimates after a 40Hz auditory stimulation in controls and depressed patients (30-50, 65-75 years). The steady-state evoked response in humans is known to be maximal in amplitude around 40Hz and involves cortical and subcortical brain areas. EEG data in controls indicate a phase shift from anterior to posterior part of the brain, with a main response in frontal brain (around Cz). Lower amplitudes are found at 31 and 46Hz. The pattern of phase vectors and amplitudes over the head are influenced by changes in frequency after monaural or binaural ear stimulation. In patients (major depression), phase vectors and amplitudes show a different pattern over the skull at 40Hz, at 31 and 46Hz, and in some patients an interaction with ear stimulation and an increasing effect of age. MEG data estimate 2 dipoles in brain, which are more in phase in controls than in patients. It is suggested that more than one generator must be responsible for the 40Hz response, which are changed during depression.

These data indicate a new possibility to study pathological changes and drug effects in human brain.

141.5

ORIGIN OF THE MAGNETIC EVOKED FIELD PRODUCED BY APPLIED ELECTRIC FIELD IN THE ISOLATED TURTLE CEREBELLUM. Y.C. Okada, L. Lopez* and C. Nicholson. Dept. of Physiology and Biophysics, New York Univ. Med. Ctr, New York, N.Y., 10016.

We applied a spatially uniform electric field (50-500 μs in duration) across the surface of the cerebellum suspended vertically in a bath of Ringer solution to stimulate Purkinje cells and others whose dendrites or axons are aligned predominantly along the field direction (Chan, C. and Nicholson, C., J. Physiol., 371:89, 1986). The component of the magnetic evoked field (MEF) normal to bath surface was monitored at 20 mm from the cerebellum with a superconducting detector (Okada, Y.C. et al., Brain Res., 412:151, 1987). The field potential (FP) was simultaneously monitored with a glass micropipette. The MEF was predominantly biphasic with the underlying current for the first component directed from the ventricular to the dorsal side and that for the second in the opposite sense. Its waveform was similar to the FP measured near the Purkinje cell layer. Both components of the MEF as well as FP were abolished either by replacing Ca^{2+} with Mn^{2+}or by bath application of tetrodotoxin (0.3-1.0 μm).

These results indicate that both the MEF and FP were produced by synaptically mediated Na-currents and not by the direct activation of Na-channels or Ca-channels in Purkinje cells (Chan et al., J. Physiol., in press) in these experiments.

The laminar profile of the FP shows that the MEF was most likely produced by synaptic inputs from the climbing fibers activated by the applied electric field.

Supported by NINCDS grant NS21149.

141.6

A RAT BRAIN GLIOMA MODEL FOR STUDYING CONTRAST ENHANCED M.R.I. D.M. Kaufman*, V.M. Runge*, S. Jacobson. Dept. of Radiology, & Dept. of Anat. & Cellular Biology, Tufts New England Med. Ctr., Boston, MA. 02111.

The introduction of contrast agents for magnetic resonance imaging (MRI) has prompted the need for developing a reliable model to quantify and compare the enhancement characteristics of these agents.

We have developed a reproducable rat gliosarcoma model which is grown in culture and was stereotactically implanted into 67 Fisher rats. These animals were scanned with MRI (Siemens 1T Magnetom) fitted with a custom designed small animal coil, 10-14 days post-implantation, both before and 10 min. after, I.V. injection (.25mmol/kg) of contrast agent. 22 animals were injected with Gd-DTPA, 19 animals were injected with Gd-DOTA, and 23 animals were injected with Gd-DO3A. T2 (TR/TE=2300/90) and T1 (TR/TE=500/25) weighted scans allowed visualization of tumor pathology and tumor enhancement, respectively. Thin 3mm slices are used to improve resolution and allow visualization of tumor on multiple slices. Pathologic correlation of excised tumor was performed and actual tumor locations were compared to the scanned images confirming the presence of the tumor.

All three contrast agents provided improved visualization of the tumor, on post-contrast T1 weighted scans, when compared to pre-contrast images. In many cases tumor could only be identified retrospectively on the pre-injection T1 weighted scans. Tumor enhancement by DTPA and DO3A are comparable (62±29% vs 59±41% respectively) while enhancement by DOTA is inferior (32±6%).

This model has proven itself as a viable means for assessing the clinical efficacy of MRI contrast agents and prompted reason for its continued use.

141.7

A MULTI-MODAL, MULTI-PLANE COMPUTER IMAGING SYSTEM FOR STEREOTACTIC NEUROSURGERY. J.X. Zhang, C. Wilson, M. Levesque*, R.M. Harper, and J. Engel. Brain Res Inst and Depts of Neurology, Neurosurgery, and Anatomy, UCLA, Los Angeles, CA 90024.

Electrode placement for localization of subcortical seizure foci in patients with complex partial epilepsy requires knowledge of 1) dysfunctioning brain tissue, as revealed by positron emission tomography (PET), 2) brain and skull structures, as revealed by computerized tomography (CT), 3) brain structures, as indicated by magnetic resonance imaging (MRI), and 4) visualization of the vascular supply, as indicated by digital subtraction angiography (DSA). Since images supplied by these displays differ on absolute size and translation and may suffer from distortion, we developed an imaging system, based on a modified Leksell stereotactic frame and a MicroVAX/GPX computer, that allows for simultaneous display from the four imaging devices in multiple windows, adjusted for common coordinates with reference to fiducial markers. Moreover, with any single imaging modality, different views (e.g., saggital, coronal, and horizontal, or, in the case of DSA, lateral or AP) can be simultaneously displayed with appropriate coordination of cursors in each view. The system has been applied to nine patients undergoing surgery for electrode placement in temporal lobe structures simultaneously with conventional ventriculography procedures.

141.8

RESPIRATORY EFFECTS OF SINGLE PULSE STIMULATION OF HUMAN AMYGDALA AND HIPPOCAMPUS. R. C. Frysinger, R. M. Harper, C. Wilson and J. Engel. Depts. of Anatomy, Neurology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

In the cat, amygdala neurons discharge phasically with the respiratory cycle, and tonic discharge rates correlate with spontaneous changes in respiratory rate. Single pulse stimuli to the central amygdala of cats produce a transient enhancement of inspiratory activation. A subset of hippocampal neurons discharge in phase with the respiratory cycle in both cats and humans. The amygdala and hippocampus have thus been implicated in control of respiration; this study was designed to assess the effect on respiration of single pulse stimulation of these two structures in the human.

We record respiratory air flow during pulse stimulation of amygdala, presubiculum, and pes hippocampi of epileptic patients three to four days following chronic depth electrode implantation. Respiratory period, inspiratory and expiratory times, inspiratory slope, duty cycle, and inspiratory, expiratory and total areas are calculated on a breath-by-breath basis off-line, and the data are partitioned into four groups on the basis of the respiratory phase quadrant of the stimulation. Analysis of variance is used to detect changes from prestimulation values.

The most pronounced effect is observed with presubicular stimulation. Pulses to this area during inspiration result in prolongation of the respiratory cycle, while pulses during expiration reduce the period of the stimulated breath. This effect may result from an inspiratory activation, resulting in prolongation of, or early switching to, inspiration. Supported by NS 02808-27.

141.9

SPINAL SENSORY AND MOTOR TRACT ACTIVATION FOLLOWING EPIDURAL ELECTRICAL STIMULATION IN THE CAT. G.Niznik*, F.Shichijo*, B.Pomeranz, F.Gentili*, E.Transfeldt* and T.Ohshima*. Dept. of Zoology, University of Toronto, Toronto, Ont. M5A 1A1.

The use of epidural stimulation as a method of monitoring spinal cord function during surgery is gaining popularity. The aim of this study was to clarify the spinal sensory and motor components of various centrally and peripherally recorded potentials. 37 adult mongrel cats were anesthetized with Ketamine and ventilated. Anesthesia was maintained with Ethrane and nitrous oxide. Bipolar stimulating electrodes were placed in the left and right dorsolateral epidural space at T7. Recording sites included the spinal cord epidurally at L3, the right L7 dorsal and ventral nerve roots, the left and right sciatic nerves and the right gastrocnemius muscle. Optimal stimulating parameters were determined by varying the stimulus intensity and frequency. Selective spinal cord lesions were made at T13. The results of these suggest that: 1) the dorsal root and sciatic potentials reflect activity in the dorsal columns; 2) the ventral root potential reflects activity in the ventral spinal motor tracts; and 3) potentials recorded from the spinal cord reflect activity in both spinal sensory and motor tracts.

141.10

EFFECT OF UPPER EXTREMITY FORCED USE ON ENHANCED FUNCTION IN CHRONIC STROKE AND TRAUMATIC BRAIN INJURED PATIENTS. S.L. Wolf, D.E. LeCraw*, L.A.Barton* and B.J. Rees*. Dept. of Rehabilitation Medicine, Emory Univ. Sch. of Medicine, Atlanta, GA 30322.

To test the clinical counterpart of the learned disuse theory, 25 chronic hemiplegic stroke and head injured patients with minimal to moderate upper extremity extensor muscle function were required to wrap their uninvolved upper extremity within a hand-enclosed sling for 2 weeks. During forced use and for 1 year thereafter, changes in force or time-based measures among 21 functional tasks were compared to values at the sixth baseline session when preintervention relearning had plateaued. Significant ($p<.05$, Friedman's repeated measures followed by Tukey multiple comparison tests) changes were seen in 19 of the 21 tasks with most persisting at 1 year followup. There were no significant differences when subjects were compared by diagnosis (brain injury versus stroke) or by predominantly involved extremity (left versus right). Results suggest that a learned disuse phenomenon may occur among select chronic neurologic patients.

Future work should be directed toward determining whether modified forms of forced use can overcome functional deficits among individuals with more profound motoric dysfunction.

141.11

THERAPY REDUCES GAPS IN EMG ACTIVITY OF CHRONIC CVA PATIENTS. S.S. Fitts, M.C. Hammond*, and G.H. Kraft*. Rehabilitation Medicine, University of Washington, Seattle, WA 98195.

A common view that recovery is complete within the first 6-12 months post-stroke may result in termination of therapy before maximum recovery is achieved. We have reported that gaps (>100 msec) in the EMG interference pattern correspond to a clinical impression of impersistent recruitment and "ratchety" movement. Thus, we predicted that gaps should be reduced after therapy which improved function in the same patients.

The 8 male and 5 female patients included 7 left- and 6 right-CVA's. All were right-handed and at least one year post-stroke (X=26.6 mo). Ten patients had therapy combining electrical stimulation with voluntary control; 3 had conventional therapy.

Patients were signaled by buzzers to maintain maximal wrist flexion or extension against an isometric restraint apparatus for 3, 6, or 9 sec. 24 trials were presented in a balanced random order. Intramuscluar EMG activity was recorded from flexor carpi radialis (FCR) and extensor carpi radialis longus (ECR).

More gaps were recorded from ECR than FCR (X's = 4.9 & 2.4, $p<.05$), which is consistent with functional deficits. More gaps were recorded before therapy than immediately or 3 mo. after therapy (X's = 4.5 vs 3.1 & 3.2, $p<.05$), which is consistent with functional gains. Improvements were not significantly greater in one muscle than the other, nor with one therapy than another.

Biometer International & Medtronic, Inc. provided therapy equipment. NIDRR grant #G008435053 supported this research.

141.12

THE EFFECT OF HYPERTENSION ON CAROTID ARTERY BLOOD FLOW CHANGES DURING A CARDIOVASCULAR STRESSOR. B. Therrien, B.L. Metzger*, B. Cimprich*, and S. Kuhnke*

Marked shifts in cerebrovascular blood flow velocity and pressure in response to straining are life-threatening in individuals with cerebrovascular disease (CVD). We examined the confounding influence of hypertension and obesity on carotid artery blood flow velocity (CABFV) during Valsalva strain. Subjects strained by blowing into a pressure gauge meter to 40 mmHg for 10 sec. CABFV was measured by noninvasive techniques during all phases of the Valsalva. Normative CABFV changes during Valsalva were established in 145 healthy, normal wt controls (30-55 yrs). This sample consisted of both hypertensive adults of average wt and fatness and obese hypertensive adults aged 30-55 yrs (n=20). During strain CABFV fell an average of 54 ± 24% from baseline. In hypertensive adults, the fall in CABFV during strain was significantly greater (>75%; $p<.0001$). In contrast to the <300% total CABFV change across the Valsalva observed in controls, the average % change observed in hypertensives from onset of strain to overshoot was >650%. Overall CABFV changes were less extreme during strain (67% vs 87%) and across the Valsalva (514% vs 990%) in obese hypertensives compared to hypertensives of average wt and fatness. These data suggest that ability to compensate for sudden change in peripheral blood flow velocity and/or cardiac output is severely compromised in the adult with hypertension.

141.13

SEX DIFFERENCES IN CEREBRAL INJURY CAUSED BY ACUTE SEVERE HYPONATREMIA. J. KUCHARCZYK, C.L. FRASER*, P. SARNAKI*, D. NORMAN* AND A.I. ARIEFF*. Dept. of Medicine and Neuroradiology, Univ. California, Sch. of Medicine, San Francisco, CA 94143.

During the past 18 months, over 100 cases of death or permanent brain damage have been reported in patients with severe symptomatic hypernatremia. Over 90% of these cases have involved young cyclic women. To determine if the cation extrusion mechanisms which normally defend against intracerebral edema are less effective in females than males, we studied Na^+-K^+ transport in synaptosomes prepared from the brains of severely hyponatremic (serum Na^+ 102-114 mM for 1 day) and normal female and male rats. A 250% greater increase in veratridine-sensitive Na^+ uptake was found in female brain synaptosomes. This sex difference was abolished by ouabain, suggesting that Na^+, K^+-ATPase pump function may be responsible for hyponatremia-induced cerebral edema. Since recent studies indicate that Na^+ K^+-ATPase pump in specific regions of the brain is sensitive to gonadal steroids and endogenous neuropeptides, we examined the influence of vasopressin administration on cerebral phosphorus metablism in normal young adult female and male rats using ^{31}P magnetic resonance spectroscopy. Females injected s.c. with 20 I.U. of arginine vasopressin (AVP) showed a rapid deterioration in cerebral oxidative phosphorylation and a significant intracellular acidosis within 30 min of injection of AVP. In contrast, there was only a slight depletion of high energy phosphates in male brains. The high circulating levels of AVP found in severe hyponatremia may therefore contribute to the genesis of cerebral edema in females.

141.14

NEUROPHYSIOLOGICAL STUDY OF THE SHORT-TERM EFFECTS OF INTRATHECAL BACLOFEN IN SPASTICITY. M. L. Latash*, R. D. Penn, D. M. Corcos, and G. L. Gottlieb. Rush-Presbyterian St. Luke's Med Center, Chicago, IL, 60625.

Six patients, with long-standing spasticity resistant to oral baclofen, received a bolus injection of lumbar intrathecal baclofen. Responses to passive joint movements, H-reflexes, ankle clonus, and Babinski's response were considerably suppressed 30-45 min after the injection and practically disappeared after 1.5 - 2 hours. Ankle clonus was seen only in patients with H-reflexes, and it disappeared when the reflex responses to stimuli of n.tibialis were absent. A decrease in clonus amplitude was accompanied by a decrease in clonus frequency. These observations support an auto-oscillation hypothesis of clonus.

Before baclofen, attempts at voluntary activation in patients with residual motor control were accompanied by uncontrolled bursts of activity in antagonist and distant muscles. Baclofen injection led to considerable improvement in selective voluntary activation of leg muscles by eliminating responses in antagonist and distant muscles. There was only a slight effect on agonist activity. These results suggest that the execution of voluntary motor commands suffered from functionally abnormal spinal circuitry rather than from changes in the descending motor commands. Intrathecal baclofen demonstrates selectivity in its action upon different spinal pathways and appears to be an effective way to treat severe spasticity. It allows voluntary motor function on a background of suppressed muscle tone and spasms.

This work was partially supported by NIH grants NS 15630, R29 NS 23593, and AM 33189.

141.15

DIFFERENTIATION OF MULTIPLE SCLEROSIS, CEREBROVASCULAR DISEASE AND NORMAL AGING WITH MAGNETIC RESONANCE IMAGING. A.L. Hunt*, W.W. Orrison*, R. Rhyne*, and G.R. Rosenberg* (SPON: L.A. Hershey). Univ. Of New Mexico Sch. of Med., Albuquerque, NM 87131.

It is difficult to differentiate multiple sclerosis (MS) from cerebrovascular disease (CVD) based on white matter lesions (WMLs) seen on magnetic resonance imaging (MRI). We studied the anatomic distribution of WMLs on MRI (1.5 Tesla) in 12 definite MS patients, 52 patients with CVD risk factors and 19 aging controls with WMLs, but no CVD risk factors. The anatomic distribution of WMLs was assigned by group consensus without knowledge of age or clinical history. Frontal, parietal and occipital lobes were the most common sites for WMLs, regardless of diagnosis. Cerebellar WMLs were somewhat more common in MS (32%) than in patients at risk for CVD (15%) or in aging controls (5%). Temporal lobe WMLs were somewhat more frequent in MS (66%) than in patients at risk for CVD (40%) or in aging controls (11%). The combination of both cerebellar and temporal lobe WMLs was significantly ($p<0.01$) more common in those with MS (33%) than in those at risk for CVD (5%) or in aging controls (0%). The presence of WMLs in cerebellum and/or temporal lobes on MRI should lead one to suspect the diagnosis of MS, rather than CVD or normal aging.

142.1

SPATIAL CODING OF MOVEMENT DIRECTION IN FRONTAL CORTICAL AREAS. R. Caminiti, P.B. Johnson, R.A. Pastore and A. Urbano. Inst. of Physiology, Univ. of Rome, 00185 Rome, Italy.

M. nemestrina monkeys were trained to make arm movements of same direction within different parts of space using different patterns of muscle activity. We studied extracellularly 169 cells of areas 4 and 6 while monkeys performed the task. Directionally tuned cells (92%) were selected and their preferred directions (PDs) computed. The spherical space where the animal performed was divided into sectors, the PDs of cells lying in each given sector expressed as 3-D vectors and their mean vector computed. These mean vectors were then compared (Watson-Williams test, $p < 0.01$) with those obtained from the PDs of the same cells studied when monkeys made movements of similar directions in other parts of extrapersonal space. In all cases these mean vectors mantained invariant their 3-D orientation, suggesting that frontal areas code direction of arm movement as a variable independent from the underlying patterns of muscle activity since these patterns differed when movements of same direction were performed in different parts of space.

142.2

NEURONAL MECHANISMS OF PREPARATION FOR MOVEMENT DIRECTION **A. RIEHLE*, J. REQUIN.** CNRS-LNF 1, Unit of Cognitive Neurosciences, 31 ch.J.Aiguier, 13402 Marseille 09, France.

Movement preparation is defined as a process which promotes selection and assembly of motor program elements. We have studied the underlying neuronal mechanisms, which result in a reduction of reaction time when movement parameters are known in advance as compared to when these parameters are not known. Monkeys were trained to perform flexion and extension wrist movements of small and large extent. The preparatory signal supplied the animals with information about either direction and extent (complete information), direction or extent (partial information), or gave no information. Single-cell recordings in the primary motor and premotor cortex during the performance of the task showed that 66% (207/313) of the neurons exhibited directionally selective activity patterns, either related to the preparation (n=71) or to the execution (n=139) of the intended movement. Two types of preparation-related neurons have been found. The activity changes of one type reflect a process which may facilitate the triggering of the programming operation after the occurrence of the response signal, while the second type may be involved in a process responsible for programming in advance movement direction during the preparatory period. A possible model of the functional interaction between both types of directionally selective, preparation-related neurons is discussed.

142.3

INFORMATION TRANSMITTED BY THE DIRECTION OF 2-D ISOMETRIC FORCE EXERTED BY THE UNRESTRAINED ARM. J.T. Massey, G. Hovey* and A.P. Georgopoulos. Bard Labs. of Neurophysiology, Dept. of Neuroscience, The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

We have measured the direction of 2-D isometric forces produced by human subjects in response to visual stimuli using a novel experimental apparatus. The visual instruction was presented to the subject by means of vivid dynamic stereograms of planes (disks) whose angle of tilt about a horizontal axis in the plane of the CRT screen varied from 15° (near vertical) to 80° (near horizontal). Targets were shown in the plane of the disk, and x-y forces were recorded. These force values were fed back onto the target plane as a cursor. Target positions varied from 4 to 60, and were distributed equidistantly on a circle of 30 mm radius. Subjects were asked to exert a force in the direction of the target when it appeared; no capture of the target was required, only crossing of a force threshold. Data from 24 naive subjects indicated that the information transmitted increased with input information and tended to reach a plateau at approximately 4.1 bits; this held for the range of plane tilts used. This is remarkably similar to the result obtained for 2-D arm movements (Georgopoulos and Massey,Exp. Brain Res. 69:315,1988).

142.4

MOTOR CORTICAL CELL ACTIVITY DURING A CONDITIONAL MOTOR TASK WITH A DIRECTIONAL TRANSFORMATION. A.P. Georgopoulos, M. Petrides, J.T. Lurito*, A.B. Schwartz, J.T. Massey and N. Porter*. Bard Labs of Neurophysiology, Dept. of Neuroscience, The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The activity of 225 cells in the arm area of the monkey motor cortex was recorded during performance of a conditional motor task which incorporated a directional transformation. In the "direct" task the animal moved a handle towards a dim light on a plane; in the "transformation" task it moved the handle 90° counterclockwise (CCW) from a bright light. The dim/bright condition and 8 stimulus locations were randomly mixed. In many cases the changes in cell activity differed from those observed when the light appeared in the same location or when the movement was made in the same direction. The neuronal population vector (Exp. Brain Res. Supl. 7:327, 1983) was computed every 20 ms during the reaction time. In the "direct" task it pointed in the direction of the movement (which was the same with the direction of the stimulus). In the "CCW transformation" task the population vector pointed initially towards the stimulus direction and then shifted gradually CCW to the direction of the upcoming movement. These findings indicate that the motor cortex is involved in the transformation of a stimulus direction into a different movement direction.

142.5

CROSSCORRELATIONS BETWEEN MOTOR CORTICAL CELLS SIMULTANEOUSLY RECORDED DURING REACHING TASKS IN THE MONKEY. J.T. Lurito*, A.B. Schwartz, M. Petrides, R.E. Kettner and A.P. Georgopoulos (SPON: V.B. Mountcastle). Bard Labs of Neurophysiology, Dept. of Neuroscience, The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

We used a multielectrode recording technique (see Soc. Neurosci. Abstr. 13:629, 1987) to record simultaneously (0.1 ms resolution) the activity of up to 8 cells in the arm area of the motor cortex of two monkeys performing reaching tasks (driven activity) or sitting quietly (spontaneous activity). Cross-correlations (CC) were computed at 1 ms and .1 ms resolution for 1240 simultaneously recorded cell pairs (driven activity; 1176 from different electrodes 0.6-4.2 mm apart and 64 from the same electrode tip). Clear and narrow CC's (up to 3 ms peak width) were observed between 77 pairs; they occurred much more frequently between pairs recorded from the same electrode (41%) than from different electrodes (4.3%). Altogether, 49/77 (64%) pairs showed a peak at zero indicating common input, 25% showed a peak near zero, and 11% showed inhibition. Directional tuning properties of cells were analyzed. Cells with different preferred directions were activated simultaneously, a finding consistent with the hypothesis of a distributed population coding of movement direction. (Supported by USPHS Grants P01 NS20868 and NS17413.)

142.6

SUPPLEMENTARY MOTOR AREA (SMA): CODING OF BOTH PREPARATORY AND MOVEMENT-RELATED NEURAL ACTIVITY IN SPATIAL RATHER THAN JOINT COORDINATES. M.D. Crutcher and G.E. Alexander. Dept. Neurol., Johns Hopkins Univ., Baltimore, MD 21205.

Single cell recordings in monkeys making limb movements to track visual targets have shown that the SMA and other precentral motor fields contain not only movement-related neurons, but also neurons that discharge during the preparation for limb movements. When directionally selective, both preparatory activity and movement-related activity are generally assumed to reflect the direction of the limb movement and not that of the target shift, even though these two variables have not been dissociated. To determine whether preparatory activity and movement-related activity are actually related to the direction of limb movement (in joint coordinates), or reflect instead the direction in which the target moves (in spatial coordinates), we trained rhesus monkeys to perform a visually guided, delayed step-tracking task that dissociated these variables. In some blocks of trials, the monkey was required to flex or extend his elbow so that the forearm moved in the same direction as the target shift, while in others he was required to move in the opposite direction. Single-cell recordings in the SMA revealed that cells with directional preparatory activity were usually related to the direction of the upcoming target shift, rather than the direction of the upcoming limb movement (20 of 23 neurons). Directional movement-related activity was also coded in terms of target location rather than the direction of limb movement for a significant proportion of cells (17/82). These results are similar to our recent findings in primary motor cortex, indicating that in both of these areas much of the preparatory activity, and a small portion of the movement-related activity, is coded in spatial rather than joint coordinates. This suggests that both areas may be involved in the highest levels of the sensory-motor transformations required for the planning and execution of visually guided limb movements.

142.7

MOTOR CORTICAL DISCHARGE DURING SINUSOIDAL ARM TRAJECTORIES. Andrew B. Schwartz. Division of Neurobiology, Barrow Neurological Inst., 350 W. Thomas Road, Phoenix, AZ 85013.

The relation between movement direction and the discharge rate of arm-related single cells recorded in the motor cortex was examined after a rhesus monkey performed two different arm-movement tasks.

During the 'direction' task, the animal was required to hold its finger in a center target on a touch-sensitive graphics monitor. This target would then disappear and one of 8 radially oriented targets appear. The monkey was trained to move its finger rapidly across the screen to the radial target for a reward. As shown previously, cells were found to have directionally sensitive discharge rates, as shown by a regression to a cosine (r^2 >.7).

In the 'sinusoid' task, three sinusoids of different amplitudes were traced horizontally, from left to right and in the opposite direction. These data were analyzed by calculating the direction of movement every 20 msec throughout the task. These directions were used to generate a predicted rate of discharge, using the relation determined in the 'direction' task. Each of the directionally sensitive cells had actual discharge rates which were modulated over the sinusoidal movement. Preliminary results show that the predicted rate of discharge calculated from the instantaneous direction of movement covaries with the actual rate. The actual discharge pattern leads the movement-derived prediction by approximately 150 msec. This interval remains constant for each of the drawn sinusoids and is independent of the spatial location of the hand on the screen. This finding as well as the consistently good fit of the prediction throughout the sine wave suggest that the directional properties of motor cortical discharge are valid for reaching movements with continually changing direction as well as for straight movements taking place in a single direction.

142.8

POPULATION CODING OF DIRECTED ARM MOVEMENTS IN SPACE BY MOTOR CORTICAL NEURONS: MATHEMATICALLY SUFFICIENT CONDITIONS FOR ACCURATE MOVEMENT. R.E. Kettner, Dept of Psychology, Indiana Univ, Prog Neural Science, Bloomington, IN 47405.

In previous work Georgopoulos, Schwartz and Kettner (Science 1986) have shown that accurate predictions of movement direction require analyses based upon large populations of broadly tuned neurons. Are there conditions which allow perfect prediction? Mathematical analyses will be presented which show that exact predictions result from populations of neurons with (1) uniformly distributed preferred directions, (2) uniformly distributed average firing magnitudes, and (3) individual neuron firing rates which are radially symmetric about their preferred direction. This population code is mathematically robust: severe violations in preferred-direction uniformity can be compensated by correction factors which are easily implemented using known brain circuits. Simulations and mathematical proofs will be presented to illustrate these points.

142.9

PRINCIPLE COMPONENT ANALYSIS OF THE RESPONSES OF PRE-MOTOR CORTICAL NEURONS TO MOVEMENT IN TWO DIMENSIONS. T.M. Amos*, C.M. Vermeersch*, S.-K. Park and T.J. Ebner (SPON: W.R. Roberts). Depts. Neurosurgery and Physiology, Neuroscience Grad. Prog., Univ. of MN, Mpls., MN 55455.

Two monkeys were trained to perform a task requiring movement in the horizontal plane, moving from a central start box to one of 6 equally spaced targets (60°) arranged around the central target. Distances to the targets varied with each set of six. The targets were displayed on a video screen and the monkey moved a cursor between targets using a manipulandum. During the task single unit responses from the premotor cortex were recorded. The responses of a single cell to a series of targets were then used to obtain a covariance matrix from which the principle components (eigenvectors) were obtained. Many cells showed similar first components, suggesting there are groups of cells with the same basic response pattern. Results indicate that over 80% of the variance in responses could be accounted for by the first component. Only the first few coefficients (used to reconstruct the original data from the eigenvectors) contribute to response amplitude modification for different targets. For some cells these coefficients varied with movement parameters (direction and amplitude). These findings suggest each cell has a basic response pattern which only needs to be scaled for movements in the plane. Supported by NSF/BNS-8707572.

142.10

EVALUATION OF MOTOR PARAMETERS IN THE PREMOVEMENT DISCHARGE OF PREMOTOR CORTICAL NEURONS DURING TWO-DIMENSIONAL MOVEMENTS. S.-K. Park, J.H. Kim and T.J. Ebner. Depts. Neurosurgery and Physiology, Neuroscience Grad. Prog., Univ. of MN, Mpls., MN 55455.

Our previous work has shown that the discharge properties of many neurons in the primate premotor cortex covary with different movement parameters (target distance and position). In this chronic unit study emphasis was placed on determining whether these parameters are represented in the premovement discharge of these neurons, by comparing their responses in two tasks. Two Rhesus monkeys were trained to move to various targets in the horizontal plane. In one task (delayed movement task) the animal was required to withhold the movement while the target was displayed until an additional instruction to move was given. In the second task (reaction time task) the animal was allowed to move as soon as the target was presented. In the delayed movement task the premovement component of the discharge peaked soon after target presentation and was maintained while movement was withheld. Premovement activity exhibited the same movement field as the discharge during the reaction time task. Both the position and distance were reflected in the discharge prior to the movement. These observations suggest that the discharge components of these neurons before and during movement contain comparable representations of movement parameters. Supported by NSF/BNS-8707572.

142.11

DOES AREA 5 ACTIVITY PREDICT MOVEMENT DIRECTION DURING AN INSTRUCTED-DELAY PERIOD? Donald J. Crammond* and John F. Kalaska, Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, H3C 3J7.

Arm-related area 5 cells were studied in an instructed-delay task requiring movements of the arm in 8 different directions away from a central start position. Monkeys were presented a visual signal (CUE) at the target end-point of an impending movement but had to withold the movement for a delay period of 1.2-2.8 sec, at which time the CUE signal changed colour (GO). To date, 41 cells have been recorded in one monkey. Nineteen cells showed changes in activity during the instructed-delay period, the intensity of which varied in a significant continuously-graded, unimodal fashion with the direction of the impending movement, centred on a preferred direction (PD). The PD of CUE activity typically was similar to that associated with movement toward the targets, but often different from that while holding over the target end-points. All cells with direction-specific activity during the instructed-delay period were also active during movement. Activity during the CUE period was less intense than during movement. We have not observed any area 5 cells active exclusively during the instructed-delay period. Supported by MRC Group Grant in neurological sciences.

142.12

TRAJECTORY-DEPENDENT SYNAPTIC INTERACTIONS IN PRIMATE MOTOR CORTEX DURING REACHING. H.C. Kwan, Y.C. Wong* and J.T. Murphy. Dept. Physiology, Univ. Toronto, Toronto, Ont. Can. M5S 1A8.

Even though the motor cortex has relatively direct access to the motor apparatus, its role in movement control is not clear. This study examines the possibility of patterning of synaptic interaction among motor cortical neurons as a mechanism for limb trajectory specification and control. Single unit recordings were performed in monkey motor cortex (M. arctoides) with two independently maneuverable electrodes. The task involved reaching out for one of six buttons which was lighted, depressing it to extinguish the light, and returning the arm to the resting position. A maximum of four separate units, two from each electrode, could be recorded. Limb trajectories for the target buttons were monitored by an optoelectronic device (Selspot). Cross-correlation analysis of spike trains revealed that close to 80% of correlated pairs of neurons exhibited variations in correlation strength as a function of limb trajectory. Further, analysis of spike trains from triplets provided evidence for trajectory-dependent synaptic interactions between members of the triplet, and trajectory-dependent common input shared by the same triplet. These findings are consistent with our hypothesis that specific patterns of synaptic interactions among motor cortical neurons play a role in the generation and control of limb trajectories. Supported by the MRC of Canada.

143.1

[^{35}S]TBPS BINDING SITES ARE DECREASED IN THE COLLICULI OF MICE WITH HIGH SENSITIVITY TO ETHANOL. J. Peris & N.R. Zahniser. Dept. Pharmacology, Univ. Colo. Hlth. Sci. Ctr., Denver, CO 80262.

One of the major actions of ethanol in the brain is to enhance GABAergic function. In support of this, long sleep (LS) and short sleep (SS) mice, genetically selected for different ethanol-induced sleeptimes, differ in GABA-dependent Cl^- flux and in muscimol inhibition of [^{35}S]TBPS binding in cerebellum. TBPS binds to the convulsant site allosterically linked to the $GABA_A$ receptor. Homogenate binding assays show no difference in the number of cerebellar TBPS sites however, it is possible that the density of these sites may differ in other more discrete brain areas which can be studied using quantitative autoradiography (QAR). We measured the density and affinity of the [^{35}S]TBPS binding site in LS and SS mice using QAR analysis of saturation isotherms. The number of [^{35}S]TBPS sites was highest in the colliculli (4.7±0.5 pmol/mg protein), cerebellum (4.7±0.5 pmol/mg), hippocampus (4.0±0.8 pmol/mg) and cortex (2.7±0.3 pmol/mg). Binding in striatum and brain stem was very low. The Bmax was lower in collicular areas of LS mice than in SS mice (1.8±0.3 vs. 4.7±0.5 pmol/mg) including both superior and inferior colliculus. There were no differences between lines in Bmax in any other area and in Kd in any area (120±12 nM). These data point to the colliculi as a potentially interesting area for ethanol research especially since GABAergic transmission in inferior colliculus may be reduced in rats during ethanol withdrawal (Frye et al., J. Pharmacol. Exp. Ther. 227:663-670, 1983). Supported by AA 03527.

143.2

CHARACTERIZATION OF N-(4,4-DI(3-METHYL-2-THIENYL)BUT-3-EN-1-YL)NIPECOTIC ACID (NO-328) HIGH AFFINITY BINDING TO THE BRAIN GABA UPTAKE CARRIER. P.D. Suzdak, U. Sonnewald, L. Knutsen, K.E. Anderson and C.L. Braestrup. Dept. of Pharmacology, NOVO Industri A/S,DK-2880 Bagsvaerd,Denmark. N-(4,4-di(3-mentyl-2-thienyl)but-3-en-1-yl) nipecotic acid (NO-328) is a potent anticonvulsant in mice and rats.We have shown that NO-328 is a potent inhibitor of ^{3}H-GABA uptake into a rat forebrain synaptosomal preparation (IC_{50}=67nM).Inhibition of ^{3}H-GABA by NO-328 is of apparent mixed type when NO-328 is preincubated prior to ^{3}H-GABA, inhibition is apparently competitive without preincubation. NO-328 is not a substrate for the GABA uptake carrier.^{3}H-NO-328 shows saturable and reversible binding to rat brain membranes in the presence of NaCl.Specific binding of ^{3}H-NO 328 is inhibited by known inhibitors of ^{3}H-GABA uptake, the amino acid GABA uptake inhibitors, however, are less potent than expected which indicates that the binding site is not identical to but overlapping with the GABA recognition site of the uptake carrier.The affinity constant for binding af ^{3}H-NO 328 is 18nM, Bmax=669 pmol/g original rat forbrain tissue.The regional distribution of NaCl dependent ^{3}H-NO 328 binding follows that of symaptosomal ^{3}H-GABA uptake.The development of photoaffinity ligands for the GABA uptake site and ion dependency will be discussed.It is concluded that NO-328 is a potent and selective inhibitor of GABA uptake and that ^{3}H-NO 328 is a useful radioligand for labelling the GABA uptake carrier in brain membranes.

143.3

CLONING OF THE ALPHA SUBUNIT OF THE HUMAN GABA-A RECEPTOR K.M. Garrett*, R.S. Duman, N. Saito*, B.Beer, A.J. Blume, M.P. Vitek and J.F. Tallman. Department of CNS Research, Lederle Laboratories, Pearl River, NY., and Ribicoff Research Facilities, Yale University School of Medicine, New Haven, CT.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the brain, and the GABA-A receptor is the site of action of several classes of CNS depressants. In an effort to more completely understand the mechanism of these drug actions, we have cloned the alpha subunit of the human GABA-A receptor. Based on the amino acid sequence of the bovine GABA-A alpha subunit (Schofield et al., Nature **328**: 221), we designed several oligonucleotide probes which were optimized for human codon usage. The probes were pooled and used to screen a λgt11 library derived from the cerebellum of a 7 year old child (ATCC). One positive clone was obtained, Gα-1, which hybridized strongly to 7 of the 8 probes. The sequence of the human clone shares a high degree of similarity with the DNA sequence of the bovine a subunit. Hybridization of Gα-1 to Northern blots of RNAs from various regions of adult human brain shows a 4.3 Kb RNA which appears to be most abundant in the cerebellum. Hybridization of Gα-1 to Northern blots of rat brain RNAs shows a 4.3/4.5 Kb doublet which is present in the cortex, hippocampus, and cerebellum. We are continuing to investigate the regional distribution of the alpha subunits and the potential heterogeneity of both the alpha and beta subunits.

143.4

RO15-4513: ANTAGONISTIC SPECIFICITY OF ETHANOL INTERACTIONS WITH THE GABA BENZODIAZEPINE RECEPTOR. D.M. Turner* and R.W. Olsen (SPON: R.N. Pechnick). Dept. of Pharmacology, Mental Retardation Research Center and Brain Research Institute, Univ. of California, Los Angeles, CA 90024.

Ro 15-4513 labels two sites in cerebellum: a diazepam-sensitive (DZ-S) site and a diazepam-insensitive (DZ-IS) site (Turner and Olsen, Abstr. Soc. Neurosci. 13, 962 (1987)). We have found the DZ-IS site to possess a unique pharmacology for BZ (DZ-S) receptor ligands. At 2 nM [^{3}H]Ro 15-4513, the compounds CGS 8216 and Ro 15-1788 have IC_{50}s of <100 nM; DMCM and ZK95962 <250 nM; and ZK93426 <1 μM. Other compounds that partially inhibit at 10 μM are ZK91296 ZK93423, βCCE and βCCM (>75%) clonazepam (40%) and CL218872 FG7142, Ro 5-4864 and flurazepam (<15%). The findings that both agonists and inverse agonists inhibit the binding of Ro 15-4513 and that FG7142 does not inhibit the binding indicate that the DZ-IS site is not responsible for the inhibition by Ro 15-4513 of the action of ethanol.

In studies of chloride efflux in rat hippocampal slices, Ro 15-4513 (5 μM) did not inhibit muscimol-induced chloride flux nor the enhancement of this flux by pentobarbital or the steroid alphaxalone. A slight enhancement of muscimol-induced chloride flux by 25 mM ethanol was altered by Ro 15-4513. The finding that Ro 15-4513 does not inhibit muscimol, pentobarbital or alphaxalone enhancement of chloride flux at a concentration which has previously been found to inhibit ethanol enhancement indicates the specificity of Ro 15-4513 for the ethanol effect. NIH Grant HD 06576.

143.5

DIVALENT CATIONS MODULATE $GABA_A$ RECEPTOR AFFINITY AND DESENSITIZATION. D. Mierlak* and D.H. Farb (SPON: L. Friedman). Dept. of Anatomy and Cell Biology, SUNY Health Science Ctr., Brooklyn, NY 11203.

GABA-evoked Cl^- conductance is modulated by various compounds including benzodiazepines, β-carbolines, barbiturates and convulsants. We have shown that GABA receptor desensitization is also a locus for modulation by benzodiazepines (Mierlak and Farb, 1988). Here we investigate the effect of divalent cations on GABA responses and GABA receptor densensitization using intracellular current clamp recordings from chick spinal cord cell cultures. The conductance increase induced by pressure ejection of 10 uM GABA is inhibited reversibly in the presence of Cd^{2+}, Ni^{2+} or Co^{2+}. Cd^{2+}(EC50= 15 uM) is a more potent inhibitor of GABA responses than Ni^{2+}(EC50= 300 uM). Both Cd^{2+} and Ni^{2+} produce a maximal inhibition of 80-85%.

In the presence of 50 uM Cd^{2+}, the GABA dose-response curve is shifted to the right in a parallel fashion with little effect on the maximal GABA response. The efficacy of Cd^{2+} inhibition of the GABA response is inversely related to Ca^{2+} concentration. The results suggest an allosteric decrease in affinity of GABA receptors mediated by a Ca^{2+}-sensitive site. In addition to inhibiting GABA responses, the presence of Ni^{2+} or Cd^{2+} results in an increase in the rate and extent of GABA receptor desensitization as compared to equivalent GABA responses in the absence of Ni^{2+} or Cd^{2+}. Taken together, these results suggest a modulatory role for divalent cations on control of GABA receptor affinity and desensitization.

143.6

HUMAN GLIAL TUMORS HAVE HIGH AFFINITY FOR ISOQUINOLINE DERIVATIVES BUT NOT BENZODIAZEPINES. J.M.M. Olson,* L. Junck,* A.B. Young, J.B. Penney, P.E. McKeever,* and W.R. Mancini* (SPON: J.T. Greenamyre) Depts. of Pharmacology, Neurology and Pathology and the Neuroscience Program, University of Michigan, Ann Arbor, MI 48104.

PK 11195, Ro5-4864 and flunitrazepam binding were compared in human glioma tissue to determine whether the drug specificity of human tissue was different from that of rat. Postmortem human brains with glioma were assayed for 1 nM [^{3}H]PK 11195, 1 nM [^{3}H]Ro5-4864 or 5 nM [^{3}H]flunitrazepam binding autoradiographically. Specific [^{3}H]PK 11195 binding was significantly higher in tumor than in normal brain whereas [^{3}H]flunitrazepam binding in the presence of 1 μM clonazepam (to block "central benzodiazepine receptors") and [^{3}H]Ro5-4864 binding were low and nonspecific in both tumor and normal brain. In human glioma biopsies the same results were observed. Binding was also assayed in monolayers of rat or human glioma lines grown in culture. [^{3}H]PK 11195 binding was high in both human and rat cultured cells. Specific [^{3}H]Ro5-4864 and [^{3}H]flunitrazepam binding were observed in rat glioma, but not in three human glioma lines. Thus, human glioma cells bind the isoquinoline PK 11195 but have low affinity for Ro5-4864 or flunitrazepam. [^{11}C]PK 11195 may be a more appropriate ligand for PET scanning human gliomas.

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143.7

HOMOLOGOUS AND HETEROLOGOUS REGULATION OF THE GABA/ BENZODIAZEPINE RECEPTOR COMPLEX.

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We have previously found that chronic flurazepam treatment of 7d primary cultures of chick brain cells significantly decreases GABA potentiation of ^{3}H-flunitrazepam (FNZ) binding. This effect is blocked by Ro15-1788. Moreover, chronic exposure to methylxanthines (MX) such as theophylline and caffeine reduces GABA potentiation of ^{3}H-FNZ binding. Chloroadenosine, an adenosine receptor agonist, blocks this effect of theophylline. To determine if the effects of chronic MX or benzodiazepine treatment were the result of increased GABA activity, cultures were treated approximately every 12h with 50 or 200 µM GABA for a total of 48h. While GABA potentiation of ^{3}H-FNZ binding was unaltered, ^{3}H-FNZ binding sites were reduced from 0.28 ± .05 pmol/mg in control cells to 0.2 ± .04 pmol/mg in cells treated with 50 µM GABA ($p < 0.05$) and 0.18 ± .03 pmol/mg in cells treated with 200 µM GABA ($p < 0.05$). There was no change in affinity. When cells were co-treated with 100 µM bicuculline and 100 µM GABA down-regulation was inhibited ($n = 2$). Chronic treatment with 10 µM muscimol also resulted in a down-regulation ($n = 4$). Thus, it appears that the GABA receptor complex can be regulated in a variety of ways. GABA receptor agonists act directly to cause down-regulation. In contrast, BZD positive modulators cause a decrease in GABA potentiation. MXs reduce GABA potentiation through a site that resembles an adenosine receptor.

143.8

GABA-A/BENZODIAZEPINE RECEPTORS IN THE RAT AND HAMSTER CIRCADIAN SYSTEM.

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The benzodiazepine, triazolam, produces phase shifts of circadian rhythms (Turek and Losee-Olson, 1986) which are abolished by destruction of the intergeniculate leaflet (IGL; Smale et al, 1988), the source of a secondary visual pathway innervating the principal pacemaker of the circadian system, the suprachiasmatic nucleus (SCN). The dorsal raphe (DR) is a source of serotonin innervation to both the IGL and the SCN. In the present study we investigated the localization of GABA-A/benzodiazepine (GABA-A/BZ) receptors in the circadian system using immunocytochemistry with a specific monoclonal antibody to the receptor (Vitorica et al, 1987) and localization of binding of [^{3}H]diazepam (DZP) and [^{3}H]flunitrazepam (FLU) by autoradiography.

There is no GABA-A/BZ receptor immunoreactivity in either the IGL or SCN but the DR shows a fine granular pattern of immunoreactivity. Semiquantitative analysis of autoradiograms for DZP and FLU binding reveals levels close to background in the IGL with the DR and SCN having levels of binding approximately 2.5 times greater than background. This pattern of immunoreactivity suggests that the DR is the most likely site of BZ effects on the circadian system but does not exclude the possibility that the IGL or SCN might mediate the effects.

Supported by NIH grants NS-16304 and NS-22168.

143.9

MOLECULAR WEIGHT ESTIMATION OF THE PERIPHERAL BENZODIAZEPINE RECEPTOR SOLUBILIZED FROM RAT LIVER.

A.L. Parola*[1], A.R. Buckley*[2], C.W. Putnam*[3,4,5], D.W. Montgomery*[5], D.H. Russell*[2], and H.E. Laird II[1]. Depts. of Pharm/Tox[1], Pharm.[3], and Surgery[4], Coll. of Pharmacy and Medicine, Univ. of Arizona, Tucson, AZ 85721 and VA Medical Center[5], Tucson, AZ 85723 and Dept. of Pharm/Therapeutics[2], Univ. of South Florida, Tampa, FL 33612.

Peripheral benzodiazepine receptors (PBZR) have been demonstrated in rat liver. The PBZR ligand, ^{3}H-Ro 5-4864, was used to pharmacologically characterize this site in the liver and revealed a single homogeneous receptor population ($n_H = 1$). Scatchard analysis of a saturation isotherm at 4°C gave a dissociation constant of 0.95 nM with a B_{max} = 5.2 pmol/mg protein. Analysis of digitonin solubilized receptors revealed a single homogeneous population of ^{3}H-Ro 5-4864 binding sites (n_H = 1) with k_d=2.6nM and B_{max} = 1.8 pmol/mg protein. Approximately 35% of the membrane receptor sites were solubilized and had the same rank order of potency for PBZR ligands as the membrane PBZR.

A PBZR photoaffinity ligand, ^{3}H-PK 14105, has been used to isolate the receptor protein from liver. A 5 nM solution of ^{3}H-PK 14105 was incubated with a crude liver membrane preparation in the presence and absence of 1 uM PK 11195 and irradiated. Approximately 25% of ^{3}H-PK 14105 was irreversibly bound after irradiation. The photoaffinity labeled PBZR was solubilized and run on SDS-PAGE. Autoradiography of the gel revealed a single radioactive band with an apparent molecular weight of 15 kd. This is in agreement with other estimations of the molecular weight for the PBZR from rat kidney.

143.10

DEMONSTRATION OF A DISTINCT CLASS OF HIGH-AFFINITY RECEPTORS FOR [^{3}H]ß-CARBOLINES IN MAN AND RAT.

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[^{3}H]Norharman ([^{3}H]ß-carboline) binds with high affinity (K_D 1.55 nmol/l, B_{max} 148 fmol/mg protein) to plasma membranes of rat brain (Pawlik and Rommelspacher, Eur. J. Pharmacol. 147: 163, 1988). High affinity binding sites were demonstrated for [^{3}H]harman (1-methyl-ß-carboline) in rat brain, spinal cord as well as human lymphocytes. Some evidence has been presented that both ß-carbolines act as endogenous modulators in the brain. They occur endogenously in man and rat and stimulate adenylate cyclase - suggesting activation of a second messenger mechanism (Rommelspacher et al., Arch. Pharmacol. 337: 116, 1988). The ß-carbolines have been found to elicit convulsive and conflict-augmenting effects at remarkably low doses. Quantitative autoradiography in rat brain slices revealed a distribution pattern which differs from that of other previously described receptors or binding sites (e.g. benzodiazepine, tryptamine, 5-hydroxytryptamine (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_2)) which suggests that a unique class of [^{3}H]ß-carboline binding sites exists in the rat brain. The present findings may explain the biological effects of these ß-carbolines which differ from other ß-carbolines which bind with high affinity to the benzodiazepine binding sites. The latter substances show k_i-values higher than 2200 nmol/l for [^{3}H]norharman binding.

143.11

REPEATED ADMINISTRATION OF BENZODIAZEPINE RECEPTOR LIGANDS IN THE BABOON.

CA Sannerud, JM Cook and RR Griffiths*
The Johns Hopkins Univ Medical School, Baltimore, MD 21205 and the University of Wisconsin, Milwaukee, WI 53201

Five groups of baboons received repeated administration of saline, 5.6 mg/kg midazolam (MDZ), 5.0 mg/kg flumazenil (Ro15-1788, Ro), 3.2 mg/kg 3-carboethoxy-ß-carboline hydrochloride (ßCCE) or 10 mg/kg ßCCE for 5 days. Behavioral signs of sedation and excitation were scored for one hour after intramuscular injections. Repeated MDZ resulted in tolerance to its initial sedative and ataxic effects; the significant increases in signs of abnormal postures, falls and ataxia on day 1 were attenuated by day 5. Repeated ßCCE resulted in a progressive sensitization to its convulsant properties; signs of abnormal postures and incidences of myoclonic jerks/seizure significantly increased over the 5 day exposure in the 10 mg/kg ßCCE group. Repeated Ro or saline produced no changes in behavior. In a second study, 3 groups of baboons received repeated daily injections of MDZ (5.6, 11.2 or 20 mg/kg) for 6 days. All 3 groups became tolerant to the sedative and ataxic effects of MDZ. Acute injections of 5.0 mg/kg Ro on day 5 produced a dose-dependent withdrawal syndrome, (i.e. twitch/jerk, limb tremor and abnormal postures). Ro produced an equivocal attenuation in the degree of tolerance to MDZ on day 6 with large between-animal differences in signs of ataxia and abnormal postures. Thus, tolerance to MDZ in baboons may have been partially reset by antagonist administration.

Supported by DA 01147.

143.12

PRENATAL DIAZEPAM (DZ) EXPOSURE: NEUROCHEMICAL AND BEHAVIORAL EFFECTS IN RAT OFFSPRING.

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Recently it has been suggested that benzodiazepine (BZ) exposure in utero increases stress reactivity in offspring. We hypothesized that such alterations may be mediated by changes in brain GABA or BZ receptors. Pregnant rat dams were injected with DZ or vehicle (VEH) s.c. at 5 mg/kg on gestational days (GD) 15, 16, 19, 20 and 21 and 7.5 mg/kg on GD 17 and 18. At postnatal day (PND) 70, DZ and VEH treated male offspring were exposed to 1 of 3 experimental conditions: shock, handling or no handling. At PND 90, all males were exposed to a behavioral test battery sensitive to fear. Subjects were sacrificed on PND 104 and brains were dissected for neurochemical analysis. Prenatal treatment effects on behavior were slight and not suggestive of enhanced stress reactivity. BZ receptor binding was unaltered by prenatal DZ in hippocampus, hypothalamus and thalamus. NE levels were unaltered in hypothalamus. However, GABA receptor binding in thalamus increased across levels of conditioned fear, and this increase interacted significantly with the prenatal BZ treatments. These data do not support the contention that prenatal BZ exposure directly enhances fearfulness. They do suggest the possibility of more subtle alterations in response to fear-eliciting pretreatments.

143.13

STEROIDS MODULATE THE $GABA_A$ RECEPTOR LINKED Cl^- IONOPHORE THROUGH DISTINCT SITES OF ACTION. K.W. Gee, R.E. Brinton and D. Joy*. Sch. of Pharm., USC, L.A, CA 90033

Progesterone (P) and some of its metabolites can modulate the $GABA_A$/benzodiazepine receptor (GBR) linked Cl^- ionophore (Majewska et al., Science, 232:1004, 1986; Gee et al., Eur J. Pharmacol., 136:419, 1987). The barbiturate-like actions of the most potent P metabolites prompted the hypothesis that these steroids share a common site and mechanism of action with the hypnotic barbiturates. Pregnenolone sulfate (PS) was reported to act as a GABA "antagonist" at the same site as the chloride ionophore ligand, t-butylbicyclophosphorothionate (TBPS, Majewska & Schwartz, Brain Res., 404:355, 1987). In this study, the effect of P metabolites on the binding of ^{35}S-TBPS to the GBR-linked Cl^- ionophore in rat brain suggests that they act at sites independent of any known regulatory sites on the complex. Analysis of interactions between the steroids as modulators of ^{35}S-TBPS binding indicate multiple sites of action. The interactions between the hypnotic steroids and barbiturates in similar studies show characteristics inconsistent with a competitive-type interaction. PS will allosterically modulate the dissociation of ^{35}S-TBPS in a manner that suggests PS and TBPS do not act at a common site. Whether these steroid effects are "receptor" mediated or the result of membrane-lipid perturbations remain to be determined. (Supported by a NIH Grant NS 24645)

143.14

STRESS HORMONES, ETHANOL, AND GABA INTERACTIONS IN LS AND SS MICE. B. J. Martin* and J. M. Wehner. (SPON: M.Upchurch). Inst. for Beh. Gen., Univ. of Colo., Dept. of Psych., Boulder, CO 80309.

Recent investigations have demonstrated that steroid hormones and their metabolites enhance GABAergic activity. In addition it has been proposed that ethanol's effects may be mediated via interactions with the GABA/BZ receptor. To determine the relationship between ethanol, stress hormones and their effects on the GABA/BZ receptor, GABA-enhanced ^3H-flunitrazepam (FNZ) binding was measured in long-sleep (LS) and short-sleep (SS) mice after adrenalectomy (ADX) and in vivo treatments. A time course evaluated 24 hr-14 days post-ADX demonstrated an increase in enhancement in cortex, cerebellum and hippocampus in both LS and SS mice. The greatest degree of enhanced binding appeared by one week post-surgery in cortex and cerebellum and by two weeks in hippocampus. Intraperitoneal injections of 2.5 g/kg ethanol 30 min before sacrifice significantly increased enhancement in SS but not LS cortical tissue. In cerebellum, ethanol treatment increased enhancement in both lines with the greater response demonstrated by LS mice. A similar pattern of FNZ-binding was observed after stress induced by a 20 min confinement to an open arm of the elevated plus-maze.
(Supported by AA-03527 and MH-16880).

143.15

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE ENDOGENOUS BENZODIAZEPINE LIGAND OCTADECANEUROPEPTIDE (ODN) IN THE RAT BRAIN. M.C. Tonon*[1], L. Désy*[2], H. Vaudry*[1] and G. Pelletier[2] (SPON: A. Dupont). [1]Lab. Endocrinologie, Univ. Rouen, France and [2]MRC Group Mol. Endocrinol., C.H.U.L., Quebec, Canada.

In order to study the distribution of the endogenous benzodiazepine ligand octadecaneuropeptide (ODN) in rat brain, we have developed antibodies against this peptide. By immunocytochemistry, ODN-immunoreactive material was detected in cells which have the appearance of glial cells. They were found in high concentrations in the cerebral cortex and the hypothalamus, especially in the supraoptic, periventricular and arcuate nucleus. No positive axons or terminals could be observed. Immunostained cells were also observed in the cerebellar cortex. They seem to correspond to basket cells. Immunoabsorption with synthetic ODN completely prevented immunostaining. Using a radioimmunoassay for ODN, we have observed that synthetic ODN and serial dilutions of cerebral cortex extracts gave parallel displacement curves. These studies clearly demonstrate that ODN immunoreactive material is present in large amounts in areas such as the cerebral and cerebellar cortex which are known to contain high concentrations of benzodiazepine receptors. Immunostaining appears to be mostly associated with glial cells. Whether or not these cells are involved in the biosynthesis of ODN or ODN-related peptide(s) remains to be established.

143.16

CEREBRAL GABA CHANGES IN PYRITHIAMINE-TREATED RATS: IMPLICATIONS FOR THE PATHOGENESIS OF WERNICKE'S ENCEPHALOPATHY. M. Héroux* and R.F. Butterworth, Lab. of Neurochem., André-Viallet Clin. Res. Ctr., Hôpital St-Luc, Montreal, Que., Canada H2X 3J4.

Treatment of rats with pyrithiamine (PT) results in severe neurological dysfunction. GABA content of brain tissue from PT-treated rats was reduced in thalamus (by 45%, $p<0.01$), cerebellum (by 18%, $p<0.05$) and pons (by 30%, $p<0.05$). Activities of the thiamine-dependent enzyme α-ketoglutarate dehydrogenase (αKGDH) were reduced in parallel with the GABA changes and alanine concentrations were found to be concomitantly increased. Activities of glutamic acid decarboxylase remained within normal limits as did affinities and densities of high affinity ^3H-muscimol binding sites. Thiamine administration to symptomatic animals resulted in reversal of neurological abnormalities and in normalization of GABA and alanine levels and of αKGDH activities in cerebellum and pons. These results suggest that the reversible symptoms of PT treatment result from impaired GABA synthesis in pontine and cerebellar structures. Similar mechanisms may play a role in the pathogenesis of the reversible symptoms of Wernicke's Encephalopathy in man. [Supported by MRC (Canada)]

143.17

DIAZEPAM BINDING INHIBITOR IN HEPATIC ENCEPHALOPATHY. J. Rothstein, G. McKhann, P. Guarneri, M. Barbaccia, A. Guidotti, E. Costa. Dept. Neuro., Johns Hopkins Univ., Baltimore, MD 21205 and FIDIA Georgetown Inst., Wash. D.C. 20007.

Recent theories on the neurochemical etiology for hepatic encephalopathy (HE) suggest activation of GABA systems. GABA receptor sites are modulated by benzodiazepines (BZ) and BZ receptor antagonists have ameliorated HE in several small clinical trials. The neuropeptide diazepam binding inhibitor (DBI) is an endogenous modulator of GABA systems at the BZ receptor. We measured CSF and serum DBI, ammonia and amino acid concentrations in patients with HE. Several amino acids were significantly elevated in the CSF of patients with HE including glutamine, tyrosine and phenylalanine ($p<0.05$). Cerebrospinal fluid DBI levels were elevated 4-5 fold in patients with HE ($p<0.001$), but were normal in those patients with liver disease not associated with mental status changes. Levels of DBI correlated with the clinical staging of HE ($p<0.05$) and with CSF glutamine concentrations ($p<0.05$). Normalization of mental status was shown to result in normalization of CSF DBI levels. These data and others suggest an important role for endogenous BZ in the pathogenesis of HE.

143.18

AUTOANTIBODIES DIRECTED AGAINST GABA-ERGIC SYNAPSES IN FOUR MORE PATIENTS AFFECTED BY STIFF-MAN SYNDROME. F. Folli *+, M. Solimena*0+, V. Toso +, and P. De Camilli*0. *Dept. of Medical Pharmac.-CNR Center of Cytopharmac and +Clinica Medica VII, S.Raffaele Hosp., Univ. of Milano, Italy; Div. of Neurol., Castelfranco Veneto Hosp., Italy; 0Dept. of Cell Biology, Yale University, USA.

We have recently reported the occurrence of autoantibodies directed against GABA-ergic synapses in both serum and cerebrospinal fluid (CSF) of a patient affected by stiff-man syndrome (SMS), epilepsy and type 1 diabetes mellitus. Glutammic acid decarboxylase (GAD) was identified as the major target of those autoantibodies. (M. Solimena, F. Folli, S. Denis-Donini, G.C. Comi, G. Pozza, P. De Camilli and A. Vicari. New Engl. J. Med. 1988, 318: 1012-21). We have now found that the sera of four other patients affected by SMS contain antibodies which produce staining patterns identical to those produced by the autoantibodies of the previous patient and by GAD antibodies when tested by light microscopy immunocytochemistry on rat tissue sections. An identical staining pattern was also produced by the CSF of one of the patient (the only CSF so far available to us). "Western blot" experiments are in progress to identify the protein antigens recognized by the autoantibodies. These findings strongly support the hypothesis that autoimmunity, and possibly autoimmunity directed against components of GABA-ergic synapses, might be involved in the pathogenesis of stiff-man syndrome. (Supported in part by grants from the Italian CNR and from MDA to P.D.C. We are indebted to Dr. F. Howard [Mayo Clinic] and to Drs. A. Harding and C.D. Marsden [Natl. Hosp. for Nervous Diseases, London] for referring their patients to us).

143.19

EFFECTS OF TAURINE DERIVATIVES ON GABA AND BENZODIAZEPINE BINDING IN INTACT NEURONS AND IN EPILEPTIC PATIENTS. O.Malminen* and K.Nieminen. Lab. of Clinical Pharmacology, Huhtamäki Pharmaceuticals, POB 325, Helsinki, SF-00101 Finland.

Exogenous taurine has been tested to be an effective anticonvulsant when applied topically in most animal models of epilepsy. The underlying mechanism may be the modulation of GABA neurotransmission in the brain. However, its penetration through the blood brain barrier is poor due to its polarity and lipophobic nature. We have studied the properties of some lipophilic taurine derivatives with radioligand binding techniques and also summarize data from clinical trials where epileptic patients were treated with taltrimide (MY 117, 2-phthalimidoethanesulphon-N-isopropylamide), the pharmacologically most promising derivative in preliminary animal studies.

The effects of six lipophilic taurine derivatives on GABA and BZD receptor binding to intact granule cells of neonatal rat were studied with primary cell culture. The amide and isopropylamide derivatives of phenylsuccinyl-imidotaurine, MY 135 and MY 136 enhanced muscimol binding, whereas taurine reduced muscimol binding. Five of the compounds studied, as well as taurine itself, reduced flunitrazepam binding stimulated by GABA, being thus able to interfere with the coupling between GABA and BZD receptors. The effect was not significant with taltrimide.

In previous, open clinical trials taltrimide was ineffective to suppress convulsive seizures when it was given to severe epileptic patients. It was also ineffective to reduce photoconvulsive response in EEG. In controlled, randomized, cross-over trial orally administered taltrimide was compared with sodium valproate and placebo in 17 epileptic patients on carbamazepine monotherapy : in primary generalized epilepsy taltrimide reduced the number of attacks by 49 % and valproate by 38 %. Also the subjective evaluations made by patients and physician suggest that taltrimide is at least as effective as valproate in primary generalized epilepsy. In focal epilepsy taltrimide was not more effective than placebo.

143.20

GABA OVERFLOW IN RAT GLOBUS PALLIDUS FOLLOWING NEUROLEPTIC AND APOMORPHINE ADMINISTRATION MEASURED BY IN VIVO MICRODIALYSIS. K.L. DREW, W.T. O'CONNOR*, J. KEHR* AND U. UNGERSTEDT*. Dept. of Pharmacology, Karolinska Institute, Stockholm, Sweden.

The dopamine agonist apomorphine (1.0 mg/kg,sc) decreased, while the DA antagonists haloperidol (0.5mg/kg,sc) and clozapine (20.0mg/kg,sc) increased GABA overflow in the rat globus pallidus. These data are consistent with electrophysiological studies indicating that DA receptor stimulation enhances the activity of pallidal neurons (presumably by decreasing the release of GABA in the globus pallidus).

Male, Sprague-Dawley rats were anesthetized with halothane and dialysis probes (Carnegie Medicin) were implanted into the right globus pallidus. Probes were perfused with ringer solution at a flow rate of 2.0 ul/min. Samples were collected at either 30 min or 10 min intervals. Ten ul of perfusate from each fraction was then analyzed for GABA by a novel OPA/t-butylthiol, precolumn derivitization, reverse phase HPLC procedure with electrochemical detection. Additional experiments are in progress to study GABA overflow in the substania nigra and the effects of more selective D-1 and D-2 agonists and antagonists on GABA overflow in the globus pallidus and substantia nigra.

AMINO ACIDS: GABA AND BENZODIAZEPINES III

144.1

EVALUATION OF THE ANTICONVULSANT EFFECTS OF DIAZEPAM AND MK-801 IN SOMAN POISONING. T.-M. Shih, T. Koviak*, and D. Rennix. Biochem. Pharm. Br., U. S. Army Med. Res. Inst. Chem. Def., Aberdeen Proving Ground, MD 21010.

The acute toxic effects of the organophosphorus cholinesterase inhibitor soman are tremors, convulsions and death. The purpose of this study was to establish a rat model to evaluate potential anticonvulsants in soman poisoning. To increase survival, the oxime HI-6 (125 mg/kg, ip) was given to male rats either 30 min prior to or immediately after various subcutaneous doses of soman. In both treatment modes, soman at a dose of 1.6 LD50 produced 100% occurrence of convulsions. Pretreatment with HI-6 showed the least variability in the onset of convulsions and mortality, and was chosen as the mode for studying the anticonvulsant effects of diazepam and MK-801. Various doses of both compounds were given with or without the concomitant administration of the atropine sulfate (16 mg/kg, im) 30 min prior to soman (1.6 LD50, sc) challenge. Without atropine, diazepam (1.25 to 10.0 mg/kg, im) did not prevent soman-induced convulsions nor did MK-801 at or below 2.5 mg/kg, but above this dose MK-801 markedly potentiated soman-induced toxicity and death. In the presence of atropine, the anticonvulsant ED50s for diazepam and MK-801 were 134.2 and 36.8 µg/kg, im, respectively. The findings indicate that in soman poisoning diazepam and MK-801 are effective anticonvulsants only in the presence of atropine sulfate.

144.2

EFFECTS OF CENTRALLY ADMINISTERED Ro 15-1788 ON BETA-CARBOLINE-3-CARBOXYLIC ACID ETHYL ESTER INDUCED ALTERATIONS IN LOCOMOTOR ACTIVITY AND ANXIETY IN RATS. L.J. Wichlinski and R.A. Jensen. Biopsychology Lab., Dept. of Psychology, Southern Illinois University, Carbondale, IL 62901.

This experiment was aimed at replicating our previous finding of enhancement of locomotor activity following a 10.0 ug dose of B-CCE administered i.c.v. to rats. A second goal was to block this expected enhancement with the benzodiazepine receptor antagonist Ro 15-1788, also given i.c.v. Such antagonism would confirm that central benzodiazepine receptors mediate this effect.

Ro 15-1788, 0.25 ug or 1.25 ug, or vehicle was given i.c.v. five minutes before either vehicle or 10.0 ug B-CCE (n = 5-8). Locomotor activity was then electronically monitored for 30 minutes. B-CCE enhanced locomotor activity once again, here during the 15-20 min time block after infusion. Neither dose of Ro 15-1788 significantly attenuated this effect. However, the two B-CCE groups pretreated with Ro 15-1788 did not differ significantly from vehicle-treated animals. Following activity testing, the rats were placed in an elevated plus maze, a behavioral measure that detects anxiogenic activity. B-CCE failed to produce anxiogenic effects, although the B-CCE-treated rats were highly sensitive to touch. Five of the B-CCE-treated rats, regardless of pretreatment condition, showed almost complete suppression of activity during the first 5 minutes following drug administration, an effect not seen in any non-B-CCE-treated rats.

144.3

REGULATION OF GLUTAMIC ACID DECARBOXYLASE IN THE CEREBRAL CORTEX, D.L. Benson, P.J. Isackson, A.J. Tobin and E.G. Jones Department of Anatomy and Neurobiology, University of California, Irvine, 92717 and Department of Biology, University of California, Los Angeles.

Antisense RNA probes prepared against cat and human cDNAs for glutamic acid decarboxylase (GAD) were used to localize the sites of synthesis of GAD in the cat and rat cerebral cortex, hippocampus, brainstem, striatum, thalamus and hypothalamus and in different regions of monkey cortex. Consistent with various immunocytochemical studies, 25-30% of the neurons with these regions contained GAD mRNA. Northern blot analysis of RNA prepared from different regions of rat, cat, and monkey brains demonstrated the presence of two apparent GAD transcripts which could be resolved on formaldehyde agarose gels. Both transcripts were present in all brain regions examined.

GAD immunoreactivity has been previously shown to decrease in ocular dominance columns of the visual cortex of monocularly deprived macaque monkeys. RNA prepared from visual cortex of these monkeys does not show a major change in levels of GAD mRNA relative to normal visual cortex from nondeprived monkeys Potential regulation of GAD at the mRNA level in monocularly deprived monkeys is currently being more rigorously examined with in situ hybridization.

144.4

EFFECT OF DIAZEPAM (DZ) ON ELECTROACUPUNCTURE (EA) -INDUCED CHANGES IN BRAIN REGIONAL GABA. S.Chakrabarti* and M.K.Poddar. Dept. of Biochemistry, University of Calcutta, 35, B.C. Road, Calcutta 700 019, India.

EA(10Hz, 1 volt; 15 min) or DZ (5 or 20 mg/kg, i.p.) -induced increase of GABA level in pons-medulla (PM) and thalamus (T) but not in other regions of adult male albino rats was potentiated in T and reduced in PM when animals were exposed to both EA and DZ. Cotreatment with EA and DZ significantly reduced GAD activity in only PM although EA or DZ alone was ineffective. EA-induced rise in hypothalamic (H) and fall in spinal (S) GAD activity did not change with DZ. DZ alone increased GAD activity in only S. EA or DZ-induced rise in GABA-T activity in T, H and S was potentiated in H and S but reduced in T during their cotreatment. Cotreatment of EA and DZ normalized the EA-induced rise of PM and cortical (C) GABA-T activity. DZ alone reduced GABA-T activity in only C. EA-induced rise in glutamate (Glu) level in C and PM was increased with both EA and DZ. DZ alone reduced Glu level in only C. DZ-induced increase of GABA turnover (TO) in all the regions was greatly enhanced when DZ and EA were cotreated. EA alone stimulated GABA TO of S, without affecting other regions. These results suggest that EA or DZ-induced characteristic and region specific change in GABA system is either potentiated or abolished when the subject is co-exposed with EA and DZ.

(Supported by ICMR, New Delhi, India).

144.5

LATENCIES CORRESPOND FOR GABA DEPLETION AND VACUOUS CHEWING BEHAVIOR ELICITED BY INTRANIGRAL ISONIAZID. S.E. Bachus. Dept. Pharmacol., Georgetown Univ., Wash. D.C., 20007.

We have previously reported[1] that intranigral isoniazid (INH) elicits vacuous chewing movements in rats after a latency of 20 min. I have measured nigral GABA levels at 10, 20, 30 and 40 min after microinfusion of 1.02 µmol INH/ 1 µl/6 min through guide cannulae in free-moving rats, with the enzymatic-fluorometric assay of Okada et al.[2]

At 10 min after INH, GABA, at 93.5% of control values, was not yet significantly reduced. GABA levels were significantly reduced ($p<.0005$) by 20 min, at 70.1% of control values, and remained low at 30 min (61.0% control) and 40 min (61.2% control). Thus, the latency for significant depletion of nigral GABA by INH appears to match that for elicitation of vacuous chewing movements.

We also noted[1] that rats receiving intranigral INH under ether anesthesia responded with vacuous chewing behavior only after a longer latency. In another group of rats, I compared nigral GABA levels 20 min after intranigral INH infused either through guide cannulae, or under ether anesthesia. GABA levels after INH were markedly higher in ether-treated rats (83.2% control; $p<.01$) than in rats with guide cannulae, whose GABA levels resembled those reported above (71.4% control). Ether had no effect on basal GABA levels. We are continuing to investigate whether ether interferes with the mechanism of action of INH, or affects GABA turnover rate.

[1]Exp. Neurol. 100:459, 1988

Supported by HHS grant MH43651

[2]Exp. Brain Res.13:514, 1971

144.6

QUANTAL RELEASE OF AMINO ACID TRANSMITTERS IN THE MEDIAL NUCLEUS TRACTUS SOLITARIUS (M-NTS) ANALYZED USING ISOLATED ADULT NEURONS. J.A. Drewe and D.L. Kunze. Baylor College of Medicine, Houston, TX, 77030.

The baroreceptor afferents synapse in the M-NTS, primarily on 10-15 µm diameter bipolar neurons. Neurons of this morphology were enzymatically dissociated from adult guinea pig M-NTS. We previously reported that spontaneous transient excitatory amino acid (EAA)-mediated synaptic currents (EPSC) and GABA-mediated synaptic currents (IPSC) could be recorded in this preparation. EAA activate non-specific cation conductances ($pNa=pK=pCs$), while GABA activates a Cl conductance ($pCl>>pMeSO_3$). Whole cell patch clamp recordings were made using a normal bath solution. $Cs\text{-}MeSO_3$ was used internally (E_{Cl}=-91 mV). The amplitudes of the spontaneous currents appeared to fluctuate in a quantal fashion. To limit the range of amplitudes, we studied the spontaneous GABA-mediated currents in nominally 0 Ca^{2+}. This decreased the amplitudes encountered to less than 15 pA. At 0 mV the miniature IPSC (mIPSC) were separated into 1 pA bins and the majority occurred at 3-5 pA (90mV from E_{Cl}). An 80 pS conductance change was found for the miniature EPSC. The frequency of the mIPSC was increased by exposure to a 0 K^+ extracellular solution which blocks the Na pump and produces presynaptic depolarization. This is a first report of quantal EPSC and IPSC in an adult mammalian CNS preparation where events of this magnitude are resolvable. Supported by DHHS 36840.

144.7

THE EFFECT OF SACRIFICE BY DECAPITATION OR MICROWAVE IRRADIATION ON RAT BRAIN AMINO ACIDS. J.M. Miller*, T.N. Ferraro, R.S. Jope, O.C. Snead and T.A. Hare. Depts. Pharmacology and Neurology, Thomas Jefferson Univ., Phila., PA 19107 and Univ. of Alabama, Birmingham, AL 35294.

Decapitation has been the principal method of animal sacrifice for neurochemical studies. Modern focused beam microwave irradiation (FBMI) instruments allow sacrifice of small animals with concomitant inactivation of brain enzymes, thereby eliminating enzyme-mediated post-mortem metabolic changes.

Over 30 amino acids and related compounds were measured in the substantia nigra, striatum, hippocampus and cortex of male Sprague-Dawley rats sacrificed by decapitation (n=5) or by FBMI (n=5). After homogenization in perchloric acid, supernatants were analyzed using a fully validated triple column ion-exchange amino acid analyzer with postcolumn o-phthalaldehyde derivatization and fluorometric detection.

Ala, GABA, Ethanolamine and NH_3 concentrations were significantly lower in all four brain regions of the FBMI group. Val, Leu, Tyr and Phe levels were also significantly lower in the substantia nigra, hippocampus and striatum of the FBMI group. The FBMI group showed significantly reduced Asp levels in the substantia nigra and hippocampus as well as Gly levels in the striatum, hippocampus and cortex. The only amino acid levels significantly higher in the FBMI group were GSH levels in the striatum and substantia nigra and Glu in the substantia nigra.

These results document the substantial impact of method of sacrifice on the baseline concentrations of brain amino acids.

144.8

STUDIES OF THE FORMATION OF γ-HYDROXYBUTYRIC ACID (GHB) FROM GABA AND 1,4-BUTANEDIOL (BD) IN BRAIN. O.C. Snead, R. Furner*, & C.C. Liu*, Department of Pediatrics & Neuropsychiatry Research Program, University of Alabama at Birmingham School of Medicine, Birmingham, Alabama 35233.

GHB is a naturally occurring compound in brain which has the ability to produce absence-like seizures in animals. Although GABA is thought to be the main parent compound for GHB, there is some evidence that 1,4-BD is also a putative precursor for this substance. The relative formation of GHB from GABA and 1,4-BD was explored by following the formation of ^{13}C-GHB in rat brain after intracerebroventricular (icv) administration of either ^{13}C-GABA or ^{13}C-1,4-BD.

^{13}C-GHB was readily formed in brain after the icv administration of the stable isotope of either GABA or 1,4-BD. The turnover times of GABA- and 1,4-BD-derived GHB were 2.04 and 1.40 nM/G/h respectively. The conversion of ^{13}C-GABA to ^{13}C-GHB was completely blocked by GABA-T inhibitors. The 1,4-BD->GHB pathway was not sensitive to inhibition by either pyrazole or ethanol indicating involvement of some enzyme other than alcohol dehydrogenase in this conversion. These data confirm the GABA->GHB pathway in brain, demonstrate conclusively that the brain is capable of converting 1,4-BD to GHB, and support the hypothesis that a 1,4-BD->GHB pathway is operative in brain (Barker et al, Biochem Pharm 34:1852, 1985).

144.9

DOPAMINE MODULATES GABA RELEASE IN THE PARS RETICULATA OF THE RAT SUBSTANTIA NIGRA. B. Florán*, A. Arias*, A. Sierra*, D. Martínez-Fong* and J. Aceves* (SPON: J. Hernández). Dept. of Physiology. Centro de Investigación del IPN. México, D.F. México.

Although there are D-1 DA receptors on striatonigral GABA terminals innervating nigra reticulata cells, the role of these receptors is not clear. Here we present evidence showing that these receptors are involved in the modulation of GABA release by DA coming from DA dendrites. The experiments were done in slices of nigra reticulata of normal rats in comparison with rats with degeneration of the DA cells induced by 6-OHDA. The slices were loaded with tritiated GABA in the presence of β-alanine (1 µM). The superfusion solution contained nipecotic acid (10 µM). Release was evoked by a continuous high (15 mM) K depolarization. In normal rats, both the GABA-B agonist, Baclofen (100 µM), and the D-1 antagonist, SCH 23390 (1 µM), inhibited the GABA release, but the D-2 antagonist, Sulpiride (10 µM), stimulated the release. In the DA-denervated slice, the effect of these drugs was lost, but DA (100 µM) markedly enhanced the release; this enhancement was blocked by SCH 23390 but not by Sulpiride. The results suggest a reciprocal DA-GABA interaction in nigra reticulata: DA, stimulates GABA release, and, GABA inhibits DA release.

144.10

Ca^{2+}-DEPENDENT and Ca^{2+}-INDEPENDENT [^{3}H]GABA RELEASE FROM HIPPOCAMPAL NEURONS AT DIFFERENT TIMES IN CULTURE Katherine M. Harris and Richard J. Miller Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637

γ-aminobutyric acid (GABA) release from neurons has been shown to have both Ca^{2+}-dependent and -independent components. GABA may be released by the reversal of the electrogenic Na^+/GABA cotransport process. Hippocampi dissected from fetal (E 18) rats were dissociated, grown in primary culture and used experimentally after 8, 15 or 22 days in vitro. Cultures of hippocampal neurons accumulated and stored [^{3}H]GABA. [^{3}H]GABA was released when the cells were depolarized with either 50mM K^+ or 30µM veratridine. Veratridine stimulated release was 4-fold higher than 50mM K^+ stimulated release at 8, 15, and 22 days in vitro. The amount of [^{3}H]GABA released, increased with time in culture. Release evoked by either 50mM K^+ or 30µM veratridine was more Ca^{2+} sensitive at 15 days in vitro than at 8 days in vitro. The amount of [^{3}H]GABA released by 100µM N-methyl-D-aspartate (NMDA) or 100µM kainate did not change with time in culture. Release evoked by 50mM K^+, 30µM veratridine, 100µM NMDA, or 100µM kainate could be inhibited by GABA transport inhibitors, nipecotic acid, SKF 100330-A, SKF 89976-A or SKF 100561. The SKF inhibitors were more effective than nipecotic acid at blocking release. The increased Ca^{2+} sensitivity of [^{3}H]GABA release with time in culture may reflect an increase in synaptogenesis in vitro.

144.11

EVIDENCE THAT VAGINOCERVICAL STIMULATION RELEASES AMINO ACIDS INTO SUPERFUSATES OF THE SPINAL CORD. D.B. MASTERS, C. BEYER, ^F. JORDAN*, J.L. STEINMAN AND B. R. KOMISARUK. INST. OF ANIMAL BEHAVIOR AND ^DEPT. OF CHEMISTRY, RUTGERS UNIVERSITY, NEWARK, N.J. 07102 USA.

We hypothesized that inhibitory amino acids are released endogenously in the spinal cord in response to vaginocervical mechanostimulation (VS) and thereby produce analgesia. This hypothesis was tested by HPLC analysis of the amino acid content of superfusates of the spinal cord before, during and after VS (400g force) in the urethane-anesthetized rat. Artificial cerebrospinal fluid was continuously superfused over the spinal cord through the intrathecal space surrounding the sacral-lower thoracic region. Automated pre-column derivatization with ortho-phthalaldehyde (OPA) was performed to achieve less than 1.0 picomole fluorescence detection.

VS significantly increased (n=8; $p \leq 0.01$; ANOVA) the levels of the following inhibitory amino acids from baseline levels (mean % increase ± S.E.M.): glycine (48.8±10.9), taurine (40.9± 9.3), alanine (23.8±5.6), and serine (40.2±10.6). VS also significantly increased the levels of the following excitatory amino acids: aspartate (265.7±161.4) and glutamate (109.9 ±33.7). Levels of other amino acids were also increased significantly by VS: glutamine (44.2±13.1), threonine (37.4±10.3), and arginine (36.5±9.4). Superfusion with KCl (34.0 mM) significantly increased aspartate (77.4±26.8), glutamate (39.4±15.0), GABA (199.2±65.8), glycine (80.9±17.0), taurine (30.1±13.0), alanine (16.1±8.0), and arginine (15.4±7.2).

Thus, inhibitory and excitatory amino acids are released into the spinal cord by VS and may mediate its effects. The observed effect of KCl provides supportive evidence that the amino acid release is a consequence of intraspinal neuronal activity. Preliminary results with microdialysis of the sacral dorsal horn support the above findings.

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144.12

THE IMIDAZOBENZODIAZEPINE RO 15-4513 LOWERS THRESHOLD FOR KINDLED SEIZURES IN THE BASOLATERAL AMYGDALA OF THE RAT. M.A. Bixler and M.J. Lewis. Dept. of Psychology, Howard University, Washington,D.C. 20059.

RO 15-4513 is reported to block the anticonflict and behavioral intoxicating effects of ethanol (Suzdak, P.D. et al., Science, 234: 1243, 1986). Based on evidence that it may also induce convulsive behavior (Miczek, K.A. and Gold, L.H., Psychopharm., 81: 183, 1983) and EEG activity indicative of seizure (Britton, K.T. et al., Science, 239: 648, 1988), the present study investigated the effect of RO 15-4513 (6.0 mg/kg, i.p.) on threshold for seizures in fully kindled rats. Bipolar electrodes were implanted in the basolateral amygdala (-2.2AP, +4.7L, -8.5V). Following recovery, daily stimulation (200 microamperes, base-to-peak) was administered until stage 5 seizure was observed. Threshold determinations were then conducted on an A-B-A design. RO 15-4513 treatment was found to significantly lower seizure threshold relative to the level observed following the initial vehicle pretreatment.

Supported in part by NIAAA grants AA06263 and RR08016.

144.13

EFFECTS OF GABA ANTAGONISTS ON THE ELECTRIC ACTIVITY OF HYPOTHALAMIC ARCUATE (ARC) AND VENTROMEDIAL (VMN) NUCLEI NEURONS IN VITRO. S. Ogawa, L.-M. Kow and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

To examine the possibility that neurons in the ARC and VMN are tonically inhibited by intrinsic GABAergic neurons, extracellular single-unit activity of ARC and VMN neurons was recorded from hypothalamic tissue slices prepared from female rats. In one type of experiment, slices were perfused with a GABA antagonist, picrotoxin (PTX; 0.25mM), or vehicle. In the ARC, the percentage of spontaneously firing neurons (57% of 152 for PTX vs 55% of 130 for vehicle) and their firing rates ($\bar{x}$±SEM spikes/sec, 1.24±0.14 vs 1.62±0.21) were not affected by PTX. In the VMN, the percentage of spontaneously firing neurons was actually lower (Chi-square test, $p<0.02$) with PTX (55% of 86) than with vehicle perfusion (69% of 84), although their firing rates were not different (1.91±0.18 vs 2.19±0.23). In another type of experiment, bath applications of bicucullin methiodide (BMI; 0.125mM) and PTX (0.125mM) were found to affect the firing rate of some ARC and VMN neurons, indicating interruption of tonic GABA action. The effect of BMI was often different from that of PTX. Thus, intrinsic tonic GABA inhibitions appear to exist in the ARC and VMN, but GABA may act through more than one mechanism.

144.14

PREDICTABILITY, CHRONICITY, AND TYPE OF STRESS: DIFFERENTIAL EFFECTS ON PICROTOXIN-INDUCED SEIZURES AND OPEN FIELD BEHAVIOR IN THE RAT. T.D. Wolinsky*, E.E. Coons*, and D.J. Nutt*. (SPON: D. Quartermain) NYU, Dept. of Psychology, NY, NY 10003 and NIAA, Bethesda, MD 20892

Using an animal model of depression (Katz, et al, Neurosci Biobev Rev, 1981) we previously reported that chronic varied, unpredictable stress (3 weeks) reduces the convulsant potency of the GABA antagonist picrotoxin (7.5 mg/kg, i.p.). Acute swim stress had an effect in the same direction but of a lesser magnitude. Relative to no-stress controls, we now compare the effects on seizures of chronic varied, chronic restaint and acute restraint stress. Behavioral depression was assessed by open field behavior 1 hour after the last treatment. Picrotoxin was administered the following day.

Chronic varied stress provided greater protection from picrotoxin-induced seizures than did other treatments. Both chronic stress groups showed the same degree of behavioral depression in the open field. Unlike acute swim animals, the acute restraint group exhibited more severe seizures relative to the other groups and the greatest attentuation of activity in th open feild. The severity of seizures in chronic restraint animals was less than acute restraint, but greater than chronic varied and no-stress groups.

These results demonstrate that different schedules of stress presentation influence the magnitude and direction of changes in seizure susceptibility in the rat, and by inference, changes in GABA activity. This suggests a role for the GABA/benzodiazapine system in adaptation to stress and in stress-induced depression.

144.15

EFFECTS OF THE β-CARBOLINE, NORELEAGNINE, ON ACTIVITY AND STARTLE HABITUATION IN PREWEANLING RATS. P.S. McGuire*, J. Tizzano, J.A. Johnson*, B.S. Martin* and J. Buelke-Sam. (SPON: J. Numberger) Dept. Pharmacol. and Toxicol., Ind. Univ. Med. Cen., Indianapolis, IN 46223, and Toxicol. Div., Lilly Res. Labs., Greenfield, IN 46140.

This study was designed to evaluate the actions of noreleagnine (NOR), a water soluble β-carboline, on developmental locomotor activity and startle habituation of preweanling rats. Six litters of SD rats and 15 litters of CD rats were tested; 1 pup/sex/litter was assigned to each dose group (0, 2, 4 or 8 mg/kg/day NOR ip). Dosing occurred on days 13-20 and testing was initiated 30 min later. The SD pups were tested in SDI home cage activity monitors for 30 min on days 13-20. The CD pups were tested in SDI Figure-8 mazes for 30 min on days 14, 16, 18 and 20, and again on day 21 with no drug pretreatment. On days 13, 15, 17 and 19, the CD pups were tested in SDI startle chambers. Each 50-trial session was made up of alternating 5-trial blocks of auditory (120 dB) and tactile (20 psi air puff) stimuli presented at 8-sec intervals. A dose-related increase was found in home cage activity in SD pups of both sexes on days 13-17 with significant increases on day 15 in males and day 13 and 15 in females. Figure-8 maze activity showed a dose-related decrease on days 16-20 in CD pups of both sexes. Activity levels on day 21 were comparable in all groups. Startle amplitude was increased by NOR treatment in a manner dependent on dose, sex, age and eliciting stimulus. Greater enhancement was seen: 1) in tactile than in auditory startle, 2) in females than in males, 3) at days 15 and 17, 4) at 4 mg/kg in males and 8 mg/kg in females. These results suggest that NOR affects early activity and reactivity in a manner consistent with its purported benzodiazepine/GABA inverse agonist properties.

144.16

ELECTROCONVULSIVE SHOCK (ECS) SELECTIVELY ALTERS THE PROFILE OF GABA LEVELS IN RAT BRAIN. T.N. Ferraro, G.T. Golden and T.A. Hare, Thomas Jefferson Univ., Phila. PA 19107 and VAMC, Coatesville, PA 19320.

ECS is a clinically effective antidepressant therapy with an unknown mechanism of action. Recent reports suggesting GABAergic involvement in the pathogenesis of depression have led us to study the effects of ECS on brain GABA levels. Male Wistar-Furth rats were subjected to ECS via earclip electrodes (EE) (n=6) or corneal electrodes (CE) (n=6) given once/day (120 V, 0.5 sec) for 10 days. A group of naive rats served as control. Current was measured and seizures were scored during each ECS trial. Rats were sacrificed by decapitation 24 hr after the last ECS, brains rapidly dissected according to a timed protocol and 8 discrete regions frozen on dry ice. GABA was measured in per-chloric acid extracts using an ion-exchange/fluorometric method. Results showed that rats receiving ECS via CE were subjected to significantly more current compared to rats treated with EE while exhibiting behavioral seizures of a similar duration. Compared to control, GABA levels were increased in all brain regions examined from rats treated with EE, except hippocampus; rats treated with CE exhibited significant GABA increases in hippocampus, frontal cortex, hypothalamus and olfactory bulbs. These results suggest that the mode of ECS delivery can selectively alter regional brain GABA levels. This effect may be a function of current intensity or localization.

144.17

SEIZURE THRESHOLDS IN DIAZEPAM-SENSITIVE AND -RESISTANT MICE. E.J. Gallaher* and S.E. Gionet* (SPON: JC Crabbe). VA Medical Center and Depts. of Pharmacology and Medical Psychology, Oregon Health Sci. Univ. Portland, OR 97201.

To facilitate the study of benzodiazepine (BZ) mechanisms, we developed diazepam (DZ)-sensitive (DS) and DZ-resistant (DR) mouse lines by selective breeding based on duration of rotarod impairment (Gallaher et al., Psychopharmacol., 93:25, 1987). Here we ask if the anti-convulsant BZ effect also segregated into the two lines.

We injected vehicle or DZ (po in corn oil; 0.3 to 300 mg/kg) into male DS, DR, and DC (control) mice. At 30 min we infused pentylenetetrazol (5 mg/ml, 1 ml/min) into tail veins to produce a myoclonic jerk. We saw no differences between lines in baseline seizure threshold (mg/kg PTZ±SEM, 8/group) or in dose-dependent protection by DZ. Higher DZ doses did not protect further.

Dose\Line	DS	DC	DR
Vehicle	56± 7	58± 7	60±11
1 mg/kg	59± 8	63± 6	74±20
10	112±19	93±10	85±11
100	137±11	141± 7	125±11

These data suggest that the anti-convulsant effect did not segregate during selection, indicating an underlying mechanism different from that which causes sedation.

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144.18

EFFECTS OF DIAZEPAM AND ETHANOL ON RATS IN A RADIAL MAZE. C.H.M. Beck and E.A. Loh. Dept. of Psychology, University of Alberta, Edmonton, AB, Canada T6G 2E9.

Ethanol reduces the variability of behavior of rats in the acquisition and extinction of learned responses (Devenport, L.D., Behav. Neurosci., 98, 979, 1984). To determine the degree to which the reduced variability could be due to sedation, we compared sedation and behavioral variability in a study of the effects of diazepam and ethanol on extinction of food reinforced performance in an eight-arm radial maze. Eight groups (n = 6) of male Sprague Dawley rats were given one of diazepam (0.0, 1.5, 3.0, or 6.0 mg/kg, ip, -30 min) or of 10% ethanol (0.0, 1.0, 1.5, or 2.0 g/kg, ip, -15 min) for 18 sessions of acquisition and 5 sessions of extinction. Both diazepam and ethanol reduced the variability of behavior in extinction as indicated by more reentries into arms, higher probability of repeating a specific angle of turn between arm choices, and more frequent retracing of the same route. Sedation effects of the drugs were noted in slower running speeds, reduced frequency of rearing, and increased time spent immobile instead of sniffing the environment. Covariance and correlational analyses revealed that animals which showed reduced behavioral variability were more sedated.

144.19

BEHAVIORAL EFFECTS OF THE INTRACRANIAL ADMINISTRATION OF BICUCULLINE. P. Roy*, N.E. Goeders, S.I. Dworkin and J.E. Smith. Dept. of Psychiatry, LSU School of Medicine, Shreveport, LA 71130.

The amino acid neurotransmitter GABA is an inhibitory neurotransmitter in the central nervous system involved in the reinforcing effects of abused drugs. The contingent administration of morphine has been shown to increase GABA turnover in several brain regions and to decrease turnover in the nucleus accumbens (Smith et al., Pharmacol. Biochem. Behav., 16, 509-519, 1982). This study investigated the reinforcing effects of the GABA antagonist bicuculline. Rats were implanted with a chronic unilateral injection cannula into either the ventral tegmental area (VTA) or the preoptic region of the diagonal band (PO). The rats were connected to an electrolytic microinjection transducer system and placed in standard operant conditioning chambers. The subjects were given the opportunity to self-administer 100 pmole of bicuculline delivered in a 100 nL volume over 7.5 sec directly into the VTA or PO. Sessions were 3 hours in duration and were scheduled every third day. The subjects appeared to quickly acquire the lever press response that resulted in contingent intracranial injections of bicuculline. However, it was subsequently determined that the intracranial injections of the drug were producing nonspecific increases in general motor activity that included responding on the lever.

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PEPTIDES: RECEPTORS II

145.1

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF RAT LIVER VASOPRESSIN V_1 RECEPTORS EXPRESSED IN XENOPUS LAEVIS OOCYTES. G.S.F. Ruigt, J. Brands, S. Geveling, J. Meyerink, R. van Rooijen and L. van Wezenbeek (SPON: F.Jenck). CNS Pharmacol., Organon, Oss, The Netherlands.

As a preliminary stage to the cloning and identification of the rat brain vasopressin receptor we report here the successful expression of rat liver V_1 receptors in Xenopus oocytes. Rat liver mRNA was isolated from 25 grams of tissue and was fractionated by preparative PAAG electrophoresis. Stage IV-VI oocytes were either injected with about 25 ng mRNA solution (0.5 mg/ml) or an equal volume of distilled water. After incubation intracellular voltage changes in response to superfusion of 10^{-9}M Arg-vasopressin were recorded in intact oocytes. Responses were only observed in cells injected with the 20S mRNA fraction and consisted of a delayed (1-4 min), longlasting (3-15 min) depolarising oscillation of the membrane potential. These oscillations gradually faded out in frequency, but less in amplitude, towards the end of the response. The vasopressin responses showed pronounced desensitization after repeated administration of vasopressin which could not be prevented by prolonged intermittent washout or by pretreatment of the oocytes with conA. DDAVP was ineffective in concentrations up to 100 μM, while vasotocin was as active as vasopressin. This indicates that the receptor is of the V_1 type, which was confirmed by complete block of the response by low doses (0.1 μM) of a selective V_1-antagonist.

145.2

MEASUREMENT OF VASOPRESSIN RECEPTORS IN THE SEPTUM OF MALE AND FEMALE RATS. M. D. Brot, M. A. Miller*, and D. M. Dorsa, GRECC, VA Medical Center, and Depts of Med, Pharm, and Psychology, Univ of WA, Seattle, WA 98108

Sexually dimorphic patterns of vasopressin (AVP) innervation have been reported in the rat brain, with the greatest differences occurring in the limbic system. Both the number of AVP neurons and the density of staining of fiber pathways is greater in male than in female rats. This pattern of innervation suggests that AVP receptor number and/or affinity might also display sex-related differences, as AVP receptor number has been shown to be influenced by the level of endogenous ligand in other models such as the Brattleboro rat.

In order to determine the existence of gender-specific AVP receptor characteristics, receptor binding assays were performed using membranes prepared from the septum of intact male and female adult Long-Evans rats. When receptors were labeled with the specific V_1-receptor antagonist ^{125}I-d(CH_2)$_5$-tyr(Me)AVP, Scatchard analysis revealed no difference in either the affinity [(K_d,nM) male: 0.48±.09, female: 0.53±.08], or number of binding sites [(B_{max}, fmol) male: 7.6±1.07, Female: 11.6±2.3].

These data suggest that septal AVP receptor density is equivalent in the sexes, even though AVP innervation is clearly dimorphic. Future studies will address the specificity of this observation to the ligand used, and the role of steroids in modulation of receptor density.

145.3

VASOPRESSIN RECEPTORS IN THE HUMAN BRAIN: LOCALIZATION BY QUANTITATIVE RECEPTOR AUTORADIOGRAPHY. R.E. Brinton, B.S. McEwen and A. Biegon▲ Rockefeller University, 1230 York Ave., NY, NY 10021 and ▲Weizmann Institute of Science Rehovot, Israel.

Vasopressin (AVP) has been found to influence memory function in human subjects (Weingartner et al., Science, 211:601, 1981). Based on the behavioral effects of AVP, we explored the existence of AVP receptors in human brain using light microscopic autoradiography to determine their localization. Binding parameters for labeling AVP receptors were those previously described in Brinton et al., (PNAS, 81:7248, 1984) and in Biegon et al., (Neurosci. Lett, 44: 229, 1984). Recognition sites for ^{3}H-AVP were detected in both hippocampus and cortex. In the hippocampus, AVP recognition sites were detected in each of the pyramidal cell layers (16.4 ± 0.8; 14) and in the dentate gyrus (21 ± 0.9; 14). Binding sites were also detected in the occipitotemporal cortex, (18.7 ± 0.7; 13). Values represent mean ± SEM; n, fmoles ^{3}H-AVP specifically bound/mg protein. Localization of AVP recognition sites in sagittal and coronal sections from post-mortem human brain will also be presented.

These studies were supported by the Rockefeller-Weizmann Foundation.

145.4

CHARACTERIZATION OF ARGININE-VASOPRESSIN BINDING (3H-AVP) SITES IN THE CINGULATE GYRUS OF THE RAT PUP. P. Szot, M. Swank*, K. Myers* and D. M. Dorsa. GRECC, VAMC and Depts. of Med. and Pharm, Univ. of WA, Seattle, WA 98108.

CNS vasopressin receptors have been shown autoradiographically to undergo dramatic changes during development. The caudate nucleus, dorsal hippocampus and cingulate gyrus (CG) display intense labeling by 3H-AVP in early postnatal stages, with little labeling in the adult.

3H-AVP equilibrium binding was performed on crude membranes prepared from CG of Long-Evans rat pups (7-10 days) and adults (90 days). Analysis of saturation isotherms yielded Bmax values of 13.0±1.3 and 21.5±3.0 fmol/mg protein, and Kd values of 0.89±0.1 and 4.5±0.9nM for pup and adult, respectively. Both the Bmax and Kd of the 3H-AVP site was significantly increased in the adult CG compared to the pup. Competition experiments using various AVP analogs were performed on crude membranes prepared from pup CG and adult septum, an area in the CNS where V1 receptors are located. The pharmacological profile of AVP analogs in the pup CG differed from that observed in the adult septum, especially when displacement potency of receptor antagonists was examined. The differences between pup CG and adult septal receptor specificity and affinity suggest that the pup CG 3H-AVP site is a subtype of the V1 receptor. (Sponsored by VA and NS 20311.)

145.5

COMPARISON OF EFFECTS PRODUCED BY CHOLECYSTOKININ (CCK) AND A CCK ANTAGONIST ON CELLS IN THE RAT NUCLEUS ACCUMBENS (NAc) AND FRONTAL CORTEX (FC). R.J. Kasser, X.T. Hu, and R.Y. Wang. Depart. of Psychiat. and Behav. Sciences, SUNY at Stony Brook, Stony Brook, NY 11794.

Based on receptor binding studies, CCK receptors have been classified into two subtypes: Type A (sulfate-dependent, like-pancreas) and Type B (sulfate-independent, like-brain). In the present study, the techniques of extracellular single cell recording and iontophoresis were used to examine and compare the effect of CCK-8S, CCK-8US and CCK-antagonizing effect of LORG (a selective Type A CCK receptor antagonist) on neurons in the NAc and FC. CCK-8S excited medial NAc neurons. Furthermore, it potently facilitated excitation induced by glutamate. Neither CCK-8US nor CCK-4 mimicked the action of CCK-8S. Moreover, in the NAc, LORG potently and selectively blocked the action of CCK-8S. In contrast to results from the NAc, CCK-8US mimicked the action of CCK-8S; it excited 10 of 16 FC cells tested. In addition, LORG blocked CCK-8S induced effects only in 1 out of 11 FC cells tested. Unexpectedly, LORG facilitated both CCK-8S and CCK-8US induced effects and independently activated 3 of 6 FC cells tested. These results suggest that the CCK receptors in the NAc and FC most closely resemble Type A and Type B, respectively. (Support by USPHS Grants MH-41440, MH-41696, MH-00378 to R.Y.W. and MH-43893 to R.J.K.)

145.6

HETEROGENEITY OF PERIPHERAL TYPE CCK-8 RECEPTORS. S.McCreedy*, S.Augello-Vaisey*, R.Simmons*, J.Comstock*, J.Rosamond*, & J.Blosser. Pharmaceutical Div., Pennwalt Corp., Rochester, NY 14623.

CCK-8 receptors have been pharmacologically distinguished as central or peripheral types. Recently, it was reported that analogs of CCK-8 in which DAla or DTrp was substituted for Gly at position 4 had greatly diminished potency and efficacy to contract gallbladder (GB) but retained activity comparable to CCK-8 to stimulate pancreatic exocrine function in vivo in dogs (Biomed.Res.6:111,1985). To explore these potential differences in CCK-8 receptors, we prepared a series of CCK-8 analogs with DAla substituted for Gly and compared their potencies in vitro to contract guinea pig GB with their potencies to compete with the binding of ^{3}H-L364,718, a CCK-8 antagonist, to rat pancreatic membrane receptors (PMR). In contrast with reported findings in GB in vivo, DAla containing analogs possessed full agonist activity in vitro. The potencies of (DAla^{4})CCK-8, Succ-(DAla^{3})CCK-7, and Desamino-(Tyr^{1},DAla^{3})CCK-7 to contract GB was 1/5 to 1/15 that of their respective Gly containing analogs. CCK-8 and Gly containing analogs had an IC50 of 15-25 nM with an apparent Hill coef. (n) of 0.5 for PMR. In contrast, the binding affinity of DAla containing analogs were 100-200 fold less (n=0.65). These findings may reflect differences in the pharmacological specificity of pancreatic and GB receptors, or alternatively, differences in selectivity of high and low receptor affinity states.

145.7

IDENTIFICATION OF CCK-8 ANALOGUES WITH HIGH AFFINITY AND SELECTIVITY FOR THE BRAIN CCK RECEPTOR. R.J. Knapp*, S.N. Fang*, G.K. Lui*, V.J. Hruby*, and H.I. Yamamura (SPON: J. Lai). Depts. of Pharmacology and Chemistry, Univ. of Arizona, Tucson, AZ 85724.

A series of sulfated and unsulfated cholecystokinin (CCK 26-33) analogues have been synthesized and their affinities measured at the CCK receptors in guinea pig pancreas and cortex. We present data here for the analogues: methyl-Nle^{31} CCK 29-33 (8701), methyl-Nle^{28,31}CCK26-33 (8702), and methyl-Nle^{28},Nle^{31}bis sulfate CCK 26-33 (8712). Guinea pig pancreatic membranes were prepared using the procedure described by Steigerwalt and Williams, Endocrinology, 109: 1746(1981), while guinea pig cortical membranes were prepared using the procedure of Saito et al., J. Neurochem., 37:483(1981). Binding studies with both tissues were carried out using the same assay buffer and conditions as described by Steigerwalt and Williams (op. cit.) except that Bolton-Hunter labeled CCK 26-33 was used as the tracer at a concentration of about 50pM. The results obtained by nonlinear regression analysis are as follows:

Analogue Number	Pancreas IC-50 (nM + SEM)	Cortex IC-50 (nM + SEM)	Ratio P/C
8701	8,004 ± 858	3.34 ± 0.32	2396
8702	958 ± 103	0.23 ± 0.01	4146
8712	29,073 ± 5,073	3.19 ± 0.17	9099

145.8

DISTRIBUTION OF CHOLECYSTOKININ (CCK) BINDING SITES IN SYRIAN HAMSTER BRAIN. M.O. Miceli and M. Steiner. McMaster University and St. Joseph's Hospital Research Institute, Hamilton, Ontario, Canada L8N 4A6.

Previously reported species differences in the pharmacological characteristics and CNS distribution of CCK receptors prompted us to map and characterize brain CCK binding sites in the Syrian hamster, a species with a CNS distribution of CCK immunoreactivity that differs from that of other rodents. Autoradiograms of brain sections incubated in 34 pM ^{125}I-Bolton Hunter CCK-8 were generated on LKB Ultrofilm. Binding specificity was assessed in adjacent sections incubated in the presence of 1 µM unlabeled CCK-8 or desulfated (DS) CCK-8. High binding densities were distributed in the cerebral cortex, olfactory bulbs and nuclei, area postrema and the interpeduncular, inferior olivary, cochlear, and solitary nuclei. Moderate binding densities were present in the striatum, amygdala, hippocampus, hypothalamus, zona incerta, habenula, thalamic parafascicular and paraventricular nuclei, central gray, and medial vestibular nucleus. Specific binding was not detected in the cerebellum. Binding sites with a low affinity for DS CCK-8 were noted in the solitary complex and in regions of the hypothalamus (supraoptic, paraventricular, dorsomedeial and accessory magnocellular nuclei) where such specificity has not been demonstrated in other species. Our observations provide further evidence of species differences in CCK receptor distribution and specificity. (Supported by St. Joseph's Hospital Foundation)

145.9

PERIPHERAL-TYPE CHOLECYSTOKININ (CCK) RECEPTORS IN CHP212 NEUROBLASTOMA CELLS: ASSOCIATION WITH INOSITOL PHOSPHOLIPID HYDROLYSIS. Ronald W. Barrett*, Michael E. Steffey* and Caroline A. W. Wolfram* (SPON: C. Briggs). Neuroscience Research Division, Pharmaceutical Discovery, Dept 47W, Abbott Laboratories, Abbott Park, IL 60064

[^{125}I]BH-CCK8 and [^{3}H](-)L364718 membrane binding assays were used to identify and characterize CCK receptors in a human neuroblastoma cell line (CHP212). Based on ligand binding properties, CCK receptors in these cells are similar to those found in pancreas and differ from the predominant subtype of CCK receptor found in brain. Binding of [^{125}I]BH-CCK8 was reduced by guanyl nucleotides. A substantial difference in the Bmax for the radiolabeled agonist ([^{125}I]BH-CCK) and antagonist ([^{3}H](-)L364718) was noted. These observations are consistent with CCK receptors existing in G-protein coupled and uncoupled states. Similar to its action in pancreatic acinar cells, CCK8(S) stimulated the formation of [^{3}H]inositol phosphates in CHP212 cells (EC50=6 nM; Max. response= 4.5 x basal). This stimulation of PI hydrolysis was not diminished by treatment with pertussis toxin. The intrinsic activity of CCK analogs in stimulating PI hydrolysis was substantially less than their intrinsic activity in pancreatic acinar cells. The CHP212 neuroblastoma cell may serve as a useful model for peripheral-type CCK receptors found in the CNS.

145.10

ONTOGENY OF CHOLECYSTOKININ RECEPTORS IN THE RAT BRAIN. D. Pelaprat*, I. Dusart* and M. Peschanski (SPON: B. Scatton). U.161 INSERM, 2 rue d'Alésia, 75013 PARIS (FRANCE).

The ontogeny of cholecystokinin (CCK) binding sites in the rat forebrain and midbrain was studied by in vitro receptor autoradiography, using (125I) Bolton-Hunter CCK_8. In the majority of structures, the densities of sites were low in the prenatal period and over the first week after birth, increased until the third week, and decreased over the fourth week to reach adult levels. However, both the rate of increase and the extent of the decrease varied in large proportions among structures. For instance, labeling became detectable at day 10 in neocortex, at day 7 in paleocortex, and at birth in endopyriform nucleus. On the other hand, the decrease over the fourth week was negligible in cingulate cortex, small (32%) in the thalamic reticular nucleus, and reached 70% to 100% in various layers of hippocampus. These different timetables may depend mostly on the differential growth of cells expressing the CCK receptor gene within the developing brain. Finally, the absence of CCK binding sites in most of the regions during the prenatal and the early postnatal periods precludes a major role of this peptide in the embryonic development of the rat brain.

145.11

HELODERMIN AND (D-CL-PHE^{6}-LEU^{17})VIP ACT ON VASOACTIVE INTESTINAL PEPTIDE RECEPTORS IN CEREBRAL BLOOD VESSELS. D.N.Krause[1], I.Jansen[2*], and L. Edvinsson[2*]. [1]Dept. Pharmacol., Univ. of Calif., Irvine, CA 92717 and [2]Dept. Exp. Res., Univ. of Lund, Malmö Gen. Hosp., S-214 01 Malmö, Sweden.

Cerebrovascular receptors for vasoactive intestinal peptide (VIP) were characterized using several newly-described VIP-related peptides. Helodermin, a peptide isolated from the venom of the lizard Heloderma Suspectum which shows a high degree of sequence homology with VIP, was found to potently displace ^{125}I-VIP from high affinity receptor binding sites in pig cerebral arteries. It was more potent than its analogues, helospectrin I and helospectrin II, and peptide histidine isoleucine (PHI) and the proposed VIP antagonist (D-Cl-Phe^{6}-Leu^{17})VIP. A similar order of potency was found when the above peptides were tested on specific ^{125}I-VIP binding to intracerebral microvessels. The potencies for VIP receptor binding correlated with the ability of the helospectrin analogues and PHI to induce dilation of precontracted ring segments of pig cerebral arteries. The putative VIP antagonist was found to act as an agonist at cerebrovascular VIP receptors and did not antagonize the VIP response, in contrast to what has been found in non-vascular tissues.

145.12

CROSS-LINKING OF LABELED VIP TO RECEPTORS IN NERVOUS AND IMMUNE TISSUES. A.I. Kook*, J.B. O'Neill, C.B. Pert* and J.M. Hill. Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892.

VIP has a broad range of biological functions due to the presence of its receptors in a wide variety of tissues throughout the body.

Recent evidence suggests that VIP binds to multiple receptor subtypes, and that the AIDS virus may bind to at least one of these. AIDS is a neuroimmune disorder in which HIV infects cells by binding first to a 60 kD surface protein (T4) found in cells of the CNS and immune system.

In this study ^{125}I-VIP was bound to membranes prepared from the brain, thymus, and T lymphocytes. Binding was competed with VIP, GTP, secretin, and peptide T; an amino acid sequence from HIV envelope protein which is homologous to a portion of VIP.

Brain tissue, especially cortical and hippocampal membranes have a major ~60 kD band and also a band at 47-48 kD. In preliminary studies, T lymphocytes also show a 60 kD band. Thymocytes have a major band at 47-48 kD. VIP, secretin, GTP, and peptide T displace the 47-48 kD band to varying degrees. The 60 kD band is partially displaced with VIP, displaced to a greater extend by peptide T, but not at all by secretin.

These results suggest that T4, the 60 kD surface protein to which HIV binds, is actually the 60 kD VIP receptor found in brain and T lymphocytes. HIV is presumably able to occupy the VIP receptor because the peptide T region of the envelope protein is homologous with a portion of VIP.

The functional aspects of these receptors are not as yet understood, but these results suggest VIP may have more than one mechanism of cell activation.

145.13

INTERACTIONS BETWEEN MONOAMINES AND VASOACTIVE INTESTINAL PEPTIDE RECEPTORS ON ASTROGLIA IN PRIMARY CULTURE. L.Rönnbäck[1,2] and E.Hansson[1]. [1]Institute of Neurobiology and [2]Department of Neurology, University of Göteborg, Göteborg, Sweden.

Many of the proposed functions of astrocytes in developing and mature brain are dependent on the ability of the cells to recognize and respond to specific neuronal signals. Evidence from a large number of laboratories indicate that astroglia cultured from immature brain exhibit a wide variety of membrane receptors with eg. cyclic AMP or inositol-trisphosphates as second messengers. Interactions between different receptors or second messenger systems have recently been observed. In this poster we demonstrate the presence of a VIP (vasoactive intestinal peptide) receptor with cyclic AMP as second messenger on cultured astrocytes. We also present data supporting an interaction between the second messenger systems for VIP and α and β adrenoceptors. The functional implications are at present unknown, but it might be that the peptide can modulate the response of the second messenger of catecholamines on astroglial cells through intramembrane mechanisms.

Two questions should be addressed. Even if the cultures used are well characterized both to their cellular content (no neurons or mature oligodendrocytes present) and to their degree of differentiation, they represent an immature system cultivated in the absence of neuronal contacts for 2 weeks. Thus, it might be questioned whether or not mature astrocytes in brain exhibit receptors and receptor interactions similar to those expressed by cultured astroglia. Second, what cellular events, beyond activation of second messengers, are influenced by receptor stimulation? Studies along these lines are in focus of our considerations.

145.14

ELECTRON MICROSCOPIC LOCALIZATION OF MU OPIOID RECEPTORS IN THE RAT LOCUS COERULEUS BY IN VITRO RADIOAUTOGRAPHY. E.Moysé*, K.Leonard*, A.Beaudet (SPON:O.Calvillo)Dpt.Neuroanatomy,Mtl. Neurol.Inst.,McGill Univ.,3801 University, Montréal,Canada.

Mu opioid binding sites were visualized by light and electron microscopic radioautography in slices of rat pontine tegmentum incubated in vitro with the met-enkephalin analog 125I-FK 33-824, in the absence(total binding)or in the presence(non specific binding)of 1μM non radioactive naloxone. In light microscopic radioautographs from slices incubated with 125I-FK alone,the labeling was intense throughout the locus coeruleus(LC),where it appeared evenly distributed over nerve cell bodies and neuropil.The addition of non radioactive naloxone to the incubation medium reduced the grain density by 72% in both compartments.At the electron microscopic level,almost 50% of the grains arising from specifically labeled 125I-FK binding sites were detected inside neuronal perikarya and dendrites,with a selective enrichment of the endoplasmic reticulum and Golgi apparatus. Most of the remaining grains were associated with neuronal interfacesinvolving the plasma membrane of intrinsic perikarya and dendrites.Among these,axosomatic and axodendritic appositions showed the highest concentration of label over random.Only a few of the labeled interfaces,however,exhibited synaptic specialization in the plane of section.These results provide a morphological support for direct non-jonctional action of opioid peptides on the perikaryon and dendrites of LC neurons. SUPPORTED BY MRC.

145.15

FUNCTIONAL COUPLING OF OPIOID RECEPTORS IN HUMAN NEUROBLASTOMA CELLS. Syed M.I. Kazmi, Cia Barlas* and Ram K. Mishra. Neuropharmacology Lab., Depts. of Psychiatry and Neurosciences, McMaster Univ., Hamilton, Canada, L8N 3Z5.

The profile of opioid receptor occupancy and functional response under pertussis toxin (PT) catalyzed ADP ribosylation of guanine nucleotide inhibitory protein (Ni) and phorbol ester (4β phorbol, 12β myristate, 13α acetate, PMA) induced stimulation of protein kinase C was studied in human neuroblastoma, SH-SY5Y cells. PMA (10^{-7} -10^{-6}M) treatment (1-2 hours) to SH-SY5Y cells caused a selective decrease (30-50%) in opioid agonist binding by reducing the agonist high affinity state of the receptor. Pertussis toxin also caused time- and dose-dependent decrease in opioid agonist binding. The effect of sodium ions on agonist binding was potentiated in PT-treated cells. Opioid agonist, DADLE, caused a significant increase in low K_m GTPase activity and a reduction (40-50%) in forskolin-stimulated cyclic AMP levels; the effects could be completely reversed by naloxone while δ-selective antagonist, ICI-17184, was effective only at concentrations higher than 10^{-7}M. Pertussis toxin completely abolished the effects of DADLE on low K_m GTPase and cyclic AMP levels, whereas PMA shifted the agonist response curves to the right. PT-catalyzed ADP ribosylation of 41K protein (α_i of N_i) appeared to decrease in PMA treated cells. These results would facilitate in elucidating the mechanisms of the regulation of opioid receptor G-protein coupling in human neuroblastoma cells.

145.16

ELECTRON MICROSCOPIC LOCALIZATION OF DELTA OPIOID RECEPTORS IN RAT BRAIN. F. Pasquini*, A. Beaudet, P. Bochet*, J. Rossier and B.P. Roques* (SPON: M. Frankfurt). Neuroanatomy Lab, Montreal Neurol. Inst., 3801 University St., Montreal, Canada, H3A 2B4; CNRS, 91190 Gif sur Yvette, France; Univ. René Descartes, 4, ave. de l'Observatoire, 75270 Paris, France.

The distribution of delta opioid receptors, selectively labeled in vitro with the photoaffinity probe ^{125}I-azido-DTLET was analyzed by electron microscopic radioautography (EM RAG) in sections of rat neostriatum. Glutaraldehyde-prefixed vibratome-cut striatal slices were incubated with 0.7 nM ^{125}I-azido-DTLET, irradiated with a single U.V. flash, postfixed in aldehydes and OsO_4, dehydrated in ethanols, embedded in Epon and thin-sectioned for EM RAG processing. Between 25 and 30% of the bound radioactivity, 75% of which corresponded to specific binding, was retained in tissue after histological processing. Fifty percent probability circle analysis of EM radioautographs revealed that a significant proportion of the specifically bound ^{125}I-azido-DTLET molecules was intraneuronal. Specifically, 16% of the labeled binding sites were found in dendrites, 12% in perikarya and 4% in axon terminals. The remaining binding sites were associated with axo/dendritic (20%), dendro/dendritic (7.0%) and axo/axonic (4%) interfaces. Only a small proportion (≈ 1.0%), however, was found at the level of synaptic specializations. These results suggest that endogenous as well as exogenous delta ligands primarily act non-junctionally on the plasma membrane of dendrites in the neostriatum. Supported by MRC.

145.17

CHANGES IN OPIOID (OP), NEUROTENSIN (NT) RECEPTOR DENSITY AND NEUTRAL ENDOPEPTIDASE (NEP) IN PARKINSON AND HUNTINGTON BRAINS. W.H. Rostène, A. Dubois*, J.M. Zajac, F. Agid*, P. Kitabgi and B.P. Roques. INSERM U.55, 266, 289 Paris, LERS Bagneux and CNRS Nice, France.

Several data suggest a functional relation between OP, NT systems and the dopaminergic (DA) nigrostriatal neurons (NS). In order to determine the effects of the degeneration of the NS on OP and NT receptors, sections of human basal ganglia and mesencephalon obtained from postmortem control, Parkinson (PK) and Huntington (HU) brains were incubated with ligands specific for mu (3HDAGO), delta (3HDETLET), kappa (3HEKC) receptors as well as for NEP (3HHACBO-Gly) and NT receptors (125I-NT). Films were then processed for computerized quantitative autoradiography. In the substantia nigra from controls, high densities of Kappa, NEP and NT binding sites were observed. In PK, no change in OP receptors was detected whereas NT binding site densities decreased by 50%. In HU, a decrease in both NEP and NT binding sites was observed. In the basal ganglia high densities in mu, delta, NEP and NT binding sites were found. In PK, OP receptor density was not different from controls whereas a 50% reduction in NT binding sites was observed in the caudate nucleus and putamen. In HU brains, a decrease in all OP binding sites and NEP was noticed with no change in NT receptors. These data suggest a different localization of OP and NT binding sites on the human NS and that NT receptor density represents a good index of the integrity of this DA pathway. (Sup. by Fondation de France and Réseau INSERM).

145.18

CELLULAR LOCALIZATION OF MU OPIOID RECEPTORS IN THE LOCUS COERULEUS OF THE RAT. D. Marcel, P.D. McNeely and A. Beaudet (SPON: B.E. Jones). Neuroanatomy Laboratory, Montreal Neurological Institute, 3801 University Street, Montreal, Quebec, H3A 2B4, Canada.

The cellular localization of radioautographic labeled mu opioid binding sites was investigated in rat locus coeruleus (LC) using a combined immunohistochemical/neurotoxin lesioning approach. In light microscopic radioautographs from sections incubated with the met-enkephalin analog ^{125}I-FK 33-824 (^{125}I-FK), high ^{125}I-FK binding densities were detected throughout the LC. Processing of serial adjacent sections for tyrosine hydroxylase (TH) immunohistochemistry revealed that within the LC, the distribution of ^{125}I-FK-labeled binding sites was in perfect register with that of constituent noradrenalin cells. Moreover, intracerebroventricular injection of the catecholamine neurotoxin, 6-hydroxydopamine, virtually suppressed ^{125}I-FK labeling in the region while concomitantly reducing both the intensity of TH-immunostaining and the number of TH-immunopositive cells. In contrast, intracerebroventricular injection of the serotonin neurotoxin, 5,6-dihydroxytryptamine, did not affect ^{125}I-FK binding in LC, despite a massive decrease in endogenous 5-HT, as recorded locally by HPLC. The present results suggest that within the LC, mu opioid binding sites are mainly associated with the perikarya and dendrites of NA neurons and not with afferent 5-HT axons. These receptors are likely to subserve opioid/noradrenalin interactions documented in LC.

145.19

CROSSLINKING OF κ RECEPTORS IN THE GUINEA PIG CEREBELLUM WITH ^{125}I-DPRO^{10}DYNORPHIN (1-11). Y-H. Yao*, J. Guerain*, J-C. Meunier*, J.M. Hiller*, T.L. Gioannini*, J. Cros*, and E.J. Simon. (SPON: O. Keren). Depts. of Psychiatry and Pharmacology, NYU Medical Center, New York, NY 10016 and Laboratoire de Pharmacologie et Toxicologie Fondamentales, CNRS, 31077 Toulouse, CEDEX, France.

DPro^{10}dynorphin (1-11) (DProDyn) was radioiodinated and used as a crosslinking probe to determine the size and number of κ binding proteins present in the guinea pig cerebellum, a tissue with a preponderance (>80%) of κ receptors. Dissociation constant and binding capacity for the binding of the iodinated peptide were found to be 0.31±0.02nM and 141±15 fmol/mg protein, respectively, in good agreement with previous results in guinea pig brain. Competition studies indicated displacement of ^{125}I-DProDyn by unlabeled DProDyn, bremazocine and U50,488, with IC_{50}'s of 0.18, 0.25, and 20nM, respectively. DPDPE did not compete and DAGO was effective only at very high concentrations (IC_{50} of 2.8 μM). When guinea pig cerebellar membranes were bound with labeled ligand, washed and crosslinked with BSCOES, SDS-PAGE followed by autoradiography revealed two major bands of apparent mol. wts. of 55 and 35kDa. Only the 55kDa band was completely eliminated when binding was carried out in the presence of U50,488, DProDyn or naloxone. The 35kDa band was decreased in intensity by these competing ligands but not eliminated. The 55kDa band was unaffected by DPDPE and only slightly reduced by high concentrations of DAGO. We therefore conclude that the major κ binding protein in guinea pig cerebellum has an apparent mol.wt. on SDS-PAGE under reducing conditions (100mM DTT) of 55kDa. (Supported by grant DA-00017 to EJS from the National Institute on Drug Abuse).

145.20

LOCALIZATION OF OPIATE RECEPTOR SUBTYPES IN THE FOREBRAIN OF THE MARMOSET. L.S. Brady, E. Johnson McClure* and M. Herkenham. Unit on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

The common marmoset, *Callithrix jacchus jacchus*, is a small new world primate. In fresh-frozen sections from adult males, μ and δ receptors were labeled with [3H]D-ala2, N-me-phe4, gly5-ol-enkephalin or [3H]D-ala2, D-leu5-enkephalin, respectively. κ Receptors were labeled with [3H]bremazocine (BRM) or [3H]U-69,593 (U-69). Dense μ binding was found in the supraoptic and paraventricular nuclei, median eminence and medial thalamus, moderate binding in the ventrobasal hypothalamus, bed nucleus of the stria terminalis and basal amygdala and low binding in the striatum, hippocampus and cortex. Dense δ binding was seen in the striatum, dentate gyrus molecular layer and homogeneously throughout the amygdala and cortex. Dense κ binding (BRM) was present in the supraoptic and paraventricular nuclei, median eminence, medial thalamus, cortical layer VI and claustrum, moderate binding in the striatum, globus pallidus and basal amygdala and low binding in the hippocampus. In contrast, κ receptors labeled by U-69 were dense only in the median eminence, cortical layer VI, globus pallidus and claustrum. The localization of dense μ and κ (BRM) binding in the hypothalamus is consistent with effects of opiate antagonists on neuroendocrine function. δ Binding is localized in large areas of cortex and striatum, suggesting a ubiquitous role in higher functions. U-69 labels a subset of structures showing dense BRM binding; most of these structures are μ receptor poor. Thus, each opiate receptor subtype shows distinct distributions in the marmoset forebrain, similar to patterns seen in rhesus and squirrel monkey brains.

146.1

LOCALIZATION OF ATRIAL AND BRAIN NATRIURETIC PEPTIDES IN THE RAT BRAIN. H.R. Holmes, K.M. Hurley, and C.B. Saper. Depts. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637

Brain natriuretic peptide (BNP) is a recently discovered peptide hormone isolated from porcine brain that has biological activities similar to atrial natriuretic peptide (ANP). We have used immunohistochemistry to examine the localization of BNP-like immunoreactivity (BNPir) in the rat brain. BNPir innervation tends to avoid areas, such as the anteroventral periventricular nucleus, that contain large numbers of ANPir cell bodies. In contrast, BNPir fibers innervate certain structures, such as the subfornical organ and area postrema, that participate in control of water balance and blood pressure, but do not have much ANPir innervation. Other areas of the brain that contain ANP binding sites, but little ANPir innervation, such as the olfactory bulb and cerebellar cortex, receive substantial BNPir input. BNPir cell bodies are found in several areas that contain no ANPir neuronal perikarya, such as the cerebral cortex and both the parasympathetic and sympathetic preganglionic cell groups, in the medulla and spinal cord. Many sensory afferent fibers, in the vagus nerve as well as trigeminal and spinal sensory roots, are also BNPir. None of these structures are ANPir. BNPir is more widely distributed in the brain than ANPir. However, the two peptides appear to belong to a family of neuromodulators that is involved in the regulation of blood pressure and fluid volume at a central nervous system level.

HRH is a Chicago Heart Assoc. Senior Research Fellow.

146.2

COMPARATIVE ONTOGENY OF CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P IN THE RAT SPINAL CORD. D. Gledic*, H. Gilbertson* and S. Jeftinija (SPON: R. Bowman). Dept. Vet. Anatomy, Iowa State University, Ames, IA 50011.

Substance P (SP) and calcitonin gene-related peptide (CGRP) immunoreactivities (IR) have been shown to be present in the dorsal root ganglion (DRG), dorsal horn (DH) and ventral horn (VH) of the adult rat spinal cord. In addition, SP and CGRP are co-localized in some DRG neurons and primary afferents. Although the ontogeny of SP-IR in the rat spinal cord has been studied (Senba, et al., 1982, J. Comp. Neurol. 208:54-66), very little is known about the development of CGRP-IR in the spinal cord. To provide additional information about the development of CGRP system and the interaction between SP and CGRP, the first appearance and distribution of SP and CGRP-IR at all levels of the rat spinal cord from gestational day 14 (E14) to postnatal day 25 (P25) have been systematically investigated. SP-IR was first found in cells of the ventral part of the dorsal horn at E16. At the same age CGRP-IR was seen for the first time in cells in the motoneuron region, and was present in those cells from that time on. The first appearance of SP and CGRP-IR in DRG neurons was at day E17. SP-IR fibers in the DH first appeared at E15, and CGRP-IR fibers appeared at E17. CGRP-IR was present in motoneurons at all ages examined. No CGRP-IR was seen in DH interneurons. SP positive interneurons in the dorsal horn disappeared at birth. Supported by DHHS/NIH Grant 2 SO7RR07034 and USDA PL95-113, section 1433.

146.3

COEXISTENCE OF PEPTIDES IN PRIMARY AFFERENT NEURONS IN CAT SPINAL CORD. M.G.Garry, L.D.Aimone†, T.L.Yaksh† and V.S.Seybold §. Dept. of Cell Biol. and Neuroanat., § Grad. Program in Neurosci., Univ. of MN, Minneapolis, MN 55455; † Dept of Neurologic Res., Mayo Fnd., Rochester, MN 55905.

It is generally assumed that a substance which is observed in the perikaryon of a neuron will also be localized to the axons of that neuron. In the present study, the peptide immunoreactivity (IR) of the central axonal processes of primary afferent neurons was investigated in the L6 spinal segment of unilaterally ganglionectomized (L4-L7) cats (n=3). Simultaneous visualization of two antigens by immunofluorescence was used to detect peptides. The difference in immunoreactivity between the ganglionectomized and the intact dorsal horns of the spinal cord was attributed to the contribution of primary afferent neurons. Substance P (SP) and calcitonin gene-related peptide (CGRP) coexisted in varicosities within laminae I and II of the dorsal horn on the control side. The proportions of coexistence of SP and CGRP in primary afferent axons were calculated to be similar to those which we observed in colchicine treated L6 dorsal root ganglia (DRG) of cat. Somatostatin (SOM)-IR varicosities were observed primarily within lamina II of the dorsal horn and coexisted minimally with CGRP. The minimal coexistence of SOM and CGRP calculated for primary afferent axons contrasts with our observations in the DRG. Bombesin (BOM)-IR varicosities were observed in moderate density in laminae I and II on the control side and were calculated to arise largely from primary afferent axons. In contrast to the lack of BOM-IR observed in the DRG, these data indicate that BOM-IR may be contained in primary afferent axons. Furthermore, BOM-IR coexisted with SOM-IR in varicosities within lamina II. In summary, these data suggest: a) the coexistence of some peptides in varicosities of primary afferent neurons within the dorsal horn of cat do not parallel the patterns of coexistence observed in the DRG; and b) BOM-IR is present in varicosities of primary afferent neurons but occurs in a form or concentration undetectable by immunohistochemistry in the L6 DRG of the cat. This work is supported by USPHS grants NS 17702 and T32 DA 07234.

146.4

THE VIP IMMUNOREACTIVITY IN THE NORMAL AND ISOLATED RAT LUMBOSACRAL SPINAL CORD. K. Chung and W.T. Lee*. Dept. of Anat. and Neurosci. and the Marine Biomed. Inst., Univ. of Tex. Med. Br., Galveston, TX 77550.

Previous studies on vasoactive intestinal polypeptide (VIP) in the mammalian spinal cord indicate that VIP is located in neurons; either in dorsal root ganglion cells or in neurons within the spinal cord. There is, however, a brief mention of an occasional extremely dense VIP immunoreactivity within the ependymal cell layer. The present study extended the VIP distribution in normal and isolated rat lumbosacral spinal cord by an immunohistochemical method. The most prominent VIP immunoreactivity in normal rat spinal cord was found in ependymal and subependymal cells and their processes. The basal processes of ependymal and subependymal cells project to pia mater or capillaries in the spinal gray matter. Sparse VIP fibers and terminals were localized in the marginal zone and lamina II of dorsal horn and the central gray matter. The VIP immunoreactivity in isolated spinal cord was increased dramatically in lamina II. In addition, many VIP-immunoreactive neurons were found in all areas of spinal gray matter. VIP neurons can be divided into three groups based on the morphology of their somata: small bipolar; medium bipolar; and medium multipolar neurons. This study is supported by grants from the National Institues of Health NS11255, the Muscular Dystrophy Association, the Marie Hall Endowment and Bristol-Meyers.

146.5

SUBSTANCE P- AND CGRP-IMMUNOREACTIVE NERVE FIBRES IN LYMPH NODES RELATED TO THE LOWER RESPIRATORY TRACT OF THE GUINEA PIG. Ch. Heym*, W. Kummer* and R. Kurkowski*. Dept. of Anatomy, University of Heidelberg, D-6900 Heidelberg, FRG.

The presence of substance P-receptors on T-lymphocytes (Payon, DG. et al., J. Clin. Invest.,74:1532,1984) and the biochemical characterization of authentic substance P in lymphatic tissue (Gepetti, P. et al., Reg. Pept.,18:321, 1987) prompted us to investigate the distribution of substance P and its common cotransmitter CGRP in lymph nodes of the guinea pig lower respiratory tract.

Double labelling experiments revealed colocalization of substance P and CGRP in virtually all intranodal nerve fibres. Immunoreactive (IR) fibres were present in varicose nerve fibres in the capsule, in the medulla and in the paracortical zone. Medullary fibres predominated in the hilus and preferentially were located in close contact with blood vessels. No substance P- or CGRP-IR nerve fibres were visible in follicles. Ultrastructurally, varicosities of unmyelinated substance P-IR fibres in addition to small clear vesicles contained few large dense core vesicles.

In view of the characteristic peptide combination a sensory origin of the observed substance P-/CGRP-IR fibres is suggested. This assumption is in line with the loss of extractable substance P-IR from rat thymus following capsaicin administration (Gepetti, P. et al., Reg. Pept., 18:321,1987). (SPON: R.H. Whitworth, Jr.)

146.6

INTERACTIONS BETWEEN CHEMICALLY IDENTIFIED NERVE FIBERS AND NEURONS IN FEMALE RAT PARACERVICAL GANGLIA. R.E.Papka and H.H.Traurig, Dept. Anat.& Neurobiol., Univ. KY., Lexington, KY 40536

Some neurons of the paracervical ganglia(PG) project to reproductive organs, sensory fibers traverse the PG and send collaterals to principal neurons, and there are other terminals, probably efferent, in the PG. The present study examines the circuitry of the PG to determine if there is specific chemical coding between identified nerve terminals and principal neurons. We used [a] adjacent cryostat sections; one immunostained for a nerve fiber marker and one immunostained for a cell body marker and [b] the immunostaining(for a cell body marker), elution, reimmunostaining(for a terminal marker) method of Tramu et al. on single cryostat sections. Overlap of staining was compared. Cell body markers were acetylcholinesterase(ACHE), tyrosine hydroxylase(TH), neuropeptide Y(NPY) and vasoactive intestinal peptide(VIP). Nerve terminal markers were substance P(SP), neurokinin A (NKA), calcitonin gene-related peptide(CGRP), cholecystokinin(CCK-8), enkephalin(ENK) and atrial natriuretic factor(ANF). To date, it appears that most cell bodies receive input from nearly all types of terminals, but in different proportions. (Supported by NIH Grant NS-22526).

146.7

SEGMENTAL ORIGINS AND POSSIBLE VASCULAR DESTINATION OF PEPTIDE CONTAINING SENSORY FIBERS IN BULLFROG SYMPATHETIC GANGLIA. J. P. Horn and W.D. Stofer Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Paravertebral sympathetic ganglia 9 & 10 of the bullfrog contain sensory fibers that are immunoreactive for substance P(Sub P) and calcitonin gene-related peptide(CGRP) [*Soc. Neurosci.Abstr.*, 13:297, 1987]. We now report experiments to establish quantitatively the segmental origins of these sensory neurons and to test the hypothesis that some of these fibers innervate blood vessels.

In 3 frogs, the sympathetic chain was cut at the level of ganglion 9 and exposed to 1% fast blue. After survival times of 12 to 28 days, the dorsal root ganglia were removed and serially sectioned. Retrograde transport of the dye labeled an average of 313±168(s.d.) sensory neurons in DRGs 4-8. Counts were corrected for split cells. The distribution of labeled cell profiles, expressed as a percent of total labeled profiles in each animal, was: DRG4: 5±1%, DRG5: 9±1%, DRG6: 19±3%, DRG7: 29±12%, DRG8: 38±15%.

To estimate the percentage of sensory neurons which project to sympathetic ganglia 9 & 10 and contain immunoreactivity for Sub P and CGRP, 2 other frogs were similarly labeled with fast blue and 119 sections from DRGs 4-8 were double labeled with a rabbit anti-CGRP serum and a rat anti-Sub P antibody. Cells containing fast blue were then scored for immunofluorescence. Of 735 dye-labeled cell profiles, 80% contained both Sub P and CGRP-like immunoreactivities, 7% contained only Sub P-IR, 4% contained only CGRP-IR and 9% contained no immunoreactivity. This pattern of staining was seen at all segmental levels.

One target that is functionally innervated by C neurons in sympathetic ganglia 9 & 10 is the descending aorta[*Neurosci.*, 22:S336, 1987]. Axons from these ganglia enter the aorta directly by way of small postganglionic rami communicantes. We therefore examined double labeled sections of rami and aorta to look for sensory fibers. Indeed both structures contained many axons that were positive for Sub P and CGRP-IR. Supported by NIH grant NS21065.

146.8

A RARE CLASS OF BULLFROG SYMPATHETIC NEURONS THAT ARE ENCRUSTED WITH SUBSTANCE P CONTAINING SYNAPTIC BOUTONS. W.D. Stofer and J. P. Horn Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Paravertebral sympathetic ganglia of the bullfrog contain a rich plexus of fibers that are immunoreactive for substance P(Sub P). Previous studies demonstrate that these fibers are of sensory origin and that most of them also contain calcitonin gene related peptide-like immunoreactivity(CGRP-IR). When examined at the light level, these fibers have not been found to make obvious synaptic contacts with postganglionic sympathetic neurons.

We now report a rare class of sympathetic neurons in paravertebral ganglia 2, 9 and 10 that are contacted by substance P containing synaptic boutons. The postganglionic neurons contacted by such fibers tend to have relatively large cell bodies and are heavily invested with immunoreactive boutons on the soma and axon hillock. In order to estimate the number of such cells and to identify them, ganglia 9 and 10 were serially sectioned at 10 μM and every other section was double labeled with antibodies to Sub P and neuropeptide Y(NPY). In 2 animals, we found a total of 68 neurons covered with Sub P containing boutons. None of these neurons contained NPY-IR. Given that NPY-IR is a marker for C cells and that B cells tend to be larger than C cells, it appears most likely that the contacted cells are a subset of sympathetic B neurons. When the adjacent serial sections were double labeled for Sub P and CGRP-IR, we found that all Sub P containing boutons on ganglion cell bodies were also positive for CGRP-IR. In another abstract at this meeting, we report that 80% of the sensory neurons projecting to ganglia 9 & 10 contain both Sub P and CGRP-IR. Since neurons containing both of these peptides are not present in the intermediolateral cell column of the spinal cord, we conclude that the ganglionic fibers containing Sub P and CGRP-IR are of sensory origin and that such fibers make morphologically recognizable synaptic contact with approximately 1% of the sympathetic B neurons in ganglia 9 and 10.

This work was supported by NIH grant NS21065.

146.9

ELECTRON MICROSCOPIC EXAMINATION OF VASOACTIVE INTESTINAL POLYPEPTIDE IN THE BABOON LUMBOSACRAL SPINAL CORD. A. Brady*, W.C. de Groat and I. Nadelhaft. VA Medical Center and Depts. of Neurol. Surgery, Pharmacol. and Behav. Neurosci., University of Pittsburgh, Pittsburgh, PA 15261.

Light microscopic studies of the distribution of vasoactive intestinal polypeptide (VIP) in the sacral spinal cord of rhesus monkeys and of humans demonstrated thick linear deposits of reaction product (2-16 microns diameter) that were interpreted as axonal bundles. These bundles were concentrated in Lissauer's tract (LT) but were also seen in parts of the gray matter. Similar studies in cats and rats revealed reaction product only in what appeared to be single axons. We studied the characteristics of VIP axonal bundles in LT of the baboon sacral spinal cord using immunohistochemical techniques at the electron microscopic level. VIP reaction was found in unmyelinated fibers in Lissauer's tract but was never detected in myelinated fibers. The VIP fibers were located within bundles together with fibers which were unlabelled. The reaction product was contained in synaptic vesicles measuring approximately 1200 angstroms in diameter and in much smaller "particles" whose boundaries were not well defined. Round profiles of about the same size were devoid of reaction product. It is concluded that VIP axonal bundles in LT of primate spinal cord are heterogeneous structures containing VIP and non-VIP axons.

146.10

SOMATOSTATIN (SS) AND NEUROPEPTIDE Y (NPY) CONTENT OF SUPERIOR CERVICAL GANGLION NEURONS PROJECTING TO THE SUBMANDIBULAR GLAND AND IRIS. L.L. Wright and J. Luebke. Dept. Anatomy, BUSM, Boston, MA 02118

The present study was done to determine whether postganglionic neurons of the rat superior cervical ganglion (SCG) that project to the submandibular gland (SMG) or iris differentially contain SS-like immunoreactivity (ir) and NPYir. Six adult male Sprague Dawley rats were anesthetized and injected bilaterally with the retrograde tracer fluorogold into the anterior chamber of the eye or the SMG. One week following injection, SCGs were removed and frozen sections of the SCGs were processed for immunocytochemical localization of SSir and NPYir using the Vector ABC-glucose oxidase technique. Control sections incubated in the absence of primary or secondary antiserum showed no immunoreactivity. Sections were examined and photographed with fluorescence microscopy to demonstrate the fluorogold, then brightfield exposures of the same fields were taken to demonstrate the presence of single and double labelled neurons. The figures below represent the percentage of fluorogold labelled neurons that displayed immunoreactivity.

IRIS:SS	IRIS:NPY	SMG:SS	SMG:NPY
43%	23%	66%	32%

Supported by NIH NS21577 and a BRSG from BUSM.

146.11

PEPTIDERGIC INNERVATION OF THE MAMMARY GLAND. M. Eriksson, T. Hökfelt and K. Uvnäs-Moberg. Depts. of Pharmacology and Histology, Karolinska Institutet, S-104 01 Stockholm, Sweden.

The blood flow in mammary tissue and the tone of smooth muscle around the milk ducts are factors of importance for milk secretion and milk ejection. How the vascular and non-vascular smooth muscle in this region is regulated is not fully known. In the present study the distribution of regulatory peptides is demonstrated in the mammary gland with help of immunohistochemical techniques. Calcitonin gene-related peptide (CGRP) and substance P immunoreactivity were localized within and beneath epidermis, around blood vessels and in the smooth muscle of the milk ducts. These two peptides have been associated with sensory information from the periphery as well as with dilatation of vessels and contraction of smooth muscle. Vasoactive intestinal peptide (VIP) immunoreactivity was found in fibers around vessels and in smooth muscle. VIP is related to relaxation of vascular and non-vascular smooth muscle. Neuropeptide Y (NPY) is a peptide associated to sympathetic nerve fibers and is known for its contraction of vascular and non-vascular smooth muscle. NPY was localized around the vessels and in the smooth muscle of the mammary tissue.

Conclusion. Oxytocin and prolactin are known to stimulate milk secretion and milk production. Demonstration of regulatory peptides in the mammary gland indicates that local mechanisms may contribute to the control of milk secretion.

146.12

LOCALIZATION OF VASOACTIVE INTESTINAL PEPTIDE (VIP)- AND PEPTIDE HISTIDINE ISOLEUCINE AMIDE (PHI)-LIKE IMMUNOREACTIVITIES (IR) IN PREGANGLIONIC NEURONS THAT PROJECT TO THE RAT SUPERIOR CERVICAL GANGLION (SCG). C. Baldwin, C. A. Sasek and R. E. Zigmond, Dept. of Biol. Chem. & Mol. Pharm., Harvard Med. Sch., Boston, MA 02115

The rat SCG has been shown to contain both VIP- and PHI-IR cells and fibers. These peptide-IR were shown to accumulate on the spinal cord side of a ligature of the preganglionic cervical sympathetic trunk (CST) of the SCG. Sections of spinal cord segments T1 -T3, immunostained for VIP and PHI, showed some IR cells in the intermediolateral (IML) nucleus (Soc. Neurosci. Abst. 13:1337, '87).

The present study shows 1) the localization and distribution of VIP- and PHI-IR cells and fibers in spinal cord segments T1 - T3 of colchicine-treated rats, 2) the distribution of preganglionic cells retrogradely labeled with fluorogold (FG) from the CST and 3) the colocalization of VIP- and PHI-IR cells with FG-labeled cells.

Both VIP- and PHI-IR cells were found (in descending order of frequency) in the lateral spinal nucleus of the dorsal lateral funiculus, lamina 7 (L7), nucleus proprius, substantia gelatinosa, IML, and the central autonomic region. The IML region contained about 70% of the neurons that had been retrogradely labeled with FG. One in four IML regions examined (20 μm sections) contained, on the average, one VIP- or PHI-IR cell. Of these peptide-IR cells, at least 10 % were also labeled with FG. VIP- and PHI-IR fibers were found throughout the regions examined but were most dense in the IML and L7 regions. In the IML, peptide-IR fibers were often seen apposed to FG labeled cells. PHI-IR fibers always appeared at a greater density than VIP-IR fibers, though their distributions were similar.

These studies support the hypothesis that VIP- and PHI-like peptides are localized in preganglionic neurons that project to the rat SCG and, thus, may function as neurotransmitters in that ganglion. (NS12651 and MH00162)

146.13

CALCITONIN GENE-RELATED PEPTIDE-LIKE IMMUNOREACTIVE (CGRP-LI) NERVES IN THE CORNEAS OF NEWBORN AND ADULT RATS. C. F. Marfurt, M. Jones* and D. F. Turner. Northwest Ctr. for Med. Educ., Indiana Univ. Sch. Med., Gary, IN (CFM & MJ), and Dept. Oral Biol., Univ. Mich. Sch. Dent., Ann Arbor, MI (DFT).

The density and distribution of CGRP-LI nerves were examined in corneas of newborn (1-12 days old) and young adult (2 months old) rats. Corneas were immersion-fixed and processed as whole mounts (neonates) or serially sectioned in a cryostat (adults) and then processed for CGRP immunoreactivity according to the avidin-biotin procedure. On day 1, a dense plexus of CGRP-LI nerve fibers was already present within the cornea and formed a complicated network of anastomosing branches that appeared to distribute relatively uniformly throughout both peripheral and central regions of the cornea. Modest numbers of labeled fibers were present also within the limbus, where they were found associated with blood vessels or free within the stroma. Intraepithelial nerves were not convincingly demonstrated at this stage of development; additional studies of sectioned material are underway to confirm this observation. By day 5, the preterminal and terminal regions of the CGRP-LI nerve fibers in the paracentral regions of the corneal stroma increased in branching complexity, and by day 12 terminal axons in the central half of the cornea were organized in parallel arrays of short, radially directed fibers (precursors of subepithelial leashes) beneath the epithelium. In the adult corneas, parallel leashes of multiple, beaded axons were abundant in the superficial stroma and basal epithelium of the central cornea and were present in lesser numbers in the peripheral cornea. Intraepithelial branches arose from the leashes and coursed obliquely or vertically into the more superficial cell layers. In conclusion, the results of this study have shown that a very high percentage of rat corneal nerves contain CGRP-LI at all postnatal stages of development. We hypothesize that these fibers, in addition to playing roles in corneal sensation, may also subserve important efferent functions related to corneal trophism, metabolism, and, perhaps, wound healing.

146.14

AN IMMUNOHISTOCHEMICAL AND BIOCHEMICAL ANALYSIS OF SEROTONIN (5HT) AND SUBSTANCE P (SP) COLOCALIZATION IN NUCLEUS TRACTUS SOLITARIUS (NTS)-AFFERENT PROJECTIONS IN RAT. K.B. Thor, K. Hill*, C. Harrod*, C. Helke. Uniformed Services Univ., Bethesda, MD 20814.

5HT- and SP-immunofluorescent (IF) terminals have been identified in the NTS. The current study determines the colocalization of 5HT and SP in these terminals and in NTS-afferent neurons. Dual-color IF studies, using FITC- and RITC-labelled secondary antisera, showed that a small proportion of the IF terminals in medial portions of the NTS at all rostrocaudal levels contain both transmitters; the majority of the terminals were singly-IF. The density of doubly-IF terminals in the medial NTS were similar to that seen in the ventral horn (VH) of the spinal cord. However, because of the higher density of terminals in the NTS that were IF for only 5HT or SP, the proportion of doubly- to singly-IF terminals was less in the NTS than in the VH. Micropunch dissection and radioimmunoassay showed that i.c.v. administration of 5,7 dihydroxytryptamine, which destroyed serotonergic terminals (88% decrease in 5HT levels), caused nonsignificant decreases in SP content at caudal and intermediate levels of the NTS but a 45% decrease ($p<0.05$) at rostral levels. Retrograde labelling of NTS-afferent neurons with rhodamine beads (RBs), combined with dual-color IF (using FITC- and AMCA- [amino-4-methyl coumarin acetate-] labelled secondary antisera) in colchicine-treated rats (n=4), showed that the following numbers of NTS-afferent neurons were: 1) not IF; 2) only 5HT-IF; 3) only SP-IF; or 4) 5HT- and SP-IF. The percentage of each type of cell in each area is in parenthesis.

AREA	1	2	3	4
N. Raphe Pallidus	71(47%)	11(7%)	13(9%)	56(37%)
N. Raphe Obscurus	95(62%)	20(13%)	7(5%)	31(20%)
Paraolivary N.	93(61%)	10(7%)	8(5%)	41(27%)
N. Raphe Magnus	339(74%)	50(11%)	9(2%)	63(14%)

These studies indicate that NTS-afferent neurons in the raphe nuclei that are IF for 5HT usually, but not always, are also SP-IF, and vice-versa. However, most SP in the NTS arises from non-5HT neurons.

146.15

Mediation of changes in VAS mRNA to hypophysis (HY), medullary vagal complex (MVC) and thoracic spinal cord (SC). S.Pretel and D.Piekut. University of Rochester, School of Medicine and Dentistry. The Neuroendocrine Unit.

Neurons of the hypothalamus are involved in mediating autonomic responses of HY, MVC and SC. Periventricular hypothalamic (PVH) neurons containing vasopressin (VAS) respond readily to changes in osmolality produced by substitution of drinkung water with 2% NaCl/water. It is hypothesized that information about these changes might be transmitted to autonomic centers outside the hypothalamus. This was examined by combining in situ hybridization (ISH) of a synthetic oligonucleotide probe to VAS* (Young et al.1986) with retrograde tracer labeling.

Salt loaded (7-18days) and control rats received .5-1µl injections of either fluorogold or rhodamine latex microspheres into the HY, MVC or SC (T1-3). After 3-8days survival time, the anaesthetized animals were perfused with 0.1M phosphate buffer (PO4), 4% paraformaldehyde and 20% sucrose in PO4. Brain, HY and SC were removed, briefly postfixed in 4% paraformaldehyde/ sucrose PO4, cryoprotected in 30% sucrose/PO4 and frozen in liquid nitrogen. The VAS probe, labeled with ^{35}S - deoxy ATPand terminal deoxynucleotidyl transferase, was applied to 10µ cryostat sections. After 5 days exposure with NTB2 solution, the slides were developed for autoradiography, fixed, counterstained with neutral red and coverslipped.

The tracer injections resulted in typical neuronal labeling of the PVH and supraoptic nuclei. Increased duration of NaCl intake resulted in increased amounts of VASprobe labeling, expressed by density of labeling in individual neurons and a slight increase in number of labeled neurons in the medial parvocellular (mp) area. Neurons containing VASprobe and projecting to HY, MVC or SC were seen consistantly in control and experimental animals. They were present in magnocellular and mp nuclei. In experimental animals, some projection neurons showed increased levels of VAS probe. These data suggest that changes in VAS mRNA due to changes in osmolality might be transmitted not only to the HY, but also to MVC and SC.

* Generously provided by Drs.Young and Brownstein (NIMH).

146.16

TRANSIENT APPEARANCE OF SOMATOSTATIN AND ITS COEXISTENCE WITH CGRP IN NEONATAL RAT HYPOGLOSSAL NUCLEUS. K.B. Seroogy, D.E. Millhorn and T. Hökfelt*. Dept. of Physiology, Univ. of N. Carolina, Chapel Hill, NC, 27599 and Karolinska Institute, Stockholm, Sweden.

The developmental appearance of SOM and CGRP immunoreactivities (I) in the neonatal rat hypoglossal nucleus (12N) was examined by using a double-labeling colocalization technique. At postnatal day (P) 0, high numbers of SOM-I perikarya were observed mainly in caudal aspects of 12N. CGRP-I somata were situated throughout the rostrocaudal extent of this sturcture except at extreme caudal levels. When present in the same sections, both peptide immunoreactivities exhibited a high degree of colocalization. At P7, few to moderate numbers of SOM-I cells were found as well as a corresponding reduction in the incidence of SOM/CGRP-I coexistence. By P14, only rare SOM-I perikarya were observed and by P29, SOM-I somata were no longer detected. CGRP-I neurons, on the other hand, were present throughout development. These data demonstrate that SOM is transiently expressed in cell bodies of the hypoglossal nucleus in the neonatal rat, and, when present, often coexists with CGRP. We are currently using in situ hybridization techniques to determine whether the developmental disappearance of the perikaryal SOM-I is due to changes at the level of transcription or translation. (HL 33831, SMRC 04X-2887, NATO Fellowship).

146.17

LOCALIZATION OF CALCITONIN GENE-RELATED PEPTIDE IN THE CARDIAC GANGLION OF THE FROG. Q. Chen and H.B. Peng (SPON:E.J. Elliott). Dept. of Cell Biology & Anatomy, Univ. of North Carolina, Chapel Hill, NC 27599.

Calcitonin gene-related peptide (CGRP) has been localized in the motoneuron terminals at the neuromuscular junction in skeletal muscle. Since the parasympathetic postganglionic neurons in the cardiac ganglion receive nicotinic inputs from the vagus nerve, it is of interest to know whether this peptide is also present in the nerve terminals. The interatrial septa were dissected from the frog (Rana pipiens). After fixation and permeabilization, they were double-labeled with a polyclonal antibody against human CGRP and a monoclonal antibody (SV48) against the 65kD protein on synaptic vesicles. By double immunofluorescence technique, the distribution of CGRP immunoreactivity was examined in relationship to the presynaptic boutons (identified by SV48 staining) on the postganglionic neurons. We found that all of the boutons were positive in CGRP immunoreactivity. In addition to the boutons, the CGRP antibody also stained a set of nerve fibers within the vagosympathetic trunks. The staining was abolished by preabsorbing the antibody with 2.6nM synthetic human CGRP. In contrast to the nicotinic boutons on the neurons, the muscarinic varicosities on atrial muscle fibers, identified by SV48 staining, were negative in CGRP immunoreactivity.

146.18

ULTRASTRUCTURAL EVIDENCE FOR COEXISTENCE OF CALCITONIN GENE-RELATED PEPTIDE (CGRP), TACHYKININ AND SOMATOSTATIN IMMUNOREACTIVITY IN PRIMARY SENSORY NEURONES AND IN FIBRES OF THE DORSAL HORN OF RAT. A. Merighi[1,2*], S.J. Gibson[1*], G. Fumagalli[2*], K. Valentino[3*] and J.M. Polak[1], (SPON: J. Wharton). [1]Dept. Histochemistry, Royal Postgraduate Medical School, London W12 ONN, U.K., [2]Dept. Veterinary Morphophysiology, Turin, Italy, [3]Dept. Psychiatry and Behavioural Sciences, Stanford University, CA, USA.

Rat dorsal root ganglia and dorsal horn were immunostained using immunogold techniques on Lowicryl K4M embedded material or ultrathin frozen sections. In the dorsal root ganglia peptide-immunoreactivities were consistently found in large (60-100nm diameter) electron dense vesicles in cell bodies and intraganglionic regions of axons. A number of vesicles were double-labelled. In particular colocalisation of CGRP + tachykinins, CGRP + somatostatin and tachykinins + somatostatin immunoreactivities was observed. In the dorsal horn peptide immunoreactivities were also localised to the large, dense-core secretory vesicles in fibre varicosities and terminals. Most large vesicles were double-labelled with different combinations of peptide antisera used in this study. Although primary afferent fibres do not represent the sole origin of peptide immunoreactivity in the dorsal horn, at least a proportion of the fibres are derived from primary sensory neurones. The data suggest that a number of peptides are co-stored in the same secretory vesicles in cell bodies of primary sensory neurones and transported to the central terminals. This provides possible support for co-release of peptides from terminals in the spinal cord.

146.19

CALCITONIN GENE-RELATED PEPTIDE (CGRP)- AND SUBSTANCE P-IMMUNOREACTIVE SENSORY NEURONES AND CGRP-IMMUNONOREACTIVE MOTONEURONES CONTRIBUTE TO THE AFFERENT AND EFFERENT INNERVATION OF CREMASTER MUSCLE AND SCROTAL SKIN

Kar S.,* Gibson S.J.,* Polak J.M., Dept. Histochemistry, Royal Postgraduate Medical School, London W12 ONN, U.K.

The genitofemoral nerve projects to the cremaster muscle and scrotal skin. These tissues play a part in thermoregulation of the testis. Origins of peptide-containing nerves in the cremaster muscle and scrotal skin of the rat were investigated using retrograde tracers (True blue or Fluoro-gold) in combination with immunocytochemistry. Dorsal root ganglion cells and motoneurones of spinal segmental levels L1/L2 were ipsilaterally labelled after unilateral genitofemoral nerve injection. In the ganglia, labelled cells were immunoreactive for CGRP (8%) and substance P (5%). Somatostatin-immunoreactive cells, although present, were not retrogradely labelled. In the spinal cord a number of labelled motoneurones were CGRP positive. Genitofemoral nerve section induced a loss of CGRP and substance P positive fibres in the muscle and skin. Neonatal capsaicin treatment resulted in a similar loss of immunoreactivity but in cremaster muscle some CGRP positive fibres remained, confirming a dual, sensory and motor origin for CGRP. Vasodilation in scrotal skin, mediated by release of CGRP and substance P from sensory nerves and contraction of cremaster muscle by CGRP from motor nerves, is consistent with known actions of these peptides. Thus, it is likely that both peptides contribute to temperature regulation of the testis and hence spermatogenesis.

146.20

THE ORIGIN OF CGRP TO THE DORSAL HORN IN THE RAT LUMBAR SPINAL CORD. W.T. Lee*, K. Chung and S.M. Carlton (SPON: R.B. Leonard). Department of Anatomy and Neurosciences and the Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

Immunohistochemical studies indicate that calcitonin gene-related peptide (CGRP) is found in a large subpopulation of small to medium sized dorsal root ganglion cells. Consequently, the possibility of using CGRP as a primary marker was suggested. In the present study, the origin of calcitonin gene-related peptide (CGRP) to the dorsal horn in the rat lumbar spinal cord is investigated. CGRP immunostained fibers and terminals are mapped following unilateral and bilateral dorsal rhizotomies and isolated spinal cord preparations. Unilateral multiple dorsal rhizotomies result in a drastic decrease in CGRP stained terminals on the operated side. Following bilateral dorsal rhizotomies and isolated cord preparations, very few CGRP stained profiles can be seen in the dorsal horn. The numbers of CGRP immunostained varicosities observed in the latter two preparations were not significantly different, suggesting that few if any axons descending from the brain contribute to the CGRP terminal population in the spinal cord dorsal horn. Supported by a grant from the National Institutes of Health, NS11255, the Muscular Dystrophy Association, the Marie Hall Endowment and Bristol-Meyers.

RETINA III

147.1

EVIDENCE FOR A Cl^- CONDUCTANCE IN CHICK RETINAL PIGMENT EPITHELIUM THAT MAY CONTRIBUTE TO RESPONSES ORIGINATING AT THE BASAL MEMBRANE. Ron P. Gallemore* and Roy H. Steinberg, Depts. of Physiology and Ophthalmology, UCSF, San Francisco, CA 94143.

While little is known about the transport properties of the retinal pigment epithelium (RPE) basal membrane, mechanisms for anion movement across the basal membrane appear to be present (Hughes *et al.*, 1984). To alter anion movement, we added the anion transport blocker DIDS to the choroidal bath of a chick retina-RPE-choroid preparation. Previously, we found that DIDS hyperpolarized the basal membrane and increased its resistance (Gallemore and Steinberg, 1987). In the present study, we found that a^i_{Cl} is above equilibrium across both RPE membranes and that DIDS increased a^i_{Cl}. These results are all consistent with blockade of a basal membrane Cl^- conductance. We also studied the effects of DIDS, and similar agents, on responses that originate as depolarizations of the RPE basal membrane: the light peak of the electroretinogram, the response to azide and the response to a retinal hyperosmotic load. The anion transport and Cl^- channel blockers DIDS, SITS, phenylanthanylic acid and thiocyanate each suppressed the light peak. DIDS, the most potent agent, abolished the basal membrane light-peak depolarization. Choroidal azide depolarized the basal membrane and decreased its resistance, as in cat (Linsenmeier and Steinberg, 1987). The basal membrane depolarization, recorded extracellulary as an increase in trans-tissue potential (TTP), was blocked by DIDS. Azide also increased the c-wave and suppressed the light peak. A retinal hyperosmotic load increased the TTP, increased the c-wave and decreased the tissue resistance (Shirao and Steinberg, 1987). DIDS suppressed each of these effects. We conclude that the light peak, the response to azide and the response to a retinal hyperosmotic load may each originate as an increase in a basal membrane anion conductance that may be permeable to Cl^-.

147.2

INTRAOCULAR SYNTHESIS OF SERUM RETINOL BINDING PROTEIN AND TRANSTHYRETIN. **R.L.Martone*, T.Cavallaro*, A. Dwork*, D.S.Goodman*, E.A.Schon* and J.Herbert* (SPON: R. Liem) Depts. of Neurology, Pathology, Psychiatry and Medicine, Columbia Univ., and Dept. of Neurotoxicology and Neuropathology, NY State Psych. Inst., New York, NY 10032.**

As the chromophoric group of the visual pigments, retinol plays an essential role in vision. Several retinoid-binding proteins have been described within the eye and are believed to play a role in the intraocular transport and storage of retinoids.

Retinol-binding protein is the major serum transport protein for retinol. Holo-RBP circulates in the serum in a 1:1 molar complex with transthyretin (TTR, prealbumin). We have detected, by Northern analysis, transcripts for both RBP and TTR in total rat eye RNA. Western blotting confirmed the presence of immunoreactive RBP and TTR in whole eye homogenates. In situ hybridization studies established the retinal pigment epithelium (RPE) as the unique site of synthesis of both TTR and RBP mRNA. However, immunohistochemical studies revealed a widespread but specific distribution of these proteins throughout the eye.

These findings suggest that RBP and TTR are synthesized and secreted locally by the RPE, and participate in the intraocular transport of retinol.

147.3

RETINYL ESTER HYDROLASE ACTIVITY IN THE BOVINE RETINA AND PIGMENT EPITHELIUM. A.T.C. Tsin and D. Malsbury. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78285.

Retinyl ester hydrolase (REH) activity was studied in tissue homogenates prepared from the bovine neuroretina and retinal pigment epithelium (RPE). Tritiated palmitic acid was used to prepare the substrate (all-trans retinyl palmitate) and the REH assay was carried out according to the method of Blaner et al. (J. biol. Chem., 262: 53, 1987). Within a three hour incubation period, the REH activity increased in proportion to incubation time in both retinal and RPE homogenates. REH activity also increased linearly with protein in the reaction mixture (up to 1.5 mg of retinal or RPE homogenate proteins). The pH optimum for retinal REH was 7-8 whereas in the RPE, the optimum REH activity was observed at pH 5. By varying the substrate concentration and then measuring the formation of the reaction product, we estimated that the apparent K_m of retinal REH to be 20 uM whereas it was only 3.7 uM for REH in the RPE. However, the V_{max} for REH in the retina (101 pmol/mg/hr) was similar to that in the RPE (96 pmol/mg/hr). These results suggest that bovine neuroretina and RPE may possess different REH enzyme(s) to hydrolyze retinyl esters. Supported by grants from the NIH, DOD and the San Antonio Area Foundation.

147.4

FORSKOLIN STIMULATES MELATONIN RELEASE FROM Y-79 HUMAN RETINOBLASTOMA CELLS. M.E.Pierce*, J. Harrington*, D.Barker*; and J.S.Takahashi (SPON:B.Pratt). Dept. Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

The view is emerging that the circadian synthesis of melatonin plays a key role in the regulation of certain aspects of photoreceptor metabolism including photoreceptor membrane turnover, retinomotor movements, and modulation of neurotransmitter release. Recently, Kyritsis et al. (1987) demonstrated that antibodies to hydroxyindole-O-methyl transferase (HIOMT), the final enzyme in the melatonin synthetic pathway, label Y-79 human retinoblastoma cells. Y-79 cells also express significant HIOMT activity, suggesting that this cell line may provide a model system in which to study the regulation of melatonin biosynthesis. We have used a highly sensitive radioimmunoassay to measure melatonin release from these cells. We report that although basal release from cells grown in static culture is low, the adenylate cyclase activator, forskolin (10 uM), stimulates significant melatonin release. The phosphodiesterase inhibitor, IBMX, also increases melatonin release. Similar results are also observed in cultures which have been treated with sodium butyrate, a treatment which causes Y-79 cells to differentiate. These results suggest that in human Y-79 retinoblastoma cells retinal melatonin biosynthesis and release may require a cAMP-dependent mechanism, as has been described previously for lower vertebrates.

147.5

A MODEL FOR STUDIES OF RETINAL PIGMENT EPITHELIUM PROLIFERATION IN ADULT MAMMALS. R. Butler and M.J. Hollenberg*. Anatomy Dept., McMaster Univ., Hamilton, Ont. L8N 3Z5 and Anatomy Dept., Univ. Toronto, Toronto, Ont. M5S 1A8.

In lower albino vertebrates, the RPE not only proliferates extensively following retinectomy, but also transdifferentiates into new neural retina. To study RPE proliferation in mammals, we used a congenic strain of rats in which siblings are either albino or pigmented. Between 2 hours and 40 days following retinectomy, eyes were fixed, embedded and 1 μm thick plastic sections prepared for light microscopy. Controls show that our retinectomy technique completely removes the neural retina but leaves the RPE layer intact in both albino and pigmented animals. Some isolated islands of proliferation are found in pigmented animals, but rarely exceed 4 cell layers thick. Extensive proliferation can be found in albino animals over the entire RPE surface (i.e. both central and peripheral). There are areas, however, where the RPE does not proliferate. The greatest extent of proliferation is in areas where layering is in excess of 30 cells thick. In these regions, there is a tendency towards stratification. It is not yet known whether this represents a differentiation of proliferating RPE cells or whether it is due to connective tissue.

Supported by a grant to MJH from the RP Eye Research Foundation.

147.6

REGULATION OF RETINAL MELATONIN IN *XENOPUS* G.M. CAHILL and J.C. BESHARSE. Dept. of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

In the retina, the activity of serotonin N-acetyltransferase (sNAT), the penultimate enzyme in the synthesis of melatonin, is regulated by a circadian clock and by light, resulting in high synthetic activity at night. In *Xenopus* retina however, the regulation of melatonin content and release apparently involves other mechanisms as well. Cultured eyecups have the capacity for rapid deacetylation of melatonin, producing 5-methoxytryptamine, which is then deaminated by monoamine oxidase. The deacetylation of melatonin is inhibited by eserine, which inhibits aryl acylamidase activity. When eyecups are cultured in the presence of eserine, release of endogenously synthesized melatonin into the medium is increased 7-fold at night but is unchanged during the day. Carbachol has no effect on retinal melatonin release.

We have recently found evidence that melatonin synthesis in *Xenopus* retina is limited by the availability of serotonin. Pargyline, which blocks serotonin breakdown, acts synergistically with eserine to increase melatonin release. Addition of 100 µM 5-hydroxytryptophan to culture medium results in increased production of melatonin and melatonin metabolites by eyecups. Our results indicate that melatonin levels in the retina can be regulated by precursor availability and by local degradation, as well as by circadian variation in sNAT activity.

147.7

THE EFFECTS OF VITAMIN A SUPPLEMENTATION ON RETINOL BINDING PROTEIN AND DARK ADAPTATION H. Zwick*, B. Burri*†, E.S. Beatrice* (SPON: C.T. Bennett) Letterman Army Institute of Research and Western Human Nutrition Research Center†, San Francisco, CA 94129-6800

The effect of vitamin A supplementation on a subject with abnormally high dark adaptation threshold, but normal serum vitamin A and total retinol binding protein (RBP) was investigated. Vitamin A supplementation significantly increased the subject's serum vitamin A, total and free RBP concentrations, while decreasing the subject's parafoveal and paramacula dark-adapted threshold by more than a 1000 fold. Contrast sensitivity functions during repletion showed an abrupt but delayed increase in sensitivity for very fine spatial frequencies while showing an immediate and more gradual increase for coarser spatial frequencies. The abnormally low concentration of transthyretin-bound RBP found in this subject may explain why vitamin A supplementation was required and that this retinol transport protein may differentiate foveal receptor systems. These findings suggest that many subjects appearing to have normal vitamin A status but having poor dark adaptation might actually be vitamin A deficient.

147.8

EXPERIMENTAL MODEL OF RETINAL LIGHT DAMAGE: EFFECT OF ANTIOXIDANT, STEROID, AND CHELATION TREATMENT. R.D. Glickman†, R.M. Cartledge§*, and W.R. Elliott III†*. †KRUG International, San Antonio, TX; and §USAF School of Aerospace Medicine, Brooks AFB, TX.

Light damage to the retina may be caused by activation of nonlinear, thermal, or photochemical processes. Because biochemical intermediates are involved in photochemical, and possibly to some extent, thermal light damage, we investigated the feasibility of reducing light damage in an isolated retina eyecup preparation following damaging light exposures from a laser source. The transretinal electroretinogram (ERG) was obtained from an eyecup preparation derived from the white New Zealand rabbit. The amplitude of the ERG was recorded before and after a laser exposure at the lesion threshold, with and without pretreatment with one of the following agents: ascorbic acid, D-alpha-tocopherol, 3-aminotyrosine (3-AT), dexamethasone, and EGTA. Only 3-AT provided statistically significant protection against permanent, laser-induced loss of ERG amplitude. The rate of ERG-amplitude recovery after the laser exposure, however, was the same as in untreated retinas. These results indicate 1) a significant portion of retinal light damage is due to oxidative reactions, and 2) antioxidant therapy may have clinical applications in preventing or treating ocular light damage. Research supported by Contract F33615-84-C-0600, let by the USAF School of Aerospace Medicine, Brooks AFB, TX.

147.9

VISUAL EFFECTS OF TRANSIENT AND PROLONGED SMALL SPOT LASER EXPOSURES. Robbins D.O., Zwick, H.*, Nawim, M.S.*, and Long, R.C.* Division of Biorheology, Letterman Army Institute of Research, San Francisco, CA 94129 and Department of Psychology, Ohio Wesleyan University, Delaware, OH 43015

The creation of an isolated punctate foveal lesion by a single, 15 nsec, laser (532 nm) pulse produces little if any transient or permanent changes in visual acuity, although such an exposure may hinder the ability to consistently maintain a stable acuity thereafter. Multiple exposures however, may ultimately summate to produce a significant permanent shift in baseline visual sensitivity. Exposure of the fovea or parafovea to extended periods (15 minutes) of less intense coherent light (514.5 nm) of similar dimension produces transient suppression of both visual acuity and contrast sensitivity. The magnitude of this suppression is comparable to that observed with relatively large diameter, 100 msec, retinal exposures (350 microns) when the energy levels were above threshold for damage. These data suggest that the suppressive mechanism may be relatively independent of visible damage processes although the consequences for visual acuity and contrast sensitivity may be quite similar.

147.10

TAURINE DEFICIENCY LEADS TO LOSS OF OPTIC NERVE FIBRES IN THE RAT N. Lake, N. Malik* and G. Battista*. Depts. of Physiology and Ophthalmology, McGill University, Montreal, Canada.

It has been known for several years that taurine deficiency in cats, rats, monkeys and man is associated with retinal dysfunction and cell degeneration. Pathologies of the photoreceptors have been emphasized, but the optic nerve has not been examined. We produce taurine depletion in rats by *in vivo* treatment with guanidinoethyl sulfonate (GES), an antagonist of taurine uptake. Adult female Sprague Dawley rats were examined after 40 days of treatment which reduces retinal taurine to about 50% of control. Cross-sections of the optic nerve were taken about 1.5-2.0 mm behind its exit from the eye. Axon counts and total nerve area (using a digitizing tablet) were taken from photomontages of semi-thin sections; fibre densities (counts / area) were also obtained from electron micrographs from each nerve. The fibre density and the total number of axons were significantly reduced in taurine-depleted animals compared to controls. Correlated changes in fibre diameter and/or myelination are currently being examined; analysis of more than 2000 axons per group indicates that the dispersion of fibre diameters about the mean is much higher for depleted than for control animals. Ganglion cell degeneration may be a direct effect, or transynaptic (secondary) to effects on taurine-sensitive elements at more distal retinal sites.

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147.11

CYTOCHROME OXIDASE STAINING OF THE DYSTROPHIC RAT RETINA. J.R. Cotter. Dept. Anatomical Sciences, State University of New York at Buffalo, Buffalo, N.Y. 14214.

Royal College of Surgeon rats (RCS-p+) and their congenic controls (RCS-rdy+ p+) were studied using cytochrome oxidase histochemistry. This technique has been used as a marker of neuronal activity (Wong-Riley,M., Brain Res.,171,1979) and so it was thought that the application of this technique to the dystrophic rat retina might demonstrate changes in the morphology of the retina and reveal changes in the functional state of cells in the various layers. The dystrophic animals studied were approximately 2 months of age. Except for the ora serrata, the pattern of cytochrome oxidase staining in all other regions differed markedly from that observed in normal animals. Local differences in the rate of degeneration produced different patterns of staining which may correlate with the initial development of small holes or voids in the layer of inner segments. In the dystrophic rat, inner segments were shortened. As in the normal animal, inner and outer plexiform layers stained as did horizontal cells, displaced ganglion cells and ganglion cells. In more advanced stages of degeneration, inner segments were completely lost and staining of the inner plexiform layer reduced. Such results indicate that oxidative metabolism is sustained in inner and outer portions of the retina even after substantial damage to photoreceptors.

147.12

IMMUNOCYTOCHEMICAL INVESTIGATION OF GFAP AND (Na,K)-ATPase IN THE RETINA OF CONTROL AND RCS DYSTROPHIC (rdy/rdy) RATS DURING DEVELOPMENT. H.J. Sheedlo and J.E. Turner (SPON: W.K. O'Steen). Dept. of Anatomy, Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27103.

Glial fibrillary acidic protein (GFAP) and (Na,K)-ATPase were localized immunocytochemically using paraffin section and wholemount methodology in the retina of young-adult Sprague-Dawley rats and Royal College of Surgeons (RCS) dystrophic (rdy/rdy) rats and age-matched RCS controls. This study was undertaken to assess the effects of photoreceptor cell degeneration in the retina of RCS rdy/rdy rats on the distribution of GFAP and (Na,K)-ATPase. As shown in retinal paraffin sections of Sprague-Dawley rats, GFAP-immunostained astrocytes and processes were detected in proximity to the optic nerve fiber layer (ONFL), while (Na,K)-ATPase immunostaining was distributed on rod inner segments, in the inner and outer plexiform layers and on axons in the ONFL. Less dense (Na,K)-ATPase staining was detected in the inner and outer nuclear layers. As revealed in wholemount preparations viewed from the vitreal surface, GFAP-immunostained astrocytes and processes were interspersed between blood vessels, with astrocyte endfeet contacting these vessels, while (Na,K)-ATPase immunostaining was observed at the periphery of radially-oriented nerve fiber tracts and, immediately beneath, surrounding ganglion cell bodies. Astrocytes and processes immunostained for GFAP were also observed in the retina of 11 day-old RCS dystrophic rats and (Na,K)-ATPase immunostaining in the retina of these mutant rats did not differ significantly from age-matched control rats. Results of GFAP and (Na,K)-ATPase immunostaining in the retina of RCS dystrophic rats at more advanced stages of development will be presented. Supported by NIH grant EY-04337.

147.13

ESTABLISHMENT AND ELECTROPHYSIOLOGICAL CHARACTERIZATION OF NEURORETINAL CELL LINES OBTAINED BY RETROVIRUS-MEDIATED ONCOGENE TRANSFER. D.P. Lenzi, K. Radke,*(1) E.L. Gleason and M. Wilson (SPON: A.T. Ishida). Depts. of Zoology and Avian Sciences (1), University of California, Davis CA 95616.

The study of the retina is made difficult by the many cell types present and the complexity of their connections. Acute isolation of adult retinal neurons and primary culture of embryonic retinal neurons are two widely used methods for obtaining retinal cells for electrophysiological enquiry. We describe here an alternative: the production of retina-derived cell lines.

Dissociated embryonic day 7 Quail neuroretinal cells were incubated with the avian retrovirus MH2 PA200 (RAV-1 pseudotype) that carries the *mil* oncogene. These cells have grown through 48 population doublings, while uninfected cells stopped growing after 4 doublings.

Electron microscopy shows these cells to release virus, and immunoprecipitation of the *gag-mil* fusion protein shows these cells to express the oncogene.

We have begun the characterization of these cells using the patch-clamp technique in the whole-cell configuration. We have measured current responses to voltage steps and to the application of putative retinal transmitters. Of 27 clones generated by limiting dilution, 4 have been studied. Some but not all cells tested within these clones (1) responded to 1mM glycine with an increased conductance, and (2) show large inward currents at short times after the application of depolarizing steps. Also, most cells recorded from showed inward rectification.

147.14

THE α AND β SUBUNITS OF BOVINE RETINAL CYCLIC GMP PHOSPHODIESTERASE ARE DISTINCT BUT RELATED POLYPEPTIDES. J. J. Erdos*, H. Tamir*, A.B. Fawzi* and J. K. Northup* (SPON: M.E. Bowers). Depts. of Pharmacology and Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06510

Purified bovine retinal cyclic GMP phosphodiesterase (PDE) was used to raise polyclonal antisera. Four of four immunized rabbits developed titers of 1:10,000 or greater by immune-spot test. All four sera recognized both the α and β subunits of PDE and recognized only these subunits in Western transblots of crude retinal extracts. Antisera 3 and 4 recognized the native conformation of PDE more strongly than denatured PDE.

The α and β subunits were resolved and isolated by SDS PAGE using 11% separating gels for Cleveland mapping. Using 2 μg of α or β subunits, digestion with trypsin (1 μg) and chymotrypsin (1 μg) yeilded proteolytic fragments with a high degree of homology. Papain (3 μg) yeilded distinct terminal digestion products. *Staphylococcus aureus* V8 protease over a range of 5 -1000 ng yeilded a set of nearly identical intermediate digestion products and distinct terminal fragments.

Reactive epitopes of the α and β subunits were determined by Western transblot analysis of *Staph. aureus* V8 protease digests. Antisera 1, 2, and 4 revealed distinct epitopes unique to the α and β subunits. The data suggests that the α and β subunits of cyclic GMP phosphodiesterase are related but distinct proteins. The differences in terminal fragments and epitopes between the subunits make it unlikely that the subunits arise from simple post-translational protein modification and suggests that the subunits may be distinct gene products.

147.15

CHARACTERIZATION OF A ZINC METALLOTHIONEIN IN BOVINE RETINA, M. Ebadi and T. Takahashi*. (Spon: A.M. Earle) Dept. of Pharmacol., Univ. Neb. Coll. of Med., 42nd St. and Dewey Ave., Omaha, NE 68105

Mammalian retinas, which contain photoreceptor systems and initiate electrical impulses on illumination, possess the highest concentrations of zinc in any known living tissues. For example, nocturnal animals with keen vision, such as foxes, have been reported to exhibit a zinc level of 138,000 ppm or 13.8% by weight in the iridescent layer tapetum lucidum of the choroid. Furthermore, zinc deficiency has been implicated in impaired night vision and zinc excess has been detected in inherited retinal dystrophy. The conversion of retinol to retinaldehyde, which in turn is necessary for the formation of rhodopsin (visual pigment), is a zinc-dependent process and is impaired in zinc deficiency state.

In order to study the metabolism of zinc in the retina further, we have measured its subcellular distribution in the bovine retina and found it to be nonuniform, exhibiting the following concentrations (;g zinc/mg protein): rod outer segment (0.230), pre-pellet (0.090), pellet 1 (0.119), pellet 2 (0.091), and post-pellet 2 (0.038). In addition, the bovine retina contains a low molecular weight metallothionein-like protein which exhibits an elution volume (Ve/Vo) of 1.9 on gel permeation chromatography, and produces only one isoform, which on reverse phase HPLC exhibits a retention time of 16.22 min and is similar to that produced by zinc-induced hepatic metallothionein II. The precise compartmentation of the retinal metallothionein-like protein is being investigated at this time. (Supported in part by a grant from USPHS ES-03949.)

148.1

VALIUM UNMASKS STIMULATION-INDUCED FEEDING.
T. Bushnik-Harris and C. Bielajew. School of Psychology, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5.

Electrical stimulation of many of the same medial forebrain bundle sites induces self-stimulation and feeding. However, some animals with positive self-stimulation placements fail to demonstrate feeding when stimulated but do appear highly aroused and interested in food, while others may eat but will require relatively high stimulation thresholds. In two rats with self-stimulation electrodes in the medial forebrain bundle, feeding was reliably observed only in one animal. The frequencies required to support half-maximum performance for a wide range of currents (80 - 1000 μA) were determined for self-stimulation and stimulation-induced feeding. These frequency-intensity trade-offs were collected following the administration of valium, a benzodiazepine, or the vehicle. Valium elicited feeding in one subject and significantly reduced the thresholds for feeding in the other, without having any effect on self-stimulation thresholds in both. During the three weeks of testing, in which valium was administered on alternate days, lowered thresholds for eating in one animal and the observation of eating in the second animal were drug-dependent. These results suggest that valium unmasks feeding that may be inhibited by emotional factors without influencing the thresholds for rewarding brain stimulation.
Supported by NSERC Grant #U0514 to C.B.

148.2

HYPOTHALAMIC STIMULATION THAT EVOKES FEEDING DOES NOT RELIABLY AMPLIFY THE HEDONIC EVALUATION OF TASTES. K.C. Berridge, and E.S. Valenstein. The University of Michigan, Department of Psychology, Ann Arbor, MI 48109.

Taste-reactivity measures of natural responses were used to examine the effects of feeding-eliciting brain stimulation upon motor activation, taste perception, and palatability evaluation. After implantation with LH stimulating electrodes and chronic oral cannulae, rats were screened for hypothalamic stimulus-bound feeding.

Positive feeders were videotaped, and their natural responses subsequently analyzed in slow-motion detail, in three conditions: in an empty chamber for motor reactions; in a chamber with food pellets and water available to verify stimulus-bound feeding; and in a chamber where taste solutions of different palatability (sucrose, HCL, or quinine) were continually infused for 1 min into their mouths. LH stimulation was delivered in alternating 15 sec-ON and 15 sec-OFF bins.

Hypothalamic stimulation elicited moderate oromotor activity and locomotion when delivered alone, and stimulation-bound feeding when food was available. Stimulation did not potentiate the positive palatability reactions to food or infusions of preferred sucrose. We conclude that LH stimulation induced feeding through a psychological system that is separable from food hedonics.

148.3

A STIMULUS-RESPONSE ANALYSIS OF INGESTIVE TASTE REACTIVITY. P. Breslin* and H. Grill (SPON: D. Jameson). Dept. of Psych., Univ. of Penn., Phila., PA 19104

Examination of oral motor responses in the 60 sec following a 0.25 ml pulse of intraoral sucrose stimulation reveals that oral responding increases linearly as a function of increasing concentration. It was also found that responding decreases post stimulus. The exponential decay constants for responding over time also shows significant differences as an inverse function of concentration; weaker solutions decaying more quickly than stronger ones. Stronger solutions stimulate greater central excitation resulting in the slower decay of post stimulus elicited behavior.

The modulatory effect of a metabolic manipulation on this stimulus-reponse system was also investigated. When rats were examined for enhancements of their post stimulus oral motor response in the food deprived state, responses were enhanced at 48-h deprivation but not at 24-h. This difference is taken to mean that the potentiation of taste elicited oromotor behavior is greater under conditions of more extreme deprivation, is consistent with deprivation data from the blowfly (Dethier et al., J. Comp. Phys. Psych., 60: 303-313, 1965).

148.4

ANORECTIC EFFECTS OF AMMONIA INFUSION. T. Foley-Nelson, L. Cao*, J.L. Nelson, J.E. Fischer* and W.T. Chance. Dept. of Surgery, Univ. Cincinnati Med. Ctr., Cincinnati, OH 45267.

Our research concerning the mechanisms of cancer anorexia suggests that ammonia released from tumor tissue may be an important cause of this phenomenon. To better evaluate this hypothesis we infused various concentrations of equal parts ammonium acetate and bicarbonate (pH = 7.9) into the external jugular vein of freely-moving Fischer 344 rats. Catheters were implanted into 12 anesthetized rats, while an additional 5 rats served as operated controls. After 3 days of continuous infusion of saline at 2 ml/hr, 6 of the cannulated rats were begun on ammonium infusion (0.1 M). The concentration was increased each of the next 3 days by 0.1 M to a final concentration of 0.4 M, after which the rats were sacrificed. In another experiment 12 rats were infused with either saline or 0.2 M ammonium salts for 5 days and sacrificed. Intake of rat chow was decreased significantly by concentrations of ammonium greater than 0.1 M (25 to 90% decrease). Significant increases were observed in brain glutamine (422 & 158%), methionine (150 & 120%), tyrosine (178 & 109%), phenylalanine (466 & 134%) and tryptophan (197 & 109%) as well as in striatal DOPAC (34 & 22%), HVA (47 & 47%) and 5-HIAA (168 & 56%) following the respective infusion of 0.4 M or 0.2 M ammonium salts. These results demonstrate that hyperammonemia can cause anorexia and induce neurochemical alterations that are similar to those observed in anorectic tumor-bearing rats.
[Supported by NICR grant 86B34 to WTC]

148.5

CANCER ANOREXIA AND HYPERAMMONEMIA. W.T. Chance, T. Foley-Nelson, L. Cao*, J.L. Nelson and J.E. Fischer*. Dept. of Surgery, Univ. Cincinnati Med. Ctr., Cincinnati, OH 45267.

Although the etiology of cancer anorexia remains unspecified, recent experiments suggest that it may be mediated by a factor secreted by the tumor into the venous circulation. Based upon experiments investigating tumor metabolism, we hypothesized that this factor was ammonia. Therefore, we assayed plasma ammonia concentrations enzymatically prior to and after the development of anorexia in rats bearing methylcholanthrene (MCA)-induced sarcomas (sc). Amino acid concentrations were determined in plasma and brain using an automated analyzer. Analyses of amine neurotransmitters and metabolites were also conducted on the corpus striatum. Although plasma ammonia concentration was elevated prior to the onset of anorexia (134±11 vs 68±8 nmol/ml), it was even more concentrated after anorexia developed (240±15 nmol/ml). It correlated negatively with food intake ($r = -0.70$, $p < 0.01$) and positively with tumor size ($r = 0.70$, $p < 0.01$). In the brain the percent increase of glutamine (29&52), methionine (88&188), tyrosine (67&192), phenylalanine (86&192), and tryptophan (45&67) was significant ($p < 0.01$) in both groups of TB rats, with the anorectic rats exhibiting a greater ($p < 0.01$) increase in each of these compounds. Striatal levels of dopamine, DOPAC and HVA were increased only in the anorectic TB rats, suggesting that the anorexia may be associated with increased dopamine metabolism and amino acid derangements that are secondary to cerebral detoxification of ammonia. [Supported by NICR grant 86B34 to WTC]

148.6

NEONATAL GASTROSTOMY FEEDING MUST OCCUR EARLY TO INDUCE ACCELERATED WEIGHT GAIN IN RAT PUPS. J. Diaz, P. Garvie and D. Fordham . Department of Psychology, University of Washington, Seattle, WA 98195.

Gastrostomy reared rat pups overfed formula milk via chronic intragastric fistulas not only become obese pups but in addition defend these obese weights as adults (J. Nutr. 112, 1339, 1982; J. Nutr. 117, 1259, 1987). The present studies were undertaken to determine if there are short critical periods for this induction of obesity.

On postnatal Day 9 or Day 7, Long-Evans hooded rats were assigned to one of three groups for each day: 1) animals that were reared in litters of ten under normal conditions (MR); 2) animals gastrostomy fed enough milk formula to match the weight gains of their MR siblings (WM); and 3) animals gastrostomy fed as much volume as the WM group but a formula diet with 20% W/V added fat (OB). Animals were gastrostomy fed for four days beginning either on Day 7 or Day 9. After these four days the animals were returned to their mothers and weaned at Day 21. All the animals were sacrificed on Day 48 and organ weights were recorded. The results indicate that both the Day 9 and Day 7 OB animals did not show accelerated weight gains; nor did Day 9 nor Day 7 WM animals show increased adiposity.

These data suggest that four days of gastrostomy feeding is not sufficient and/or must begin very early in life to induce obesity in rat pups.

148.7

REPEATED VMH-AREA STIMULATION LEADS TO REDUCED WEIGHT GAIN IN THE RAT. Peter A. Pawson. Division of Biol. Sci., National Research Council Canada, Ottawa, Canada.

We have determined hypothalamic sites (the posterior AH and the anterior VMH) where acute stimulation leads to marked increases in energy expenditure as measured by whole animal oxygen consumption (Soc.Neurosci.Abstr. 13:1163). In the course of these studies I noted that rats that underwent repeated testing had a decreased rate of weight gain. One element of the VMH-lesion induced obesity syndrome is believed to be an associated decrease in energy expenditure, leading to obesity even in rats that are restricted to a normal rate of food intake. This suggests that regular VMH-area stimulation would lead to increased energy expenditure and reduced weight gain in normophagic rats. To test this hypothesis 5 rats were stimulated every third day over a 15-day period. Each stimulation session was composed of 20 trains over a 6 hr period; each 50 Hz train (400 ms on/600 ms off) of 300 microamperes cathodal current lasted 60s. While both control and stimulated rats had identical food intake over the period, the stimulated rats had a reduced net weight gain (33.2 ±18g.) versus unstimulated controls (62.8 ±8g.). The data indicate that increased neuronal activity in the VMH region leads to decreased weight gain and perhaps to differences in overall body composition.

148.8

PERMANENT ALTERATION IN LICKING PATTERN OF RATS DUE TO NEONATAL LEAD EXPOSURE. P.M. Martin and R.B. Mailman. Biological Sciences Research Center, Departments of Psychiatry and Pharmacology, Chapel Hill, NC 27599-7250.

Administration of lead acetate p.o. (200mg/kg/day for 8 consecutive days) to Long-Evans rat pups younger than 21 days of age results in a permanent increase in lithium-induced polydipsia during adulthood, although 24 hr water consumption of these animals do not differ from controls in the absence of lithium (Mailman, R.B., Psychopharmacol., 80:143-149, 1983). In the present experiment, a detailed analysis of drinking behavior was made to test the hypothesis that lead alters drinking without changing overall intake. Animals were given either lead or sodium acetate (n=7) on days 2-9 of age and at approximately 60 days of age were placed in test chambers during two separate 24 hour sessions. Tongue contacts to the sipper tube of a water bottle mounted outside the chamber were recorded via a PC microcomputer, and bout analysis was used to characterize the temporal organization of drinking. Mixed design analyses of variance showed that lead treated animals exhibited more licks per burst ($F=5.122$; $df=1,12$; $p<0.05$) and shorter interburst intervals ($F=5.15$; $df=1,12$; $p<0.05$) than did controls, although they did not differ in total number of drinking bouts, number of lick bursts per bout, or interlick interval. Animals then were sacrificed, and concentrations of dopamine, norepinepherine, serotonin, and their metabolites in various microdissected brain regions were measured by HPLC. Results suggest that brief neonatal lead exposure results in perseverative drinking, possibly through perturbation of dopaminergic motor areas. (Supported, in part, by Grant ES01104).

148.9

EFFECT OF FOOD TEXTURE ON ORAL-MOTOR BEHAVIOR. E.G. Gisel. School of Physical and Occupational Therapy, McGill University, Montreal, Quebec, H3G 1Y5.

Oral-motor behaviors change rapidly in human infants from 6 months to 2 years of age. During this weaning period children make the transition from the ingestion of liquids through sucking to the ingestion of solids through chewing. Although little is known about the influence of food texture on oral-motor development, children are generally offered a diet of increasingly resistive textures during this phase. Therefore, the purpose of our study was to examine the effect of three different textures of food (solid, viscous, puree) on chewing duration, as well as to describe tongue movements during chewing and swallowing. Fifty-two children from 6 months to 2 years were studied using video analysis. The influence of taste was controlled by using sweet flavors.

Preliminary data indicate that chewing time decreases for a comparable volume of food as the texture becomes softer. Chewing time also decreases for all textures resulting in greater chewing efficiency acrosss age. Within the same age groups, puree elicits more immature tongue patterns than the other textures. Therefore, different food textures elicit unique oral-motor patterns. It was shown earlier that the severity of a feeding problem in children with neurologic deficits (cerebral palsy) can be identified through the use of different textures of food. Supported by NHRDP #6605-2430.

148.10

ADJUSTMENT OF INTAKE PATTERNS IN FOOD DEPRIVED RATS TO VARYING TRIAL LENGTH IN A DISCRETE TRIALS PARADIGM *M.L. Volkov*, A.K. Bayer*, and S.M. Feldman.* Department of Psychology, New York University, NY, NY 10003.

A discrete trials paradigm was employed to study the intake curve and time course of the first meal following a 24 hr. fast in rats. We divided the meal into constrained bouts (trials) which permitted the sampling of intake and its components. Previously we have shown through the use of this paradigm that intake rate declines over the course of a session as a function of decreasing bite size, with constant bite frequency (Volkov, et al., *Soc. Neurosci. Abstr.*, **13**:334, 1987). In the present study we investigated the effect of trial length (defined by food availability) on the deceleration of the intake curve. Deprived subjects were adapted to trial-structured meals of wet mash (Purina rat chow and water, 3:2, w/w) over a 7 day training period. Experimental sessions consisted of feeding trials of either 15, 30, 45 or 60 sec. duration, with a constant interval of 2 min. separating trial initiation. Food intake was measured following each trial and sessions were recorded on videotape for subsequent analysis of latency, duration and bite frequency.

Results showed that the slope of the trial intake curve was a negatively accelerated linear function of trial duration. The time course of the meal also depended upon trial length, with the shortest meals consumed under the 60 sec. condition. Interestingly, cumulative intake was the same under all conditions. In addition, intake rate was an inverse function of trial duration, which indicates an alteration in either bite size or bite frequency.

148.11

FURTHER CHARACTERIZATION OF EFFECTS OF EXOGENOUS INSULIN IN THE SPINY MOUSE - MEAL FREQUENCY AND STOMACH EMPTYING. D. A. Czech and R. Prince*. Department of Psychology. Marquette University, Milwaukee, WI 53233.

Expt 1: Spiny mice were injected s.c. with 2 doses of insulin (10 & 30 U/kg) and saline vehicle in a within-S design. Frequency of entry and time spent in a food hopper were monitored with photodetector electronics and a microcomputer for 6 hr. Amount eaten was also recorded. A feeding bout (meal) was defined as at least 1 min of nearly continuous time in the feeder followed by a post-meal interval of 15 min. ANOVA and t-tests showed that meal frequency was significantly higher for both insulin conditions relative to control condition ($p< 0.05$). Meal duration was not significantly altered by insulin.

Expt 2: Mice were injected s.c. with insulin (30 U/kg) or saline following overnight food deprivation, given access to powdered chow, and visual monitoring was begun. Mice were sacrificed 0, 25, 50 & 75 min after eating a meal (meal end defined as 3 min without eating). Under ether anesthesia, the stomach was removed and its contents rinsed into a preweighed tube. It was centrifuged, the supernatant drawn off and remaining material dried to a constant weight. Dry weights were converted to percent of dry weight of 0-delay controls. A 2-factor ANOVA design showed both main effects and the interaction to be significant. Evaluation of the interaction revealed that weight of stomach contents was significantly lower in insulin treated mice than in saline controls at all non-0 delays ($p <.05$, 1-tail).

148.12

CHEMICAL COMPARISON OF TWO SATIETINS (SAT) WITH ALPHA1-ACID GLYCOPROTEIN (AAGP). V.E. Mendel) and M. Palieschekey*. Depts. of An. Physiol. and An. Sci., Univ. of Calif., Davis, CA 95616.

Isolation of human SAT (hSAT) by ultrafiltration of plasma (PM-10 microfilter) and precipitation with trichloroacetic acid (TCA) followed by passing the supernatant through a sizing chromatography column (Sephadex G-15) plus an affinity chromatography column (Concanavalin-A) and, finally a weak anion exchange HPLC column (Synchropak AX300), using a linear gradient of 0-60%B in 25 min. (A:0.02M Tris Acetate, pH8; B: A+1M NaAcetate, pH8), resulted in three peaks. The major peak (PKC) corresponds to the 45 Kd protein standard. This peak was eluted from the column at 0.46M salt and represents 57% of the protein (PKA = 31%, PKB = 12%). The HPLC profile of comparably prepared rat SAT (rSAT) is like hSAT except that PKA is the major peak.

A literature search revealed that PKC at 45 Kd corresponds to a major plasma protein (44Kd), namely, alpha1-acid glycoprotein (AAGP). Thus, we have compared SAT to AAGP. Pure AAGP and SAT when precipitated with (TCA) have nearly identical HPLC elution patterns but both hSAT and rSAT also have a 68 Kd band corresponding to albumin on SDA-Page gel. Biological activity of satietin, therefore, may be only an artifact of preparation.

148.13

REPEATED EXPOSURE TO ACTIVITY-INDUCED SELF-STARVATION: EFFECTS ON FOOD INTAKE, RUNNING, AND SURVIVAL. Y. Beaulieu* and R. Eikelboom, Psychology Dept., Queen's Univ., Kingston, Ontario, Canada, K7L 3N6.

Rats placed on daily 23 hr food deprivation with concurrent activity wheel access (the Activity-Induced Self-Starvation (AISS) procedure), display a reduced food intake relative to home-cage controls on the same deprivation schedule (Routtenberg, JCPP, 66: 234-238, 1968). This decrease is accompanied by marked daily increases in running and, unless interrupted, the severe weight reduction that ensues leads to death. However, in the present experiment, animals were returned to ad libitum feeding in the home-cage after 23% weight loss (days until removal was defined as survival time). We hypothesized that repeated exposures to AISS with intervening recovery periods would result in increasing survival times (i.e., slower weight loss). Over 3 exposures, animals increased their food consumption and running, resulting in longer survival times (8, 11.8, and 14.6 days respectively). Experience with an activity wheel prior to AISS increased survival time (10.6 days) as running did not show the expected increase. Deprivation experience, with recovery, before AISS resulted in marked increases in survival time (18.6 days) as food consumption was elevated while running was low. These results, in contrast to Routtenberg, suggest a major role for novelty in AISS and imply that, given enough experience, animals may adapt to this procedure.

148.14

HERITABILITY OF FISCHER-344 NACL AVERSIONS. S.I.Sollars*, E.E. Midkiff* & I.L.Bernstein, Depts of Psychology, University of Washington, Seattle, WA 98195 and Pacific University, Forest Grove, OR 97116.

Fischer-344 (F-344) rats fail to prefer NaCl solutions to water at any concentration, and avoid NaCl solutions preferred by other strains, including the Buffalo (BF) rat. These findings implicate a genetic mechanism in the F-344's salt aversion and the present study examined patterns of heritability of this behavior. Two inbred strains, F-344 and BF, were crossed and offspring from 8 litters examined. In half the breeding pairs, F-344 was the maternal genotype. NaCl preference of male offspring was examined in 24-hr, two bottle preference tests with water and NaCl solutions. Twelve pairs of F1 animals were randomly mated to produce an F2 population and the males received similar preference testing. For the purposes of analysis preference data of the BF strain were used to establish 99% confidence intervals around the mean NaCl preference at each concentration. This lower limit of NaCl preference provided a basis for excluding F1 and F2 animals from the norm of BF salt preference or classifying them as similar to the F-344. One of 33 animals in the F1 generation had preference scores below this cutoff. No differences in NaCl preference scores were evident between F1 animals based on maternal genotype. Thus, F1 males show NaCl preference which is similar to BF rats. Also, the gestational and suckling environment provided by F-344 dams appears not to be sufficient to produce NaCl aversion. In the F2 generation, 16 of 56 animals (29%) displayed NaCl preference scores which fell below the criterion levels on all NaCl concentrations and therefore their preference was more like F-344 rats. These results are consistent with a simple genetic model where a single, recessive gene carries the trait of NaCl aversion in F-344 rats although additional studies would be needed to confirm this.

148.15

THE EFFECT OF IN VIVO TREATMENT WITH GABA MIMETICS ON THE LEVEL OF GABA BINDING IN HYPOTHALAMUS. P.C. Madtes Jr. and V.S. Wahby. Dept.Biol. Point Loma Nazarene Col., San Diego, CA 92106 and VA Medical Center, N. Chicago, IL 60064.

Opposing GABAergic mechanisms may be acting in appetite regulation, one to suppress appetite in the lateral hypothalamus (LH) and the other to inhibit satiety in the ventromedial hypothalamus (VMH). Since eating behavior influences obesity and the GABA systems may, at least in part, control eating behavior, we studied the effect on in vivo treatment by GABA mimetics on the level of GABA binding in the brain. Young (5 wk and 10 wk) CD rats (Charles River) were lightly anesthetized with ether and treated by intraperitoneal injection 48 hr and 24 hr prior to sacrifice with one of the following: saline; THIP (GABA agonist) (7.5 mg/kg); picrotoxin (GABA antagonist)(1 mg/kg); or nipecotic acid (GABA uptake blocker)(20 mg/kg). The animals were sacrificed and 4 brain areas (cerebral hemispheres, cerebellum, LH, and VMH) were rapidly removed and stored frozen until assayed for both high- and low-affinity ^{3}H-muscimol binding. All 3 drugs lowered high-affinity binding in VMH in 5 wk and 10 wk rats and low-affinity binding in 10 wk rats. These data suggest that treatment with GABA mimetics may be useful in studying eating behavior.

148.16

Blood glucose and insulin responses to glucose infusions in the free-feeding rabbit. P.J. Geiselman, L. O'Farrell, D.S. Gray*, A. Acevedo-Cruz*, and L.J. Jakobsen*. Dept. Psychol. and Neurosci. Prgm., UCLA; Div. Diabetes, USC Sch. Med., Los Angeles, CA 90024.

Rabbits were given duodenal infusions of 0.3M glucose or 0.15M NaCl at a fast rate (3ml/min) or a slow rate (1ml/min).

Fast intraduodenal glucose infusion resulted in a sharp increase in blood insulin levels followed by a precipitous decline in blood glucose levels when food was not presented. However, when food was present, rabbits increased their food intake and, thus, did not show the precipitous decline in blood glucose levels.

After slow intraduodenal glucose infusion, blood insulin levels showed only a moderate increase, which failed to decrease glycemic levels. When food was present in this condition, rabbits suppressed food intake.

Thus, insulin levels that are sufficient to produce a precipitous decline in blood glucose levels are associated with hunger. However, moderately increased insulin levels that are insufficient to produce a significant reduction in glycemic levels appear to mediate satiety.

Supported by NSF grant BNS-8709982 to PJG.

TROPHIC AGENTS II

149.1

MOLECULAR INTERACTIONS OF HEPARAN SULPHATE PROTEOGLYCANS ASSOCIATED WITH SENSORY NEURONS IN VITRO. K.E. Dow and R.J. Riopelle. Queen's University, Depts. of Pediatrics and Medicine, Kingston, Canada K7L 3N6.

Proteoglycans on the cell surface and in the extracellular matrix have been implicated in cell adhesion and in the regulation of neurite outgrowth.

Dissociated sensory neurons in vitro displayed Alcian blue positive labelling on cell bodies and processes in the presence of 0.3 and 0.7 M $MgCl_2$ but not at 1.0 M $MgCl_2$, suggesting the presence of chondroitin sulphate (CS) and heparan sulphate (HS). In conditions where there was no apparent contribution of released proteoglycans (PGs) to neurite outgrowth , outgrowth was inhibited if the sensory neurons were coincubated with HS glycosaminoglycan (GAG) and if the cells were treated prior to seeding with heparitinase. Pretreatment of cells with HSGAG or with the monoclonal antibody HNK-1 (Leu 7) which recognizes a carbohydrate domain on cell adhesion glycoproteins, also resulted in inhibition of neurite outgrowth on laminin. Inhibition by HNK-1 (Leu 7) and HSGAG was not additive and the inhibitory effect produced by coincubation with HSGAG was eliminated by pretreatment with heparitinase or with HNK-1 (Leu 7) and/or HSGAG. These data suggest that HSPGs associated with sensory neurons bind by non-covalent interactions to distinct GAG binding domains on laminin and on the oligosaccharides of cell adhesion glycoproteins and promote neurite outgrowth by bridging between the cell adhesion glycoproteins and laminin.

149.2

THE IDENTIFICATION OF PHOSPHORYLATED PROTEINS ASSOCIATED WITH EPIDERMAL GROWTH FACTOR (EGF)-INDUCED NEURITE OUTGROWTH IN CULTURES OF CNS NEURONS. J.R. Moskal and R. S. Morrison. Albert Einstein College of Medicine, Bronx, NY 10467.

The stimulation of cyclic AMP-dependent protein kinase and the down regulation of protein kinase C appear to be key events in EGF-induced neurite extension. Thus, we have begun to examine protein phosphorylation patterns using 2D-SDS PAGE in primary cultures of rat cortical neurons treated with dibutyrl-cyclic AMP (dBcAMP: 1 mM), phorbol-12-myristate-13-acetate (PMA: 320 nM), and EGF (10 ng/ml). Autoradiograms were analysed using PDQUEST (Protein Data Bases, Huntington Station, NY). EGF treatment induced the phosphorylation of 7 proteins not found in control cultures. EGF treatment also led to the disappearance of 13 phosphorylated proteins found in the control cultures. In the dBcAMP-treated cultures 16 proteins with significantly altered phosphorylation patterns compared to controls were identified. Two of these proteins (Mr=80.1, pI=5.37 and Mr=17, pI=6.66) also were found in EGF-treated cultures and showed a similar pattern (increase) of phosphorylation. In PMA-treated cultures one protein (Mr=41.1, pI=5.36) was found that was present in EGF-treated cultures and showed an opposite (decrease) phosphorylation pattern. These results (1) demonstrate that EGF is capable of inducing a large number of protein phosphorylation changes, many of which are different than those induced by either dBcAMP or PMA and (2) suggest that the specific phospho- proteins with Mr=80.1, 41.1, and 17 are involved in regulating neurite extension.

149.3

SUPPRESSION OF PROTEIN KINASE C ACTIVITY POTENTIATES THE NEUROTROPHIC ACTION OF EPIDERMAL AND BASIC FIBROBLAST GROWTH FACTORS. R.S. Morrison, J.L. Gross*[1] and J.R. Moskal. Albert Einstein College of Medicine, Bronx, NY 10467, [1]Dupont Co., Wilmington, DE 19898.

The addition of either epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF) to primary cultures of CNS neurons enhances cell survival and induces neurite outgrowth and arborization. The purpose of this study was to examine the role of protein kinase C in a signal transduction pathway utilized by EGF and bFGF. Previous work has suggested that some growth factors initiate their action by stimulating PKC. The role of PKC in neurite outgrowth was studied in primary cultures of cerebral cortical neurons established from the brains of neonatal rats. The activation of PKC by phorbol dibutyrate (PDBu), in EGF-treated neurons significantly **depressed** total neuronal survival as well as neurons bearing long processes. The addition of 4-beta-phorbol, a very weak tumor promoter, did not inhibit survival or process outgrowth. However, when neurons were first **pretreated** with PDBu to down regulate PKC, the action of the growth factors was dramatically potentiated. The potentiation was dependent on the duration of PDBu pretreatment, reaching an optimum after 24 hr. Moreover, the addition of a PKC **inhibitor (H7)** promoted neurite outgrowth when added alone or in combination with EGF or bFGF. Therefore, we conclude that the neurite extension induced by binding EGF and bFGF to cortical neurons does not require stimulation of PKC for signal transduction. In fact, down regulation of PKC may be a consequence of growth factor binding to cortical neurons.

149.4

SURVIVAL ACTIVITY OF HYDRA HEAD ACTIVATOR PEPTIDE FOR PERIPHERAL NEURONS IN CULTURE. A.M. Duchemin, T.T. Quach, B.K. Schrier, R.J. Wyatt. Laboratory of Developmental Neurobiology, NICHD, Bethesda, MD and Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, DC.

The head activator (HAP) is a signal for nerve cell differentiation in hydra (S. Hoffmeister and C. Schaller, Dev. Biol. 122:7, 1987). This undecapeptide, with completely conserved amino acid sequence, is also present in significant amounts in intestine and hypothalamus of adult and developing mammals.

We tested the activity of HAP on the survival of 12 day old chick embryo sympathetic and dorsal root ganglion neurons in culture. HAP was found to support neuron survival when applied at concentrations ranging from 0.1 to 10 pM in both cell assays. In hydra, HAP is also active at picomolar concentrations. The level of neuron survival with HAP was similar to that obtained with NGF (1 nM). Bradykinin, which has some amino acid sequence homology with HAP, was inactive as a trophic factor in these assays. HAP may have neuron maintenance or trophic function across the animal kingdom.

149.5

SOLUBLE FACTORS FROM RAT OLFACTORY BULB AND HIPPOCAMPUS SUPPORT THE SURVIVAL AND DIFFERENTIATION OF CULTURED RAT BASAL FOREBRAIN NEURONS. M. Martinic*, G. Garden*, M. P. Lambert and W. L. Klein. Department of Neurobiology and Physiology, Northwestern University, Evanston, Il. 60208.

Rat basal forebrain cells have been used to test trophic factor activities of various preparations. Soluble proteins from rat olfactory bulb and hippocampus support long-term survival (longer than 9 days) and process outgrowth in these cultures. These proteins also induce aggregation of the cells and fasciculation of neurites. Immunocytochemical labeling for NSE and GFAP shows that these cultures are primarily neuronal. Extracts from other neural areas, such as cerebellum, are able to support survival for up to 7 days, but do not induce aggregation or fasciculation. The addition of heart extract does not support survival for even 24 hours. Fetal calf serum and adult rat serum both promote long-term survival, but do not induce aggregation. Serum also induces proliferation of flat cells, not seen with neural extracts. Cells grown with extracts from the olfactory bulb and hippocampus, both targets of basal forebrain cholinergic neurons, show the highest proportions of acetylcholinesterase-positive staining.
(Supported by NIH grant NS23348 to WLK.)

149.6

BASIC FIBROBLAST GROWTH FACTOR STIMULATES THE SYNTHESIS OF THREE NEW PROTEINS IN THE GOLDFISH RETINA. M.A. Deaton*, C.A. Barba*, C.R. Flores*, A.M. Dahlberg*, G.P. Jones*, and M.C. Powanda* (SPON: D.J. Ramsay). Division of Ocular Hazards, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129.

Basic fibroblast growth factor (bFGF) promotes the survival of neurons *in vitro* and *in vivo*. Since specific newly synthesized proteins appear to be essential for neuron survival after axotomy, bFGF might promote neuron survival by inducing the synthesis of new proteins. Thus we injected *C. auratus* eyes intravitreously with 10, 100, or 1000 ng bFGF and evaluated subsequent changes in retinal protein synthesis using 2-D PAGE/fluorography of [35S]met-labeled proteins. Incubation with bFGF was for 4 or 24 hr. bFGF-treated retinae (all doses/times) synthesized 3 new proteins (i.e., not found in controls) which comprised a substantial percentage of the total resolved protein. Each has a MW less than 20 KD, and an acidic pI. Furthermore, these 3 proteins are distinct from known retinal stress/heat shock proteins. Thus bFGF can induce the synthesis of new proteins in a neural tissue.

149.7

FIBROBLAST GROWTH FACTOR (FGF) LEVELS IN THE DEVELOPING BRAIN. C.G. Caday, A. Mirzabegian*, J. Prosser*, M. Klagsbrun*, and S.P. Finklestein. Mailman Research Center, McLean Hospital, Belmont, MA, 02178.

Fibroblast growth factors (FGF) are polypeptides with potent effects on the growth and differentiation of CNS neurons, glia, and endothelial cells. Both acidic and basic forms are found in the mammalian CNS. These factors are unique by virtue of their strong binding to heparin.

We used a 3T3/BalbC cell mitogenic assay to examine the levels of FGF in the developing and mature rat brain. FGF levels were relatively low in the embryonic brain (5-10 units/mg protein) and showed a rapid rise to reach adult levels (90-100 units/mg protein) during the first month of life. Most (> 90%) of this activity bound strongly to heparin-affinity columns, confirming its identity as FGF. These results on total FGF levels parallel those reported previously for the basic form alone (Logan, A., et al., Neuroscience, 15:1239-1246, 1985).

The marked increase in FGF levels during the first month of life may play a central role in the glial and endothelial proliferation, and neuronal differentiation that is occurring during this time. Sustained high levels of FGF may play an important role in the maintenance of these cell types in the mature brain.

149.8

BASIC FIBROBLAST GROWTH FACTOR (WITH HEPARIN) INCREASES THE SURVIVAL OF RAT DENTATE GRANULE CELLS IN CULTURE. D.L. Needels and C.W. Cotman. Preclinical CNS Biology, Bristol-Myers Co., Wallingford, CT 06492 and Dept. Psychobiology, Univ. California, Irvine, CA 92717.

Basic fibroblast growth factor (bFGF) is a potent heparin-binding growth factor reported to have trophic effects on a variety of cultured CNS neurons; including those from hippocampus, cortex and spinal cord. We report here that bFGF (in the presence of heparin) also supports dentate granule neurons grown in low density cultures.

Dentate gyrus was dissected from the hippocampus of 4-5 day old rats and cultured at 17,000 cells/cm2 under serum-free conditions. Neurons steadily died over the first 5 days in culture, as determined by phase contrast microscopy. The addition of 5 ng/ml of commercial bFGF delayed, but did not prevent, this neuronal death.

Cultures grown in the presence of 2-50 ug/ml heparin had a very different appearance. Very few flat cells were present (1% of cells at 3 days), suggesting that heparin may be useful in reducing the number of glial cells in these cultures. Neurons were phase bright with few neurites. Although the addition of heparin initially increased the number of live neurons, all neurons had died by 5-7 days. The combination of bFGF plus heparin resulted in the survival of up to half of the seeded cells at 5 days, and they were much larger with neurites.
Supported by NIA grant AG00538.

149.9

CHARACTERIZATION OF NEUROLEUKIN ACTIVITY IN NEUROBLASTOMA CELL LINES.

Y Mizrachi, ME Gurney, DD Ho*. Cedars-Sinai Medical Center/UCLA School of Medicine, Los Angeles, CA 90048.

Neuroblastoma cell lines were studied for their responsiveness to neuroleukin (NLK), a mediator of the nervous and the immune systems. NLK was expressed by transfection of COS cells, and control supernatant fluids were from COS cells transfected with expression vector DNA alone. Human neuroblastoma cell lines (LA-N-1, LA-N-2, SK-N-MC) and a rat cranial neuroblastoma cell line (B35) responded to NLK by a 30-50% reduction in ^{3}H thymidine incorporation. No such response was noted for SK-N-SH, IMR-132 and B50 neuroblastoma cell lines. In addition, the responsive cells (LA-N-1, LA-N-2) showed enhanced neurite extension when grown on laminin in the presence of NLK but laminin alone was without effect. NLK shares partial sequence homology with gp120, the envelope glycoprotein of HIV-1. The reduction in ^{3}H thymidine incorporation induced by NLK was antagonized (50-75%) by HIV-1 lysates and purified gp120. These HIV-1 protein preparations had no direct effect on neuroblastoma cells in the absence of neuroleukin. Recently, NLK was shown to be identical to phosphoglucoisomerase and to have enzymatic activity. Studies concerning that point are currently in progress.

149.10

CHARACTERIZATION OF THE NEURONAL BASIC FIBROBLAST GROWTH FACTOR(bFGF) RECEPTOR.

P.A.Walicke, J.J. Feige* and A. Baird*. Dept. of Neuroscience, UCSD, and Laboratories for Neuroendocrinology, Salk Institute, La Jolla, CA 92093

Previous studies have shown that bFGF is neurotrophic, increasing neuronal survival and process growth in vitro. Serum-free cultures of fetal rat hippocampal neurons were used to characterize the neuronal bFGF receptor. Two components of [^{125}I] bFGF binding at 4°C were distinguished: one removed by 2M NaCl represented binding to glycosoaminoglycans, while the second stable to 2M NaCl represented a receptor. Scatchard analysis showed the presence of about 26,500 receptors/neuron with a Kd of 0.1 nM. Crosslinking with disuccinimidyl suberate (DSS) produced a major labeled band of 150 kD and a minor band of 100 kD. Labeled proteins of similar size were detected in cultures of neurons from several brain regions, and in membranes prepared from fetal brain. Specificity was demonstrated by blockade with cold bFGF but not a variety of other proteins. The contribution of carbohydrates to receptor structure was investigated with degradative enzymes. N-glycanase decreased receptor size by 10-15 kD, but neuraminidase, endoglycosidase H and heparinase were inactive. Wheat germ agglutinin (WGA) blocked bFGF binding and activity in the bioassay. To determine the domain of bFGF interacting with the receptor, a series of 13 synthetic peptide fragments of bFGF were tested. Two peptides from the carboxyl terminal region inhibited binding in the radioreceptor and crosslinking assays and neuronal survival in the bioassay.

149.11

S100β AND NEURITE EXTENSION FACTOR ACTIVITY: SYNTHESIS, EXPRESSION, AND SITE-DIRECTED MUTAGENESIS OF AN S100 GENE.

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The S100 family is a group of structurally similar proteins that includes calcium binding proteins, cell proliferation and differentiation markers, and the cystic fibrosis antigen. As an initial step in addressing what structural features of the S100 family are related to their specific functions and localizations, a gene coding for one of the S100 proteins, S100β, was synthesized and expressed in E. coli. The gene was designed to allow rapid, efficient changes at single or multiple amino acids by using cassette-based mutagenesis. The recombinant S100β (termed VUSB-1) is structurally and functionally similar to bovine brain S100β, including the ability to stimulate neurite outgrowth from cerebral cortex neurons. Because the neurite extension factor (NEF) activity of S100β appears to require a disulfide form of the protein, initial experiments to analyze the structural requirements for NEF activity involved mutagenesis of the cysteine residues of VUSB-1. Currently, we are characterizing the NEF activity of the mutant S100 proteins, and testing the hypothesis that S100β may be a neurotrophic factor in the central nervous system.

(Supported in part by NIH grant GM33481)

149.12

THE MAJOR NEURITE OUTGROWTH PROMOTING ACTIVITY IN MOUSE HEART CELL CONDITIONED MEDIUM (NEURONECTIN) IS A 600 kDa SIALYLGLYCOPROTEIN.

M.D.Coughlin, J.Stanisz*, D.E.Jang*. Dept. Neurosci. McMaster Univ., Hamilton, Ont. CAN L8N3Z5

Neuronectin is the major substrate-binding neurite extension factor isolated from mouse heart cell conditioned medium and has M_r 350 kDa as determined by radiation inactivation analysis (Coughlin et al., J. Neurosci. 6:1553). We have now purified this factor and determined that it is a sialylglycoprotein. Activity isolated on a DEAE column (Coughlin et al.,Dev.Biol.82:56) was purified by Sephacryl S-300 column chromatography and preparative gel electrophoresis with a final yield of 10% of initial activity and 100-fold purification. Analysis by SDS PAGE revealed a single major band of stained material at ~600 kDa. The glycoprotein contains approximately 5% sialic acid. Treatment with chondroitinase ABC, heparitinase, and neuraminidase caused no loss of activity. Western blot and dot blot analysis of neuronectin with antibodies to neuronectin, laminin and heparan sulfate proteoglycan (HSPG) core protein (gift of Dr. John Hassell) suggests that there is less than 1% cross reactivity of neuronectin with anti-laminin or anti-HSPG. Differences between radiation inactivation and SDS PAGE estimations of molecular size will be discussed.

(Supported by NIH Grant NS 19573, Medical Research Council of Canada, and the Dysautonomia Foundation.)

149.13

LAMININ-LIKE IMMUNOREACTIVITY IN ADULT RAT BRAIN NEURONS.

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Laminin (LN), a glycoprotein component of the extracellular matrix, is abundant in basement membranes of several tissues, including those of the nervous system where it is produced by Schwann and astroglial cells and occurs largely in an extracellular location. We report here the widespread presence of LN-like immunoreactivity associated with neuronal cell bodies of the adult and neonatal rat CNS. Animals were perfused with 4% paraformaldehyde, brain sections were cut on a freezing microtome and immunostained using an ABC method and nickel chloride and mild osmication to intensify the DAB reaction product. Affinity-purified polyclonal and monoclonal antibodies raised against rat and human LN caused the expected staining of the basement membranes but also a distinctive cellular staining that was not found with pre-immune sera, control antibodies or with anti-LN antisera pre-absorbed or pre-incubated with rat LN. The size and morphology of the stained cells indicated them to be neurons and the pattern of reactivity suggested an intracellularly located antigen that sometimes extended into the processes. Some regions of the brain including the cortex, striatum, thalamus and hippocampus showed heavy neuronal immunoreactivity whereas other regions including the basal forebrain, hypothalamus, cerebellum and spinal cord revealed very weak or no reactivity. The LN-like immunoreactivity in these neurons could be due to the recognition by the anti-LN antibodies of i) LN epitopes or ii) non-LN molecules containing epitopes found in LN. Supported by NSF grant BNS 17034 and NIH grant NS 25011.

149.14

LAMININ EXPRESSION IN THE DEVELOPMENTAL RAT BRAIN: AN IMMUNOCYTOCHEMICAL AND IN-SITU-HYBRIDIZATION STUDY.

C. T. Hu*, F. C. Zhou, Y. W. Hwang* and C.H.Lee* (SPON: J. F. Hyde) Dept. of Anatomy and Pathology, Indiana Univ. Indianapolis, IN 46223

Laminin has known to promote neurite outgrowth in the *culture* (review: Davis et al., TINS, 8:528, 1985). We recently provided evidence that laminin facilitates and guides fiber growth of grafted neurons in the *brain* (Zhou & Azmitia, J. Chem. Neuroanat, in press). In this study the morphology of the laminin in the brain were investigated at various developmental stage.

Brains from Sprague-Dawley rats of 16 days embryo (E16), postnatal day 1 (P1), P7, and P29 were used for immunocytochemical staining of laminin with antibodies to EHS laminin (E.Y and Biogenex Lab). Antibodies preabsorbed with EHS laminin was used as control. Four forms of immunostained laminin, small punctates, large punctates, sheath form and peri-somatic form, were observed in the E16, P1, and P7 brains. Only sheath form was detected in P29 brain. Small punctates distributed in patches in all regions of brain, whereas large punctates only exist in the hippocampus of E16, P1, and P7. Peri-somatic form of laminin was also detected in E16, P1, a nd P7. Sheath form of laminin was found on outer limiting membrane, ependyma , choroid plexus, and on blood vessels. Staining in control remains negative.

Paraffin section of E16, P7, and P29 brains were examined for laminin mRNA by in situ hybridization. A 50-base long oligonucleotide complementary to B2 region of laminin gene was labelled with alpha-^{35}S (or ^{32}P)-dATP at 3' end and used to detect laminin mRNA. Both neurons and glia of E16 and P7 brain have positive signal. Dense granules (positive signal) were observed in outer limiting membrane, ependymal cells, and endothelial cells of vessels of all the stages. The amount of dense granules were greatly reduced in P29 brain, and the granules only exist in where sheath form laminin was found. These results indicate that laminin is expressed in the developmental stage but is turned off upon maturation.

149.15

PERIPHERAL NERVE SECTIONS CONTAINED IN HOLLOW FIBERS FUNCTION AS A DELIVERY SYSTEM OF NEUROTROPHIC MOLECULES. J. E. Springer, T.J. Collier, and M.F.D. Notter, Dept. of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, New York 14642.

Schwann cells, a major support cell for peripheral nerves, are known to produce neurotrophic and neurite-promoting substances such as nerve growth factor (NGF) in vitro and in vivo. Following sciatic nerve transection in rats, mRNA encoding for NGF in Schwann cells distal to the cut increases dramatically, as does NGF synthesis. We tested whether sciatic nerve sections contained in hollow porous fibers would provide a source of NGF for NGF-sensitive cells in culture. Rat sciatic nerve sections were injected into hollow fibers (Amicon Corp) containing uniform pores with an maximum molecular weight exclusion of either 100 or 500kDa. These peripheral nerve-containing fibers were then placed into cultures of undifferentiated rat pheochromocytoma cells (PC12). Within 24 hours, PC12 cells began to differentiate and send out neurites indicating the presence of NGF or a similar substance. These nerve containing fibers continued to exhibit this NGF-like effect for up to 21 days following placement in culture medium. A similar effect was observed using only nerve fiber-derived conditioned medium.

Magnocellular cholinergic neurons in the basal forebrain are known to transport and utilize NGF derived from their target areas. When these targets are removed, these cholinergic neurons are thought to shrink and/or die, an event similar to that observed in individuals suffering from Alzheimer's disease. Exogenous NGF treatment will reverse axotomy-induced degeneration of basal forebrain cholinergic neurons in rodents. From the present results, it is suggested that transplantation of these peripheral nerve fibers may provide an alternative source of trophic support for damaged cholinergic neurons. Studies utilizing cultures of basal forebrain neurons, as well as transplantation of peripheral nerve fibers into the CNS of rats are currently under investigation. Supported by grants from the American Federation for Aging Research and PHS DA05274 (JES), and ADRDA FSA85015 (TJC).

149.16

SUSTAINED REGENERATION OF AXOTOMIZED CHOLINERGIC NEURONS USING CO-GRAFTS OF NGF-RICH TISSUE AND FETAL RAT BASAL FOREBRAIN. Dorothy Heyer (*), Timothy J. Collier, Rebekah Loy, and Joe E. Springer. (Spon: G. Thomas) Dept. of Neurobiology and Anatomy, Univ of Rochester Med Ctr., Rochester, N.Y., 14642.

Magnocellular cholinergic neurons in the basal forebrain appear to utilize nerve growth factor (NGF) for trophic support. Specifically, damage to the dorsal septo-hippocampal pathway results in a loss of cholinergic neurons in the medial septum (MS). We have recently demonstrated that transplants of NGF-rich tissue will increase the survival of these axotomized cholinergic neurons. We now report that fetal rat basal forebrain co-grafted with NGF-rich tissue provides a substrate for regenerating host cholinergic neurons. Adult female Long Evans rats received unilateral fimbria-fornix transections followed by intraventricular grafts of either male mouse submaxillary gland (a rich source of NGF), day E15 rat basal forebrain, or both. At 4 or 8 weeks following transplantation, animals were perfused and 30μm brain sections prepared for NGF receptor (NGFR) or choline actyltransferase (ChAT) immunocytochemistry.

As reported previously, grafts of male mouse submaxillary gland (but not fetal basal forebrain) increased the number of NGFR- and ChAT-positive neurons in the host MS. When animals received both grafts, numerous regenerating cholinergic neurons from the host MS were found to innervate the fetal basal forebrain graft. These NGFR- and cholinergic-positive fibers were maintained for at least 2 months following transplantation (the longest time period analyzed). It is suggested that, given the appropriate environment, i) regenerating fibers from "rescued" cholinergic neurons can be sustained within an intraventricular basal forebrain graft for up to 2 months, and ii) functional reinnervation of the target area by regenerating host neurons may be possible. Supported grants from the American Federation for Aging Research (JES) and ADRDA FSA85015 (TJC). JES is an NRSA postdoctoral fellow.

149.17

REGULATION OF NEUROTRANSMITTER EXPRESSION IN CULTURED SYMPATHETIC NEURONS BY A MEMBRANE ASSOCIATED FACTOR. V. Wong & J.A. Kessler, Depts. Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Contact of sympathetic neurons with other cell membranes regulates neurotransmitter expression. Treatment of cultured sympathetic neurons with membranes isolated from sympathetic ganglia or from spinal cord stimulates levels of choline acetyltransferase (CAT) and induces expression of the peptide neurotransmitter substance P (SP). A 29kDa membrane associated neurotransmitter stimulating (MANS) factor which reproduces the effects of neuronal contact with membranes has been solubilized and partially purified from adult rat spinal cord membranes (Wong & Kessler, PNAS 84:8726, 1987). The onset of action of MANS was rapid; treatment of freshly plated cells with MANS resulted in a 26-fold increase in CAT activity within 48 hours. Older cultures responded less rapidly and less intensely. Treatment with MANS induced levels of preprotachykinin mRNA (PPT-mRNA), which were undetectable in control cultures, indicating that pretranslational mechanisms mediate effects of the factor. Treatment with rat fibroblast conditioned medium (RFCM) in addition to saturating levels of MANS resulted in further increases in CAT activity, suggesting that soluble factors in RFCM and MANS act through different mechanisms. Spinal cord cholinergic neurons were also responsive to the factor (see Lombard-Golly, Wong, & Kessler; this volume). To help further characterize the MANS molecule, monoclonal antibodies to the partially purified factor were generated. Six clones have been identified which produced antibodies that partially blocked the cholinergic-promoting actions of the factor. Further efforts will be directed towards determining whether these antibodies are specific for the MANS factor. In toto, our observations suggest that MANS mediates the stimulatory effects of cell-cell contact on cholinergic and peptidergic expression by sympathetic neurons.

149.18

REGULATION OF CHOLINERGIC SPINAL CORD NEURONS IN CULTURE BY A MEMBRANE FACTOR WHICH MEDIATES EFFECTS OF CELL-CELL CONTACT. D. Lombard-Golly*, V. Wong and J. A. Kessler, Depts of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Neuronal contact with other cell membranes plays an important role in regulating neuronal growth and development. We have been examining the role of cell-cell contact in the development of cholinergic spinal cord neurons. Levels of the cholinergic biosynthetic enzyme, choline acetyltransferase (CAT), were stimulated by increasing cell density in cultures of embryonic rat spinal cord. Our laboratory has recently solubilized and partially purified a 29 KDa molecule (MANS) from adult rat spinal cord membranes which reproduces the stimulatory effects of cell-cell contact (Wong and Kessler,P.N.A.S. 84: 8726, 1987). Treatment of low density cultures with MANS on day 3 in vitro increased CAT activity to levels comparable to those in higher density cultures. MANS treatment of 3 day old cultures had progressively less effect with increasing cell density. Freshly plated cells were more responsive to the factor than the older cultures. MANS appeared to stimulate cholinergic differentiation rather than survival of cholinergic neurons although effects on survival could not be excluded. Effects of the factor were examined in ventral and mediodorsal spinal cord cultured separately. In both types of culture, the treatment of freshly plated cells increased CAT activity after 1.5 days in culture. However, effects on the mediodorsal cultures were transient and levels of CAT activity in treated and control cultures did not differ significantly after 14 days in vitro. By contrast, effects of treatment of freshly plated ventral cultures persisted with time in culture; after 3 weeks CAT activity in treated cultures (15.64 nmol./mg prot./hr) was about 2.5 times the level in controls (6.69 nmol./mg prot./hr). Thus ventral (motor neuron enriched) cholinergic spinal neurons are more responsive to the factor than mediodorsal (autonomic neuron enriched) populations. Our observations suggest that development of cholinergic spinal cord neurons may be regulated by cell-cell contact and that the MANS factor may mediate the stimulatory effects of cell-cell contact on cholinergic expression.

149.19

MEMBRANE ASSOCIATED SURVIVAL FACTOR FOR CULTURED CILIARY GANGLION NEURONS. J.B. Tuttle and D.J. Creedon, Dept of Physiol. and Neurosci., Univ. of Virginia Sch. of Med., Charlottesvl., VA, 22908.

Survival of avian ciliary ganglion neurons in culture depends upon an exogenous supply of trophic factor(s). Skeletal muscle, one of the normal ganglionic synaptic targets, is a well-documented provider of survival-promoting activity, yet the molecular basis of this activity has not been thoroughly investigated. To test the source of skeletal muscle support, dissociated neurons were plated into microwells containing either: medium conditioned by myotubes; membrane remnants of osmotically lysed myotubes; or, membrane remnants and conditioned medium. Neuronal counts after one, two, five, and seven days revealed that partial neuronal survival is supported by both muscle conditioned medium and the membrane remnants of cultured myotubes. Together, these two sources furnish trophic activity equivalent to live myotubes. The results suggest that both soluble and membrane-associated trophic factor(s) are involved in neuronal survival in vitro, and they draw attention to membrane contact phenomena as important events in neuronal trophic signaling. Because the membrane-associated activity can be recovered from a high-speed supernatant, comparison of this with known purified soluble factors will reveal if this form is a novel trophic factor or a novel mode of trophic exchange. Supported by the Virginia Affiliate of the American Heart Assoc. and NIH 5 T32 HL07284.

149.20

EXPRESSION OF NGF BY PERMANENT CELL LINES DERIVED FROM POSTNATAL HIPPOCAMPUS. H.J. Lee, D.N. Hammond, T.H. Large§, and B.H. Wainer. Comm. on Neurobiology, The University of Chicago, Chicago, IL 60637 and §Department of Physiology and Howard Hughes Medical Institute, Univ. of California, San Fransisco, CA 94143.

In the central nervous system, nerve growth factor (NGF) is produced by hippocampal cells and has neurotrophic effects on septal neurons, the primary source of cholinergic innervation to the hippocampal region. To examine NGF expression by hippocampal cells in an isolated system, postnatal day 21 mouse hippocampal cells have been immortalized through somatic cell hybridization to murine N18TG2 neuroblastoma cells as previously reported (Lee, H.J. et al., *Soc. for Neurosci. Abst.* 13: 701, 1987). Postnatal day 21 was chosen to maximize the probability of deriving an NGF-producing cell line since rat NGF protein and mRNA levels reach a developmental peak at this time.

We now report that several hippocampal cell line subclones synthesize significant quantities of NGF. A sensitive 2-site ELISA for NGF, modified after Weskamp and Otten (*J. Neurochem.*, 48: 1179, 1987), demonstrates that cell extracts of these lines contain over 7,000 fg NGF/mg total protein when cells are grown to 90% confluency in 10% fetal calf serum in DMEM. This level of NGF content is comparable to reported values of ~1,300 fg NGF/mg wet weight for adult rat hippocampal tissue. Using a rat probe for NGF receptor mRNA, Northern blot analysis of the parent lines displays low levels of binding.

These NGF-producing hippocampal lines display a variety of morphologies: some extend mutiple processes while others are round and relatively undifferentiated in appearance. Their hybrid nature has been documented by isoenzyme and karyotype analysis. These hybrid hippocampal cell lines may provide a useful tool to elucidate the mechanisms underlying hippocampal NGF expression and may provide a source of novel, hippocampally derived trophic factors that affect septal neurons. Supported by NIH grants 5-T32HD070009, NS 01244, and NS 25787; grants from the Alzheimer Disease and Related Disorders Association, the American Philosophical Society, and the French Foundation; and the Illinois Department of Public Health.

149.21

MEDIUM CONDITIONED BY A HIPPOCAMPAL CELL LINE INCREASES CHOLINE ACETYLTRANSFERASE ACTIVITY OF SEPTAL CELLS IN SERUM-FREE CULTURE. D.N. Hammond, H.J. Lee, B.H. Wainer, and A. Heller. University of Chicago, Chicago, IL 60637.

Target-derived cell lines may serve as useful sources of trophic factors which influence normal projecting neurons. A series of cell lines has been derived from murine hippocampus using polyethylene glycol-mediated fusion with neuroblastoma cells. Using a serum-free culture system, medium conditioned by these hippocampal cell lines has been examined for effects on the choline acetyltransferase (ChAT) activity of cells of the septal region, the major source of cholinergic innervation of the hippocampus.

Embryonic mouse septal cells are dissected and dissociated. The cells are suspended in modified N2 medium (after Bottenstein and Sato), and plated on poly-lysine-coated microtiter plates. At the conclusion of time in culture, ChAT activity is assayed directly in culture wells using a modification of the method of Fonnum. Under these conditions, ChAT activity increases over time in culture, with a peak at 7 - 10 days. The effect of NGF (100 ng/ml) on primary septal cells has also been studied using this bioassay system. ChAT activity increases by 2- to 3-fold when NGF is added to the medium. When serum-free medium conditioned by one of our hippocampal cell lines (HN10) is added, there is a 2- to 3-fold increase in ChAT activity compared to controls. These studies indicate that medium conditioned by a hippocampal cell line contains factor(s) which increase the ChAT activity of murine septal cells in serum-free culture. (Supported by grants NS01244, NS25787, and HD070009; grants from the American Philosophical Society, French Foundation, Alzheimer's Disease and Related Disorders Association; and the Illinois Department of Public Health.)

149.22

DOES NERVE GROWTH FACTOR (NGF) ENHANCE SURVIVAL OF DEVELOPING CENTRAL CHOLINERGIC NEURONS? B.H. Wainer[a], S.D. Price[a*], S.G. Nelson[a*], and W.C. Mobley[b]. The University of Chicago[a], Chicago, Illinois; 60637, and The University of California, San Francisco, 94143.[b]

Previous work in this laboratory has demonstrated increased numbers of septal cholinergic cells in the presence of target (hippocampal), but not non-target (cerebellar) cells in reaggregate co-cultures (Hsiang et al. Neuroscience.21: 333-343, '87). Further electron microscopic (EM) experiments revealed that the hippocampal target cells enhanced cholinergic cell survival; ie. in the presence of non-target cerebellar cells, large numbers of degenerating acetylcholinesterase (AChE)-positive cells were observed (Wainer et al. Soc. for Neurosci. Abs. 12: 586, '86). In other studies, addition of NGF to septal reaggregate cultures resulted in increased numbers of AChE-positive cells (Wainer et al. Neurosci. Lett. 26: S17, '86).

The present experiments were designed to determine if NGF actually increases the survival of cholinergic neurons. Septal reaggregates, prepared from E15 mouse embryos, were cultured for either 15 or 21 days and either in the presence or absence of 10ng/ml NGF. The reaggregates were then processed for AChE histochemistry and EM. AChE-positive neurons were identified by electron dense reaction product associated with the nuclear membrane and endoplasmic reticulum. The mean number of healthy cells in samples of 15-day untreated aggregates was 31 as compared to 43 for NGF-treated cultures. In the 21-day untreated cultures the mean number of healthy cells was 33 as compared to 78 in the NGF-treated cultures. At both time points the numbers of AChE-positive cells undergoing degeneration were not significantly different with respect to the presence or absence of NGF in the respective cultures. This data suggests that NGF treatment increases the numbers of AChE-positive cells observed, by enhancing expression of AChE. No evidence for an actual cell survival effect was obtained. Supported by NS 25787, the Alzheimer Disease and Related Disorders Assoc., and the Brain Res. Fndtn. of the Univ. of Chicago.

NEURONAL DEATH I

150.1

LONG-TERM NUCLEUS BASALIS LESIONS INDUCE NEURONAL LOSS OR ATROPHY IN NEOCORTEX, AMYGDALA, AND ENTORHINAL CORTEX. G.W. Arendash, J. Mazziotta*, V. Dillela*, S. Saba*, P. Mouton*, V. Panzarino*, and R. Randhawa*. Dept. of Biology, University of South Florida, Tampa, FL 33620

We have previously reported a marked neuronal loss within the fronto-parietal cortex long-term (14 months) after nucleus basalis magnocellularis (nBM) lesioning in the rat (Science 238:952, 1987). In order to quantify the time-course of this neocortical cell loss, as well as to quantify neuronal changes in other brain areas we know to be affected by nBM lesions, adult rats were infused unilaterally or bilaterally with ibotenic acid into the nBM and sacrificed 3 to 22 months later. Significant neuronal losses in fronto-parietal cortex were first evident at 5 months after lesioning and became progressively more severe (often 30% or higher) at time points thereafter. Through 14 months after unilateral lesions, no decrease in neuronal density was observed in the basolateral nucleus of the amygdala (BLA) or in layers 2 and 3 of the entorhinal cortex. However, a significant 36% decrease in mean neuronal area occurred within the BLA by this time point. Also, significant neuronal atrophy within entorhinal cortex layer 2 was present by 5 months following unilateral lesions; this entorhinal atrophy was more severe at later post-lesion time points. The progressive, protracted nature of these degenerative changes suggest a mechanism involving anterograde transneuronal degeneration, most probably initiated by a lesion-induced loss of nBM cholinergic neurons.

150.2

DISAPEARANCE OF TRANSMITTER-ASSOCIATED ENZYME STAINING DOES NOT CORRELATE WITH DEATH OF AXOTOMIZED CHOLINERGIC NEURONS B.E. Lams*, O. Isacson, M.V. Sofroniew. Department of Anatomy, University of Cambridge, England

To assess the validity of using loss of transmitter-associated enzyme staining to document the death of injured cholinergic neurons, we have compared the disappearance of staining for choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) with the death or survival of cholinergic neurons following axotomy, as documented by other means. Neurons in the vagal and hypoglossal nuclei were examined because they can unambiguously be identified using Nissl stains or retrogradely transported dyes which remain intracellularly in live cells for many weeks following transport. At 7 d or 28 d post axotomy, staining for ChAT or AChE had disappeared from >95% or >70% of vagal neurons respectively and from >50% or <15% of hypoglossal neurons respectively. In contrast, no significant losses of either vagal or hypoglossal neurons were found at 7 d or 28 d post axotomy using Nissl stains or detection of True Blue which had been transported retrogradely prior to axotomy. Longer survival times and similar studies with basal forebrain cholinergic neurons are currently under investigation. These findings show that intracellular levels of the transmitter-associated enzymes ChAT and AChE can be regulated independently of neuronal survival following axotomy, and that absence of ChAT or AChE staining is not an absolute indicator of the death of cholinergic neurons.

150.3

Numerical reduction and proliferation during development of frog trochlear motoneurons

R. Sonntag* and B. Fritzsch, Fac. Biol., Univ. Bielefeld, 4800 Bielefeld, FRG.

During vertebrate development, the proliferation of motoneurons usually ceases before a period of cell death is believed to achieve a proportional, quantitative relation between pre- and postsynaptic elements (Oppenheim, R.W., TINS 8:487, 1985). Previous studies of the trochlear motoneurons of Xenopus laevis revealed a significant decrease in number between st 42 and st 46 (Sonntag, R. & Fritzsch, B., Neurosci. Lett. 77:143, 1987); the number of muscle fibers of the sole target muscle remains constant throughout these stages. Subsequently, the number of motoneurons, nerve fibers, muscle fibers and neuromuscular endplates increases throughout larval life. ^{3}H-Thymidine injections at the onset of the phase of numerical reduction result in 25% ^{3}H-Thymidine labelled trochlear motoneurons, as identified by retrograde HRP-filling. Single motoneurons show double labelling even after ^{3}H-Thymidine injection at st 49, i.e. long after the initial numerical reduction. These data show that a continuous motoneuron proliferation is overlaid by an early phase of numerical reduction in the frog trochlear system. - This work was supported by DFG, SFB 223.

150.4

VASOACTIVE INTESTINAL PEPTIDE VIA cAMP RESCUES MAMMALIAN RETINAL GANGLION CELLS FROM TETRODOTOXIN-INDUCED DEATH. Peter K. Kaiser* and Stuart A. Lipton (SPON: A. Ganser). Dept. of Neurol., Children's Hosp. & Harvard Med. Sch., Boston, MA.

Tetrodotoxin (TTX, 1 μM) causes death in 50% of rat retinal ganglion cells (RGCs) *in vitro* during a critical period of development [Lipton, *PNAS* 1986;83:9774]. Since natural cell death *in vivo* occurs in neurons of similar type and age, and in the same proportion as that produced by TTX in culture, it is tempting to speculate that electrical activity is involved in the mechanism of natural cell death. Conditioned medium prevents TTX-induced death [Lipton, *ibid*]. Thus, a critical element for survival may represent a trophic factor related to the level of electrical activity. Here, we show in retinal cultures from 1 week-old rat pups that forskolin (100 μM) or 8-bromo:cAMP can protect against the lethal effect of TTX. cAMP is normally present in the cultures, and TTX decreases cAMP. Vasoactive intestinal peptide (VIP, 10 nM-1μM), found in some amacrine cells that synapse on RGCs, prevents *(i)* cAMP from falling, and *(ii)* the death of RGCs in the presence of TTX. The action of VIP appears specific since the related peptides PHI-27, secretin, and VIP_{10-28} are ineffective. Taken together with work on spinal cord cultures [Brenneman et al., *PNAS* 1986;83:1159], our results suggest that VIP, mediated through cAMP, has a specific trophic effect on central neurons.

150.5

THE EFFECT OF A SMALLER TARGET MUSCLE ON MOTOR NEURON DEATH DURING DEVELOPMENT. G.S. Sohal, L.R. Campbell*, S.D. Stoney, Jr. and T. Arumugam*. Med. Col. of Georgia, Augusta, GA 30912.

About half of the motor neurons produced by many neural centers die during normal development. We tested the hypothesis that target size determines the number of surviving neurons by forcing the larger trochlear nucleus (TN) of a duck to innervate the smaller superior oblique muscle (SOM) of a quail and determining the number of surviving neurons. Chimeras were produced by substituting the embryonic forebrain of a 50 hr old duck with that of a 40 hr old quail. The quail origin of the SOM was confirmed by Feulgen staining which identified the characteristic nucleoli of the quail. The smaller size of the target was confirmed by determination of myofiber number and motor endplate counts. Innervation of the target by appropriate neurons was confirmed by injection of HRP into the SOM and identification of reaction product in the cell soma of all trochlear neurons. Electrophysiologic studies demonstrated functional afferent and neuromuscular connections. The hypothesis was tested by counting the number of surviving trochlear neurons on day 20. The number of motor neurons was found not to be significantly different from those of duck and to significantly exceed those in quail suggesting that in the TN the number of surviving neurons is not determined by target size. Supported by NIH grants HD17800 and 18280.

150.6

THE PROTECTION OF SEPTAL AREA CELLS AFTER SECTION OF THE FORNIX BY NIMODIPINE

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Damage to the fimbria-fornix induces the death of cells in the medial septal complex. The death of such cells is not, however, a necessary consequence of the lesion since the direct application of nerve growth factor (NGF) to the septal area preserves many cells in this area. A calcium "overloading" of mitochondrial membranes may be involved in the pathophysiology of the cell death. In the brain nimodipine is an antagonist of "L" calcium channels and it has been shown to protect both cardiac cell and neurons from ischemia. In this study, we examined the effect of nimodipine on septal area cells after transection of fimbria-fornix. Complete unilateral fimbria-fornix transections were made in adult male rats. Immediately afterwards, the animals were injected with nimodipine (20 µg/rat) i.p. in a PEG 400 vehicle. Control animals received only the vehicle. The injections were given daily for next 7 days. At different times after surgery, the brains of the rats, especially the medial septal area were examined using histochemical staining for of acetycholinesterase (AChE) or Cresyl Violet.

One day following the transection no decrease in the number of cells was observed in the medial septal area. A substantial loss of both AChE-stained cells and Nissl-stained cells was noted at 14 days. The loss of AChE stained cells was 52% in the medial septal area of animals receiving the vehicle, but there was little change (-4%) in the drug- treated rats. In the Nissl stained sections of lesioned controls, the septal cells were decreased 21%, while in the drug treated animals, the loss was only 2%. At 7 days, the effect of nimodipine on the preservation of septal cells was not as great as at 14 days.

150.7

DIPYRIDAMOLE AND BRAIN DIALYSATE ADENOSINE CONCENTRATION DURING CEREBRAL ISCHEMIA IN NEONATAL PIGLETS.

T. S. Park*, R.Rubio* and R.M.Berne*, (SPON:J.A.Jane).
Dept. of Neurosurgery & Physiology, Univ of Virginia Sch. of Med., Charlottesville, VA 22908

We have demonstrated in neonatal piglets that hypoxia and seizures elevate brain interstitial fluid adenosine (ISF ADO). While dipyridamole (DP) inhibits transport of adenosine across the myocardial cells and elevates myocardial ISF ADO, its effects on *in vivo* brain ISF ADO under control and ischemia in the neonate are unknown. In this study, we sampled ISF ADO of ketamine-anesthetized piglets (<5 days of age) using the brain dialysis technique. Dialysis probes were placed in the bilateral frontal cortex; artificial CSF with or without DP (10^{-4}M) was infused through the probes on each side. 5-min cerebral ischemia was induced by clamping the subclavian and brachiocephalic arteries via a thoracotomy. Dialysate samples were collected every 5 min.

Results	Dialysate s DP	Dialysate c DP
control	0.20±0.03	0.45±0.10†
ischemia	0.96±0.23*	1.79±0.89*
p ischemia1	3.05±0.72*	3.58±0.67*
p ischemia2	0.65±0.14	2.04±0.56*†
p ischemia3	0.22±0.04	0.76±0.25*

Values are means±SE of dialysate ADO (µM). *=p<0.01 vs control; †=p <0.05 s DP vs c DP

These data indicate that piglet ISF ADO rises during and immediately after cerebral ischemia; DP increases resting ISF ADO and the period of elevated ISF ADO following ischemia via its *in vivo* inhibition of transport of adenosine. Supported by NS009240 & NS21045.

150.8

AN OVERVIEW OF THE PATTERN, MORPHOLOGY, AND POSSIBLE MECHANISMS OF CELL DEATH IN THE NEURAL TUBE OF 11-DAY CONTROL RAT EMBRYOS AND COMPARABLE EMBRYOS EXPOSED TO DEFICIENCIES OF ZINC, FOLIC ACID, VITAMIN E, AND TO NICOTINE AND ALCOHOL. R.S. Tuisl, I. Dreosti, I. Record, M. Joschko, A. Harding and J. Bremert. Dept. of Anatomy, University of Adelaide, and CSIRO, Adelaide, Box 498 G.P.O. S.A. 5001.

In the past decade, as part of a multidisciplinary study, we have examined the effect of maternal exposure to agents on the developing neural tube in a large number of 11-day control and experimental rat embryos. Three major observations are summarised. Firstly, in the control embryos, the incidence of cell death in the recently closed portion of the neural tube ranged from 0.26 to 0.42%. The details of cell death were indistinguishable from those in experimental animals. Secondly, the incidence of cell death was significantly higher than in the controls when a single agent was used. Thirdly, when zinc deficiency was combined with another agent the incidence of cell death increased markedly. For example, when zinc and vitamin E deficiencies were superimposed, TEM showed extensive cell death in both mesenchymal and neural tube tissues. SEM revealed collapse of surface features of embryos and open neural tubes. In yet another experiment there was a three-fold increase in the incident of cell death and malformation when zinc deficiency was combined with folic acid deficiency, than when either of the two agents was applied alone. There was evidence that cell death occurred throughout the neural tube and extended from those cells lying near the lumen to those on the mesenchymal border of the tube. In the second example the mechanism of cell death is probably synergistic rather than additive.

150.9

MORPHOLOGICAL AND IONIC CHANGES IN IRREVERSIBLE CEREBRAL ISCHEMIA: EFFECTS OF GANGLIOSIDE PRE- AND POST-TREATMENT. A. Gorio, A.M. DI GIULIO, V. DE CRESCITO*, W. YOUNG. Inst. Pharmacol. Sci., Univ. of Milano, Via Balzaretti 9, Milano, Italy, and Dept. Neurosurg., New York Univ. Med. Center, New YorK, N Y

Permanent middle cerebral artery occlusion (MCAo) caused morphological and ionic changes which were monitored up to 7 days post-lesioning. Ganglioside daily treatment 30 mg/kg was performed either beginning 1 or 3 days prior or immediately after MCAo up to sacrifice. The results show gradual Na loading and K loss with maximum between 6-24 hours. Ca gradually accumulates reaching maximum level at 7 days. Ganglioside treatment failed to protect from early ionic changes, but remarkably blocked the late Ca loading occurring between 1 and 7 days. The morphology of the interzone between ischemic dying tissue showed an intense axonal outgrowth.

150.10

AFFERENT CONTROL OF NEURONAL SURVIVAL IN THE DEVELOPING BRAIN. R. Linden and A.S. Renteria*, Inst. de Biofisica UFRJ, Rio de Janeiro, Brazil

Developmental neuron death is classically attributed to limited target space, but it has been shown that developing afferents may control neuron death. We tested the hypothesis that the afferents may determine the number of target neurons. The model was the direct projection from the rat superior colliculus (SC) to the middle division of the ipsilateral parabigeminal nucleus (Pbm). Partial deletions of the SC were obtained in newborn rats by aspiration or contralateral enucleation. The deafferented Pbm was quantitatively analyzed after the period of natural cell death. The number of neurons surviving in Pbm was linearly related to the number of neurons remaining in the SC. Lesions limited to the caudal third of SC produced no cell loss in Pbm, consistent with the lack of a tectal projection therefrom. Equivalent cell loss distributed across SC after eye removal, however, reduced the number of neurons in Pbm by predictable amounts. Thus, the number of neurons may be determined by their afferents. The results support our hypothesis of a major role of afferents in the control of quantitative matching in the developing brain. (CNPq, FINEP, CEPG-UFRJ)

150.11

LENGTHY ADMINISTRATION OF LOW-PROTEIN DIET TO ADULT RATS INDUCES CELL LOSS IN THE HIPPOCAMPAL FORMATION BUT NOT IN THE MEDIAL PREFRONTAL CORTEX. M.M.Paula-Barbosa, J.P.Andrade*,F.P.Azevedo*, M.D.Madeira* and M.C.Alves*, Dept. of Anatomy, Oporto Medical School, Portugal.

Reduction in the number of neurons have been described in adult rats submitted to perinatal protein deprivation. Conversely, it was accepted that the mature CNS was resistant to this condition. Yet, we have demonstrated that 2--month old rats fed with a low-protein diet (8% casein-ICN) for 6, 12 and 18 months showed marked cerebellar granule cell loss. Thus, we decided to extend these investigations to the hippocampal formation (HF) and to the prelimbic (PL) area of the prefrontal medial cortex and compare the results with those obtained in aged-matched controls (27% casein).

Groups of 6 rats treated as above refered were used. The numerical densities of the HF granule cells and CA3 pyramidal cells (6,12 and 18 months) and the pyramidal cells of layer III of the PL area (6 and 18 months) were determined using the unbiased disector method applied to epon embedded serial semithin sections. In the HF we found a significant decrease of both cell types after 6 months of treatment, whereas in the PL area no significant cell reduction was detected. We concluded that low-protein diet-induced neuronal loss is a marked and widespread phenomenon, although displaying regional selectivity.

150.12

CYCLOHEXIMIDE INHIBITS ECDYSTEROID-REGULATED NEURONAL DEATH IN THE MOTH MANDUCA SEXTA. S.E. Fahrbach and J.W. Truman.Dept. of Entomology, Univ. of Illinois, Urbana, IL 61801 and Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

Half of the neurons in the unfused abdominal ganglia of the moth Manduca sexta die during the first 3 days of adult life. This death is a response to the decline in blood ecdysteroids that occurs at the end of metamorphosis. We have asked whether the response to the withdrawal of the hormone requires new protein synthesis by studying the effects of treatment with cycloheximide.

The stage of degeneration of the D-IV neurons was used as an indicator of neuronal death. Moths were given single injections of cycloheximide (200 ug) or saline vehicle at 2 hr intervals from the time of adult emergence until after the onset of degeneration. Treatment with cycloheximide before the steroid hormone commitment point (10 hr before the onset of degeneration) or after the neurons had begun to die had no effect on the process of degeneration. However, treatment with cycloheximide after the steroid hormone commitment point and before the start of degeneration significantly delayed the onset of death. This treatment was effective in delaying death even when given as late as 1-2 hr before the D-IV neurons initiate degeneration. An identical pattern of results was seen when the effect of cycloheximide on the degeneration of other identified motoneurons (MN-2, MN-12) was studied.

150.13

MK-801 PREVENTS HYPOXIC-ISCHEMIC HIPPOCAMPAL NEURODEGENERATION IN NEONATAL RATS. L.M. Ford, M.H. Fogelson,* A.B. Norman and P.R. Sanberg. Lab. of Behavioral Neuroscience, Dept. of Psychiatry, College of Medicine and Dept. of Ped. Neurology, Children's Hospital, University of Cincinnati, Cincinnati, Ohio 45267.

Treatment with the glutamate N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (Merck, Sharp, Dohme) reduces hypoxic-ischemic (HI) neurodegeneration. We evaluated the ability of MK-801 to prevent hypoxic-ischemic hippocampal neurodegeneration in neonatal rats, thus resulting in improved neurologic/behavioral outcomes. Seven day old rats with and without MK-801 pretreatment were subjected to unilateral carotid ligation followed by hypoxia. At age 30 days, spontaneous alternation behavior (SAB) to evaluate hippocampal learning and memory functions were measured; mean alternation scores were: normal controls (N=14) 77%, HI/MK-801 (N=29) 74%, HI/saline (N=28) 64%. The HI/saline group demonstrated significant impairment in SAB vs. controls and HI/MK-801 groups ($p<0.01$). Histological studies revealed pyrimidal neuronal cell loss in the hippocampal CA1 region in HI/saline group, while MK-801 treatment prevented cell loss ($p<0.01$). While hippocampal lesioned rats showed defective learning and memory, MK-801 treatment was neuroprotective. Pharmacologic blockade of the NMDA receptor prevents neonatal asphyxial brain damage.

150.14

REDUCTION OF NATURALLY OCCURRING NEURONAL DEATH IN VIVO BY THE INHIBITION OF PROTEIN AND RNA SYNTHESIS. R.W. Oppenheim and D.M. Prevette.* Anatomy Dept., Wake Forest Univ., Winston-Salem, NC 27103.

Recent in vitro studies of chick sympathetic ganglion cells and in vivo studies in invertebrates suggest that occurrence of embryonic cell death requires gene expression and active protein and RNA synthesis. These observations indicate that cell death is a particular type of differentiation or cell fate and thus may be regulated by the same general mechanisms involved in the fate of all developing cells. To determine whether active protein or RNA synthesis may be necessary for the occurrence of neuronal death in vivo, we treated chick embryos with inhibitors of protein and RNA synthesis and examined their effects on the degeneration of motoneurons (MN) and DRG cells. Embryos were treated with puromycin, actinomycin-D and cycloheximide by administration onto the chorioallantoic membrane once every 2-3 hours over a 9-12 hour period on embryonic day 8. At the end of the treatment, embryos were killed, staged and processed histologically. Pyknotic motoneurons were counted throughout the lumbar region and dying DRG cells were counted in the third lumbar (L3) DRG. Based on their morphological stage as well as on other criteria (e.g., neuronal size) embryos developed normally following treatment with the above agents. At the appropriate dose, all of the agents significantly decreased the number of pyknotic cells. For example, cycloheximide (1 µg) reduced the total number of dying MN from 337 ± 24 to 53 ± 8 ($p<0.001$) and the number of dying L3 DRG cells from 463 ± 91 to 63 ± 26 ($p<0.01$). In the future it should be possible to use the techniques of molecular biology to identify the specific genes and gene products responsible for the determination of the cell death fate in developing neurons. Supported by NS 20402.

150.15

ROLE OF VOLTAGE-ACTIVATED CALCIUM CHANNELS IN THE PROMOTION OF NEURONAL SURVIVAL BY POTASSIUM. F. Collins. Synergen. 1885 33rd St., Boulder, CO 80301.

Elevated concentrations of potassium in the culture medium, sufficient to depolarize cells, have been reported to promote the survival of embryonic nerve cells in vitro. One consequence of potassium-induced depolarization would be the opening of voltage-activated calcium channels. To determine whether the activation of such channels is involved in the survival-promoting effect of elevated potassium, we have used dihydropyridine antagonists and agonists of voltage-activated L-type calcium channels.

We have observed that the survival-promoting effect of potassium on chick embryo ciliary ganglionic nerve cells is blocked by the calcium channel antagonists nitrendipine and PN200-110. The calcium channel agonist BAY K 8644, in contrast, strongly potentiates the survival-promoting effect of elevated potassium. These results indicate that activation by elevated potassium of dihydropyridine-sensitive, presumed L-type voltage-activated calcium channels is necessary and sufficient to promote neuronal survival.

151.1

Effects of Neuroleptic and Reinforcement Manipulations on Initiation Latencies in a Food-Rewarded Task. E. O'S. Hammond and A. Ettenberg. Department of Psychology, University of California, Santa Barbara, CA 93106.

Controversy exists concerning the observation of neuroleptic-induced deficits in response initiation. For some, such deficits represent a motoric incapacitation which might account for some of the behavioral data otherwise thought to support a drug-induced "anhedonia" position. Others have suggested that the major neuroleptic deficit is in the animals' ability to maintain responding and that any increases in response initiation are too small to be of behavioral significance. In the present study, the latencies to contact a food cup were measured following repeated presentations of a tone that predicted reward availability. Reinforcement reductions and haloperidol pretreatments (0.15 & 0.3 mg/kg) produced comparable results in which initiation deficits tended to increase within a session. In contrast, a nembutal-treated "motor" control group produced response latencies that were initially high but decreased over the course of the test session. These results suggest that while neuroleptics do produce deficits in response initiation, the pattern of these deficits is most comparable to that which results from a reduction in actual reward magnitude.

151.2

PARADOXICAL EFFECTS OF HALOPERIDOL ADMINISTERED DURING THE EXTINCTION PHASE OF AN OPERANT RUNWAY TASK. J.C. Horvitz and A. Ettenberg. Dept. of Psychology, University of California, Santa Barbara, CA 93106.

Neuroleptic drugs typically produce deficits in rates of operant responding. In investigations of such deficits, a paradoxical effect of haloperidol (HAL) was revealed. Male albino rats were trained to traverse a straight-arm runway for food reward during eight single daily acquisition trials. Rats then received daily non-rewarded (extinction) trials over the next four days. On the fourth extinction trial, animals traversed the runway under the influence of either 0, 0.15 or 0.30 mg/kg of HAL, i.p. Surprisingly, the group receiving the high dose of HAL (0.30 mg/kg) showed dramatic and reliable increases in running speed relative to animals receiving either the vehicle (.002 M lactic acid) or the lower dose of the drug. Having twice replicated this phenomenon, additional experiments were conducted to test incentive motivational, attentional, and motoric hypotheses which might account for this neuroleptic effect.

151.3

Effects of haloperidol on conditioned place preferences produced by electrical stimulation of medial prefrontal cortex. C.L. Duvauchelle* and A. Ettenberg. Dept. of Psychology, University of California, Santa Barbara, CA 93106

Although rats will work to obtain electrical stimulation of the prefrontal cortex, the precise neurochemical substrates mediating the rewarding effects of such stimulation remain unclear. In the present study, a Conditioned Place Preference procedure (see Duvauchelle & Ettenberg, Soc Neurosci Abstr, 1987; 13:1323) was used to investigate the effects of dopamine antagonist application on rewarding prefrontal stimulation.

Rats exhibited strong preferences for the side of a two-compartment test apparatus in which they experienced sessions of experimenter-administered 0.5 sec trains of PFC sine-wave 60Hz stimulation. Pretreatment with the dopamine antagonist drug, haloperidol (0.15 & 0.3 mg/kg IP) resulted in a dose-dependent reduction in the magnitude of observed place preferences. Preference tests were conducted 24 hours after drug-conditioning trials and, hence, were not subject to motoric or other nonspecific actions of the neuroleptic treatments. These results are consistent with the view that dopamine neurotransmission is required for the demonstration of brain stimulation reward in the medial prefrontal cortex.

151.4

DOPAMINE AUTORECEPTOR AGONIST PROPERTIES OF A NOVEL SERIES OF [3-(4-PHENYL-1-PIPERAZINYL)PROPOXY]AROMATIC COMPOUNDS. **L.D. Wise*, H.A. DeWald*, J.J. Jaen*, B.W. Caprathe*, T.A. Pugsley and T.G. Heffner (SPON: F.M. Hershenson). Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105.**

We have reported that 7-[3-(4-phenyl-1-piperazinyl)-propoxy]-2H-1-benzopyran-2-one (PD 116,795) is an orally active dopamine (DA) autoreceptor agonist (Soc. Neurosci. Abst. 13, #128.9, 1988). The unique properties of this agent led us to examine related phenylpiperazines for DA agonist activity. Compounds in which the benzopyranone of PD 116,795 was replaced with indole or benzofuran did not have DA agonist activity as assessed by DA receptor binding and inhibition of GBL-stimulated brain DA synthesis in rats. Replacement with a phenol afforded weak DA agonist activity in biochemical tests and resulted in loss of locomotor inhibition (LMI) in rats after oral administration. Replacement of the benzopyranone with aromatic systems such as aniline, chromone, or chromanone provided agents with biochemical and behavioral profiles comparable to that of PD 116,795. More potent behavioral activity (LMI ED_{50}s of 1-3 mg/kg PO) was seen with benzomorpholinone, carbostyril or quinoline analogs. These results indicate that orally active DA autoreceptor agonists can be obtained by replacement of the benzopyranone of PD 116,795 with related aromatics.

151.5

SPONTANEOUS ACTIVITY OF MIDBRAIN DA NEURONS AFTER ACUTE AND CHRONIC ADMINISTRATION OF THE SELECTIVE D-1 ANTAGONIST, SCH 23390. Jeffrey M. Goldstein and Linda C. Litwin*, Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

We previously reported that SCH 23390 (SCH) potently reversed d-amphetamine suppresion of substantia nigra (A9) and ventral tegmental (A10) DA cell firing. Since activity in this test is correlated with clinical antipsychotic (AP) properties, SCH may be a potential AP. To further explore this possibility, the effects of SCH on the population response of A9 and A10 DA cells were investigated. One hour pretreatment with SCH (0.0125, 0.025, 0.05 mg/kg sc) caused a dose-related increase in the number of spontaneously firing DA cells in both the A9 and A10 areas. There was no evidence of differential effect at any acute dose. Chronic (28 day) treatment with SCH (0.05 mg/kg sc) caused a selective decrease in the number of A10 DA cells which was found to be reversed by a subsequent injection of apomorphine, inferring that the decrease was due to depolarization inactivation. On A9 DA cells, chronic treatment with SCH caused a slight but nonsignificant increase in the number of actively firing cells. The ability of SCH to cause depolarization inactivation of A10 DA cells after chronic administration confirms and extends our previous findings that this agent may have potential AP properties. Moreover, the lack of effect of chronic SCH on A9 DA cells suggests the possibility that this agent may not induce neurological side effects.

151.6

U-65556A: A PROPOSED NOVEL ANTIPSYCHOTIC. N.F.Nichols, P.J.K.D.Schreur, and J. Szmuszkovicz*. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

U-65556A (2,3-dihydro-N,N-dimethyl-1H-phenalen-2-amine monohydrochloride) appears to have both dopamine autoreceptor agonist and postsynaptic antagonist activities. It was tested in Sprague-Dawley rats for its effects on open-field locomotor activity in several protocols (Omnitech Digiscan monitors) and on turning behavior (visual scoring).

In the exploratory activity test, U-65556A showed mixed activity. At 3 mg/kg it had a profile similar to that of autoreceptor agonists (decreased total distance, increased number of movements, modestly increased rest time), and at 30 mg/kg it was similar to postsynaptic antagonists (decreased total distance and greatly increased rest time).

In two other tests, U-65556A showed only postsynaptic dopamine antagonist activity. In rats habituated to the test apparatus, 30 mg/kg significantly antagonized both apomorphine-and amphetamine-induced hyperactivity. It also antagonized amphetamine-induced turning in rats with unilateral substantia nigra (SN) lesions.

U-65556A had no postsynaptic dopamine agonist activity. In habituated rats, it did not stimulate locomotor activity. When given to rats with unilateral SN lesions, it caused no turning activity.

In conclusion, U-65556A shows both autoreceptor agonist activity and postsynaptic antagonist activity. It is active subcutaneously, intraperitoneally, and orally. The oral and intraperitoneal potencies are approximately equal.

151.7

U-65556A, A NEW ATYPICAL ANTIPSYCHOTIC. M.F.Piercey, J.Szmuszkovicz*, W.E.Hoffmann, J.T.Lum, and W.H.Darlington*. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

U-65556A (1H-phanalen-2-amine,2,3-dihydro-N,N-dimethyl, monochloride) and its desmethyl derivative, U-64273A (1H-phenalen-2-amine,2,3-dihydro-N-methyl, monochloride) were evaluated for effects on firing rates of dopaminergic neurons in the substantia nigra pars compacta (SNPC) and on 5-HT neurons in the dorsal raphe (DR) of chloral hydrate-anesthetized rats using standard criteria (Bunney et al., JPET 185:560, 1973; Aghajanian et al., JPET 137:178, 1977). In SNPC, U-65556A and U-64273A reversed amphetamine-induced depressions of firing rates with ED50's of 150 and 2600 ug/kg, respectively. ED50's for haloperidol and clozapine were 7 and 1600 ug/kg, respectively. Haloperidol, but not clozapine, U-65556A or U-64273A increased firing rates of SNPC cells. Compared to amphetamine antagonism potencies, haloperidol and U-64273A were equipotent in reversing depression of SNPC firing by autoreceptor doses of apomorphine (100 ug/kg). Clozapine and U-65556A were weaker in reversing apomorphine (amph antag/apo antag potency ratios of 3.9 and 8.0, respectively), suggesting predelictive effects at postsynaptic sites. The related dihydrophenalene, U-68553B, was a dopamine agonist which depressed SNPC cells. In DR, U-65556A, U-64273A, and U-68553B all depressed firing rates with ED50's of 275, 1450, and 46 ug/kg, respectively. It is concluded that 1) both dopamine agonists and antagonists exist in the dihydrophenalene series, and 2) U-65556A is a novel antipsychotic with clozapine-like properties.

151.8

DISTINCTION OF THE NOVEL ANTIPSYCHOTIC CI-943 FROM DOPAMINE ANTAGONISTS AND DOPAMINE DEPLETING AGENTS. **T.G. Heffner, J.N. Wiley*, A.E. Williams* & T.A. Pugsley. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.**

CI-943 (8-ethyl-7,8-dihydro-1,3,5-trimethyl-1H-imidazo[1,2-c]pyrazolo[3,4-e]pyrimidine) is a novel antipsychotic candidate that is not a brain dopamine antagonist. We reported previously that CI-943 inhibits spontaneous locomotion, apomorphine-induced cage climbing, self- stimulation and conditioned avoidance responding in rodents and primates (Soc. Neuroscience Abst. 13:459-460). Unlike dopamine antagonists, CI-943 augments locomotor stimulation caused by amphetamine in mice and rats. But, CI-943 neither induced stereotyped behavior nor augmented amphetamine-induced stereotypy in rats, and it did not potentiate apomorphine-induced climbing in mice. Unlike reserpine, high doses of CI-943 neither depleted brain dopamine nor potentiated the locomotor stimulant effect of apomorphine in rats. In addition, doses of CI-943 higher than those necessary to reduce locomotor activity reversed reserpine-induced sedation in rats. These results indicate that the preclinical profile of CI-943 differs from that of dopamine antagonists and dopamine depleting drugs. The ability to augment effects of amphetamine and to reverse effects of reserpine suggest that CI-943 may provide efficacy for negative schizophrenic symptoms.

151.9

EFFECTS OF HALOPERIDOL ON STRESS-INDUCED CHANGES IN MESOCORTICAL DOPAMINE NEURONS. David J. Mokler and Tammy Hay*. Dept. Pharmacology, Univ. New England, Biddeford, ME 04005.

The effects of subchronic administration of haloperidol (HAL) on changes in medial prefrontal cortex dopamine neurons following acute or chronic stress were examined. Male Fischer-344 rats were exposed to an acoustic stressor (115 dB) for 2 sec/min for 2 hours for one day or eight days over a period of sixteen days. During the last ten days half of the animals received 0.5 mg/kg HAL s.c. Following the last day of chronic stress or the acute stress session animals were sacrificed. Medial prefrontal cortex was dissected out and analyzed by HPLC with electrochemical detection. Norepinephrine or 5-hydroxytryptamine were unaffected by stress or HAL or the combination. Levels of DA were increased by chronic stress, while dopamine turnover as estimated by the ratio of either of the dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) to dopamine was increased by acute stress and decreased by chronic stress. HAL increased the levels of HVA in control animals and the levels of HVA and DOPAC in acute and chronic stress. This resulted in a further increase in dopamine turnover following acute stress, but a reversal of the decrease in turnover following chronic stress. These data suggest that HAL may reverse the stress-induced changes in mesocortical dopamine metabolism following chronic stress. This may relate to the anti-psychotic efficacy of haloperidol.

151.10

EFFECT OF ATYPICAL AND TYPICAL ANTIPSYCHOTIC DRUGS ON D-1 and D-2 DOPAMINE (DA) RECEPTORS AND SEROTONIN $(5\text{-HT})_2$ RECEPTORS. **S. Matsubara and H.Y. Meltzer Case Western Reserve University School of Medicine Cleveland, Ohio 44106**

The biochemical features which distinguish atypical antipsychotic drugs (AAD) from typical AD (TAD) are unclear. We determined the affinities for D-1 and D-2 sites in striatal membranes using ^{3}H-SCH-23390 and ^{3}H-spiperone, respectively and for the 5-HT_2 binding site in frontal cortex using ^{3}H-ketanserin of 6 AAD (clozapine, fluperlapine, RMI-81582, setoperone, melperone and amperozide) and 7 TAD (haloperidol, chlorpromazine, loxapine, thiothixene, cis-flupentixol, thioridazine and mesoridazine) by log-probit analysis. Among the noteworthy findings were the following: 1) a discriminant function based on D-1, D-2, and 5-HT_2 values distinguished the AAD and the TAD; 2) the D-2 affinity contributed more than the D-1 and 5-HT_2 affinities to this discrimination and also to a stepwise discriminant analysis; 3) D-1 and D-2 affinities of the AAD were significantly lower than the TAD but there was no difference in 5-HT_2 affinities; 4) there was no overlap in 5-HT_2/D-2 ratio, the same was true for 5-HT_1/D-2 ratio with the exception of RMI- 81582. These results agree with Altar et al. (Brain Res Bull 16, 517, 1986) that a low 5-HT_2/D-2 ratio is characteristic of AAD but also highlight the low D-2 and D-1 affinities and a low D-1/5-HT_2 ratio.

151.11

ALPHA-ACID GLYCOPROTEIN IN PSYCHIATRIC INPATIENTS. A. Patel*, R. C. Young, R. Klein*, J. Kream* (SPON: C. A. Shamoian) New York Hospital-Cornell Medical Center, Westchester Division, White Plains, New York 10605.

Alpha-1-acid glycoprotein (AAG) is a major binding protein for psychotropic drugs. However, there is little available data concerning AAG in psychiatric patients. We have begun studies of serum AAG in patients admitted to an acute care psychiatric hospital (N = 55). Their ages ranged from 16 to 94 yrs. The median age was 56 yrs. The sex ratio was 19:36 (M:F). Their predominant diagnoses were affective disorders and schizophrenia. None had evidence of inflammatory disease. Serum AAG was determined by rate nephelometry. Mean serum AAG was 67.8 mg/dl and ranged from 19.9 to 144.0 mg/dl. In female patients, AAG was significantly positively correlated with age ($r = .43$, $p < .01$), but in male patients it was not ($r = -.23$, NS). There was a nonsignificant trend for higher AAG in female patients. AAG was not correlated with body weight. These preliminary data in psychiatric patients are in part consistent with relationships reported in normal subjects.

151.12

α_2-ADRENERGIC ANTAGONISTS AND LIPOPHILIC α_1-ADRERGIC AGONISTS BLOCK THE CLOZAPINE-INDUCED HYPOTHERMIA IN MICE. V.G. Haddox*, and M.K. Menon. Psychopharmacol. Res. Lab. V.A. Med Ctr., Sepulveda, CA 91343, and Dept. Psychiat. UCLA Sch. Med., Los Angeles, CA 90024.

Clozapine produced dose-related hypothermia in male Swiss-Webster (Charles River) mice. This response was promptly blocked by pretreatment (i.p., 10 min before) with the lipophilic α_1-adrenergic agonists St 587 (3 mg/kg) cirazoline (0.3 mg/kg) or SKF 89748 (1 mg/kg). The relative potencies of these three drugs roughly paralleled their α_1-agonistic potencies as determined by other procedures. Sgd 101/75, a lipophobic α_1-agonist did not antagonize clozapine hypothermia. This response to clozapine (6 mg/kg, i.p.) was also completely blocked by pretreatment (10 min. before, i.p.) with the α_2-adrenergic antagonists yohimbine (1 mg/kp), idazoxan (1 mg/kg), CH 38083 (5 mg/kg) and SKF 86646 (5 mg/kg). However, their antagonistic effects were more pronounced at 90 min after clozapine than at 30 min. It indicated that their effectivenesses were dependent upon the accumulation of NE in the brain areas resulting from α_2-adrenergic blockade. The suitability of clozapine-induced hypothermia as a model for the evaluation of α_1-agonists and α_2-adrenergic antagonists is being investigated. Supported by the Veterans Administration.

151.13

LITHIUM AND MAGNESIUM PREVENT HALOPERIDOL-INDUCED ABNORMAL LICKING PATTERNS. S.J. Lytle and K. M. Kantak. Lab. of Behav. Neurosci., Dept. of Psychol., Boston Univ., Boston, MA. 02215.

Alterations in the control of the oropharyngeal musculature were indirectly observed in mice after chronic neuroleptic administration by recording their licking patterns. Aberrant licking patterns follow a time course parallel to the human condition tardive dyskinesia. The present investigation compares the effectiveness of magnesium (Mg^{2+}) and lithium (Li^{2+}) in preventing this abnormal licking pattern. All groups increase the number of licks over 3 months, with potentiated effects measured with Li^{2+}/hal and Mg^{2}/hal treatments. The number of bouts was increased by haloperidol. The licking pattern as specified by the licks per bout indicates that haloperidol alters the licking pattern after 2 months of treatment which persists into the 3rd month of treatment. Li^{2+}/hal and Mg^{2+}/hal prevent this altered licking pattern, with Li^{2+}/hal and Mg^{2+}/hal producing equally potentiated effects after 2 months. After 3 months, Mg^{2+}/hal continues to prevent an altered licking pattern. Li^{2+}/Hal greatly increased the licks per bout at this time indicating that Mg^{2+}/hal may be more beneficial than Li^{2+}/hal in the long term prevention of TD.

151.14

THE USE OF CONDITIONED AVOIDANCE BEHAVIOUR IN PREDICTING ANTIPSYCHOTIC ACTIVITY. J.A.M. van der Heyden, M.T.M.Tulp* and B.Olivier. Dept of Pharmacology, Duphar B.V., P.O.Box 2, 1380 AA Weesp, The Netherlands.

Several behavioural animal models can be used to predict the antipsychotic activity such as attenuation of apomorphine-induced behaviour and/or inhibition of conditioned avoidance behaviour. The activity of neuroleptics in these models is most likely due to their blockade of DA receptors.

Drugs affecting the 5-HT system have similar effects and can thus be classified as putative antipsychotic drugs. We have studied the effect of several types of drugs affecting the 5-HT system on one-way conditioned avoidance behaviour in rats.

The 5-HT_{1a} specific compounds 8-OH-DPAT and ipsapirone were inactive in our procedure. Buspirone did show an inhibitory effect, which can be atributed to its strong DA receptor blockade. The 5-HT_{1b} agonists TFMPP and RU24969 both inhibited CAR without affecting the ER. The 5-HT precursor 5-HTP and 5-HT releaser fenfluramine did inhibit CAR in contrast to the 5-HT uptake inhibitor fluvoxamine. The 5-HT_2 antagonist ketanserine was inactive, as were the mixed 5-HT_2 / 5-HT_{1c} antagonists ritanserin and mianserin. The 5-HT_3 antagonists GR38032F and MDL72222 showed a no and non-specific effect, respectively. On basis of these data, it can be concluded that drugs stimulating 5-HT receptors inhibit conditioned avoidance behaviour comparable to the neuroleptics.

A recently developed 5-HT agonist, Befiperide also inhibits conditioned avoidance behaviour and strongly inhibits apomorphine-induced stereotypy without blocking the DA receptor. Clinical studies on its antipsychotic efficacy will have to confirm the 5-HT hypothesis on basis of which this drug was developed.

151.15

EVALUATION OF Wy47,384, A GAMMA-CARBOLINE ANTIPSYCHOTIC CANDIDATE, IN A PRIMATE MODEL OF PSYCHOSIS. R.F. Schlemmer, Jr. & J.M. Davis. U. of Illinois at Chicago & Ill. State Psychiatric Inst., Chicago, IL 60612.

Wy47,384, 8-fluoro-2,3,4,5-tetrahydro-2-[3-(3-pyridinyl)-propyl]-1H-pyrido[4,3-b]indole HCl, has been identified as an antipsychotic candidate in rodent screens. This study was designed as a preliminary evaluation of the behavioral effects of Wy47,384 (Wy) in nonhuman primates. Wy was tested alone & in combination with apomorphine (APO) which was used to induce a model psychosis. Four females from a social colony of 5 Stumptail macaques received 4 doses of Wy, 1-8 mg/kg, for 2 days in a cross-over design. Wy was given n.g. at 0700 & 1700 on day 1 & at 0700 on day 2. APO, 1 mg/kg, was given i.m. at 1015. Two 60 min observation sessions were conducted daily at 0900 & 1030. Wy produced a dose-dependent antagonism of APO-induced stereotypy, increased checking, & increased submissive gestures, behaviors which model positive symptoms of psychosis; but Wy failed to reverse social withdrawal, a negative symptom. Wy alone caused an increase in resting. Movement disorders were only noted at the highest dose for less than 20% of the time. This study demonstrates that Wy antagonizes a primate model of psychosis consistent with known antipsychotics. Although the results suggest that Wy may produce sedation & extrapyramidal side effects, these were only pronounced at doses higher than those indicative of antipsychotic activity. (Supported by Wyeth Laboratories).

151.16

EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION ON CLOZAPINE LEVELS IN BLOOD PLASMA AND BRAIN TISSUE. H.F. Villanueva, J.H. Porter, N. Narasimhachari, J.K. Stewart*, B. Landa* and J.L. Wiley. Depts. of Psychology, Psychiatry and Biology, Virginia Commonwealth Univ., Richmond, VA 23284.

With a chronic dosing regimen, tolerance to the rate suppressing effects of clozapine (CLZ) on operant responding develops within 6 to 10 days (Kaempf & Porter, JPET, 243: 437-445, 1987). In order to determine possible mechanisms of this tolerance the present study measured clozapine levels in blood plasma and in frontal cortex and corpus striatum following a single acute 10 mg/kg dose of clozapine (n = 6) or following 10 days of chronic dosing with 10 mg/kg clozapine (n = 5).

HPLC assays revealed that there were no significant differences in mean CLZ levels following the acute dose or the 10 days of chronic dosing in the blood plasma or in brain tissue. However, there were significantly higher levels of CLZ in the frontal cortex as compared to the corpus striatum. These results suggest that the tolerance to CLZ's effects seen in operant studies is due to a pharmacodynamic mechanism rather than a metabolic mechanism.

	Plasma (ng/ml)	Cortex (ng/g)	Striatum (ng/g)
Acute	271.5	13.41	8.25
Chronic	281.2	14.93	9.81

151.17

EFFECTS OF RACEMIC AND ENANTIOMERIC BMY 14802, A POTENTIAL ANTIPSYCHOTIC DRUG, ON NORADRENERGIC LOCUS COERULEUS NEURONAL ACTIVITY. C.P. VanderMaelen and J.P. Braselton.* Bristol-Myers Co., Preclinical CNS Research, 5 Research Parkway, Wallingford, CT 06492.

BMY 14802 is a potential antipsychotic drug which does not bind to dopamine D2 receptors, binds to sigma sites, is active in animal models of antipsychotic efficacy, and shows some similarities to haloperidol in electrophysiological studies of A9 dopamine cells (Matthews et al., JPET, 1986, 239, 124-131; Taylor et al., Soc. Neurosci. Abst., 1985, 11, 114; Taylor & Dekleva, Drug Dev. Res., 1987, 11, 65-70). Haloperidol increases locus coeruleus (LC) neuronal firing (Dinan & Aston-Jones, Brain Res., 1984, 307, 359-362). In this study BMY 14802 and its (+) and (-) enantiomers were administered i.v. to chloral hydrate anesthetized rats, and effects on noradrenergic LC neuronal firing were observed using standard extracellular single-unit recording techniques. Both enantiomers, as well as the racemic mixture of BMY 14802 produced increases in LC neuronal firing. The dose required to increase the firing rate of an LC neuron by 25% (ED_{25}) was estimated to be 0.31 mg/kg, i.v. for (-)-BMY 14802, and 0.55 mg/kg, i.v. for (+)-BMY 14802, while the ED_{25} for racemic BMY 14802 was calculated to be 0.36 mg/kg, i.v. These results are congruent with other data suggesting that BMY 14802 may be an effective antipsychotic drug with reduced propensity for extrapyramidal side effects.

151.18

THE POTENTIAL ANTIPSYCHOTIC BMY 14802 INHIBITS SIGMA BINDING BUT NOT PCP BINDING. Duncan P. Taylor, Jennifer Dekleva,* and Susan H. Behling.* CNS Biology, Bristol-Myers Company, P.O. Box 5100, Wallingford, CT 06492-7660.

BMY 14802 has been identified as a potential antipsychotic agent in behavioral testing (**Soc Neurosci Abstr 11**: 114, 1985). In addition to its low affinity for D-1 and D-2 dopamine receptors *in vitro*, BMY 14802 did not induce catalepsy in rats. We have shown that this agent has affinity for the haloperidol-sensitive site labeled with high affinity by (+)-[^{3}H]NAN and by (+)-[^{3}H]3-PPP (**op.cit. 13**:457, 1987). However, BMY 14802 has low affinity for the site labeled by [^{3}H]TCP. These findings have been confirmed and extended employing [^{3}H]DTG to selectively label sigma sites and [^{3}H]MK-801 to label NMDA-associated PCP sites.These data suggest that if BMY 14802 proves clinically efficacious, its novel mechanism of action will provide a safer alternative to currently available agents for the treatment of schizophrenia.

Agent	[^{3}H]DTG	(+)-[^{3}H]3-PPP	[^{3}H]TCP	[^{3}H]MK-801
Haloperidol	2	1.4	>1,000	18,000
(+)BMY 14802	32	28	>10,000	>100,000
(+)3-PPP	91	24	>10,000	>100,000
(-)BMY 14802	140	310	>10,000	>100,000
PCP	1200	640	34	57

Data are mean K_I (nM) for at least 3 determinations.

152.1

SEROTONERGIC DRUG ACTION AND THE TREATMENT OF OCD. W.K. Goodman, L.H. Price, G.M. Anderson*†, P.L. Delgado, J.M. Palumbo*, G.R. Heninger and D.S. Charney. Depts. of Psychiatry and Lab Medicine†, Yale University School of Medicine, New Haven, CT 06508

The serotonin (5-HT) hypothesis of obsessive compulsive disorder (OCD) has been strengthened by recent studies showing that the potent and selective 5-HT reuptake inhibitor fluvoxamine (FVX) is superior to placebo in treating OCD. To examine the relevance of FVX's effects on 5-HT function to its antiobsessional action in OCD: 1-the efficacy of FVX was compared to the relatively selective norepinephrine (NE) reuptake blocker desipramine (DMI), and 2- CSF levels of metabolites of 5-HT, NE, and dopamine (5-HIAA, MHPG, and HVA, respectively) were measured before (drug-free) and after 6-10 wks of FVX treatment. METHODS:1-DRUG TRIAL. After 1 wk of placebo, OCD outpatients (data available on N=29) were randomized to 8 wks of double-blind FVX or DMI. Raters assessed OCD with the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) and by global improvement ("responder" = "much" or "very much improved"). 2-CSF STUDY. Lumbar CSF was obtained in 12 OCD patients (incl. 5 patients from a different FVX trial) between. 10AM and 12PM. HVA and 5-HIAA were assayed by HPLC with electrochemical (HVA, 5-HIAA) and flourometric (5-HIAA) detection. MHPG was assayed by gas chromatography-mass spectrometry RESULTS: 1-DRUG TRIAL. (Interim analysis of N=29) Only 2/14 (14%) patients treated with DMI were responders; whereas, 8/15 (53%) patients treated with FVX were responders ($p<.05$, Fisher's Exact). 2-CSF STUDY. Pre- v. post-FVX CSF levels, respectively: 5-HIAA =17.1±7 v.10.9±6 ng/ml, $p<.001$ (paired t-tests); MHPG= 6.9±1.4 v. 6.3±1.5ng/ml, $p<.05$; HVA=28.±9 v. 34.4±14ng/ml, NS. All patients showed a decrease in CSF 5-HIAA with FVX (mean reduction = 36%). There were no significant correlations between changes in OCD severity (measured by the Y-BOCS (mean improvement =37%)) and metabolite levels (either at baseline or in change from baseline). CONCLUSION: The preliminary finding that FVX is superior to DMI in treating OCD suggests that the 5-HT reuptake properties of a medication are relevant to its antiobsessional efficacy. The robust decrease in CSF 5-HIAA is evidence that chronic FVX has major effects on 5-HT function in the brain. The approx. 50% response rate to FVX and the lack of clinical correlates with CSF 5-HIAA suggest the need for other approachs to assessing the pertinent neurochemical effects of FVX in OCD.

152.2

INCREASED PLASMA CONCENTRATIONS OF α_1-ACID GLYCOPROTEIN, AN ENDOGENOUS MODULATOR OF THE PLATELET [3H]-IMIPRAMINE BINDING/5HT TRANSPORT COMPLEX, IN PATIENTS WITH MAJOR DEPRESSION. C.B. Nemeroff, K.R.R. Krishnan*, D.L. Knight*, D. Benjamin* and L.R. Meyerson. Depts. Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710 and Med. Res. Div., Amer. Cyanamid Co., Mahwah, NJ 07430.

Many depressed patients exhibit a reduction in the density (Bmax) of platelet [3H]-imipramine binding sites (Raisman et al., Psychopharmacol 75, 368-371, 1981). This platelet [3H]-imipramine binding site is similar to, if not identical with, the [3H]-imipramine binding site identified on presynaptic 5HT nerve terminals and is allosterically coupled to, but distinct from, the serotonin transporter. Recently an α_1-acid glycoprotein has been isolated from human plasma which fulfills criteria for an endogenous modulator of the platelet [3H]-imipramine binding site - 5HT transport complex (Abraham et al., Biochim Biophys Acta 923, 8-21, 1987). In the present study, the plasma concentration of this α_1-acid glycoprotein was measured by radial diffusion assay in 21 drug-free patients that fulfilled criteria for major depression and 18 controls. Plasma concentrations (μg/ml) of the α_1-acid glycoprotein were significantly ($p < 0.02$) elevated in the depressed patients (mean ± SEM = 858 ± 60) when compared to the normal controls (mean ± SEM = 687 ± 35). Ten of the 21 depressed patients had plasma concentrations higher than the highest normal control. These results provide the first evidence of alterations of an endogenous modulator of [3H]-imipramine binding/5HT transport in depression (Supported by NIMH MH-40159 and MH-42088).

152.3

ACUTE AND CHRONIC EFFECTS OF MONOAMINE UPTAKE BLOCKADE ON PARADOXICAL SLEEP IN THE CAT. R.J. Ross, W.A. Ball*, P.J. Gresch* and A.R. Morrison. VA Med. Center and Depts. of Psychiatry and Animal Biology, Univ. of Penna. Schools of Med. and Vet. Med., Phila., PA 19104

The monoamines norepinephrine (NE) and serotonin (5-HT) may inhibit paradoxical sleep (PS) generation. The relatively specific NE uptake blocker desipramine (DMI) suppresses PS acutely in the cat (Hishikawa, Y., et al., Electroenceph. Clin. Neuro., 19:518, 1965). 5-HT uptake inhibition likely has a similar effect acutely (Sommerfelt, L., et al., J. Neural Transm., 68:127, 1987). Less is known about the chronic effects of monoamine uptake blockers, yet clinical experience suggests their antidepressant actions take 2-3 weeks to appear. We investigated changes in PS in the cat after single and repeated doses of DMI or the selective 5-HT uptake blocker sertraline. Six cats were implanted with standard EEG, EOG, EMG, and lateral geniculate electrodes. Sleep was recorded first under placebo then after acute (day 1) and chronic (19 days) p.o. administration of drug (0.75 mg/kg acute dose; 2.0mg/kg chronic daily dose). Three cats received only DMI, two received only sertraline, and one received both drugs (27 day "washout" period). Six-hour records were scored according to standard criteria (Ursin, R., Sterman, M.B., Brain Info. Serv./U.C.L.A., 1981). PS percentage (PS time/total sleep time) fell after acute DMI (25.0 vs. 4.0%, $p<.05$), rose again after chronic DMI (4.0 vs. 14.0%, $p<.05$), but failed to reach placebo level ($p<.05$). Likewise, there was a tendency for PS percentage to drop after acute sertraline (23.0 vs. 6.0%) and then return towards baseline after chronic sertraline (6.0 vs. 17.0%) ($p<.07$). Thus, chronic administration of both NE and 5-HT uptake blocking drugs leads to some recovery in PS despite the marked initial decline also noted by other investigators. Receptor mechanisms that may underlie these behavioral changes remain to be determined. (Supp. by VA Med Res Svc)

152.4

CAN TIME SERIES ANALYSIS OF SEROTONIN TURNOVER TEST THE THEORY THAT CONSCIOUSNESS IS A FIELD? N. Pugh*, K.G. Walton and K.L. Cavanaugh* (SPON: V. Argiro). Dept. of Chem. and Dept. of Mngmnt. and Public Affairs, Maharishi International University, Fairfield, IA 52556.

Over 30 studies in the last 15 years support the theory that consciousness in its pure form is a field. These studies, mostly sociological in nature, were inspired by Maharishi Mahesh Yogi's claim in 1960 that 1% of a population practicing the Transcendental Meditation (TM) technique would improve the quality of life for the entire population. The basis of the claim is the central theme of the ancient Vedic tradition which he represents, namely, that the unified field of natural law is nothing other than the "least excited state" of individual consciousness, "pure consciousness", a field directly accessible to the human nervous system. Many of the above studies purport to show that group practice of the TM and related TM-Sidhi techniques has a more powerful beneficial effect than individual practice. The question we addressed is whether an increase in the number involved in the group practice of these techniques at MIU might increase the rate of 5-HIAA excretion by individuals. In diet-controlled studies involving 5 and 3 subjects, nightly 5-HIAA excretion rates over periods of 91 and 50 days, respectively, were compared to the daily numbers in the group. The data were analyzed by the linear transfer function method of time series analysis modified by the use of the Akaike information criterion, a method of minimizing subjective bias in model selection. The result of the first study was highly significant at lag 0, $t\,(68) = 2.82$, $p < .0063$. (This effect at lag 0 indicates a contemporaneous, same-day increase of 5-HIAA excretion and number in the group.) The second study was highly significant at lag 0, $t\,(34) = 2.91$, $p < .0063$ and at lag 8, $t\,(34) = 4.72$, $p < .0001$. In both cases the total effect (overall steady-state gain) was positive. The results strongly suggest that increasing the size of the group increases the turnover of serotonin not only in the group members (Expt. 1) but also in individuals completely outside the group (Expt. 2).

152.5

DIFFERENTIAL EFFECTS OF 5-HT1B AGONISTS ON FEMALE AND MALE SEXUAL BEHAVIOR IN THE RAT. S.D. Mendelson and B.B. Gorzalka Dept. Psychology, Univ. British Columbia, Vancouver, B.C. V6T 1Y7 Canada

Varying doses of the 5-HT1B agonists 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and m-chlorophenylpiperazine (MCPP), and the 5-HT1A/5-HT1B agonist RU24969 were evaluated for effects on sexual behavior in ovariectomized female and intact male rats. In female rats treated with estrogen, TFMPP (0.2-1 mg/kg), MCPP (1 mg/kg) and RU24969 (0.2 mg/kg) facilitated lordosis behavior. In female rats treated with estrogen and progesterone, TFMPP (0.04-1mg/kg) and MCPP (0.04-1 mg/kg) were ineffective, that is, lordosis behavior remained at high baseline levels, whereas RU24969 (1 mg/kg) inhibited lordosis. In male rats low doses of the agonists (0.04-0.2 mg/kg) were ineffective. However, at the highest dose MCPP (1 mg/kg) reduced the number of male rats that displayed ejaculation, and TFMPP (1 mg/kg) and RU24969 (1 mg/kg) profoundly reduced the number of males that displayed mounting, intromission and ejaculation behaviors. It was concluded that activity at 5-HT1B receptors produces differential effects on female and male sexual behavior. Activity at 5-HT1B receptors facilitates sexual behavior in female rats but inhibits sexual behavior in male rats.

152.6

EFFECTS OF L-TRYPTOPHAN ON SLEEP IN THE RAT. M.J. Bakalian* and J.D. Fernstrom. Departments of Psychiatry and Behavioral Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh PA 15213.

L-tryptophan (TRP) administration has been reported to reduce sleep latency and enhance other sleep parameters in rats and man. These effects are reputed to follow from a TRP-induced stimulation of serotonin (5HT) synthesis and release in brain. Recently, several studies have shown that 5HT presynaptic reuptake blockers (e.g., fluoxetine, zimelidine) have little effect on sleep onset or total sleep time, but delay rapid-eye movement (REM) sleep onset, and reduce total REM-sleep time. 5HT reuptake blockers and TRP should produce similar effects on sleep, since both enhance 5HT synaptic transmission. We have therefore reexamined the effects of TRP on sleep in rats. Animals were prepared for EEG recording as described previously (Pastel and Fernstrom, Brain Res 436: 92, 1987). After recovery, they received saline or TRP (125 mg/kg ip) at light onset, and EEG recordings were made for 6 hr. Fluoxetine (2.5 mg/kg ip) was also tested as a positive control. Sleep onset and total sleep time were unaffected by TRP, but REM latency was increased, and REM sleep time was reduced for 3 hr. A similar, but more robust effect was seen with fluoxetine, as observed previously (above reference). In no case did TRP produce sleep-promoting effects. These results indicate that TRP does not behave as a hypnotic agent in rats. Rather, it produces effects similar to those of a 5HT reuptake blocker; viz., to suppress selectively REM sleep.

152.7

SEROTONERGIC INFLUENCES IN THE AMYGDALA AFFECT BEHAVIORAL INHIBITION IN RATS. M.M Curtis*, P.M. Saxton (Wixom) and J. Siegel. Institute of Neuroscience and School of Life and Health Sciences, Univ. Delaware, Newark, DE 19716

Behavioral inhibitory mechanisms are being investigated using a differential reinforcement of low rate of response (DRL) operant task. Rats were trained on a DRL schedule which involves an active behavioral inhibitory process. Cannulae were directed to the basolateral amygdala bilaterally for the administration of a 0.25 µl suspension of serotonin (5-HT) (40 µg); fluoxetine, a 5-HT agonist (16 µg) or *p*-chlorophenylalanine (PCPA), a 5-HT antagonist (40 µg). Control conditions involved injections of saline vehicle. An error on the DRL schedule occurred when an animal failed to withhold a bar press for 15 sec. Reinforcement was received when the animal bar-pressed at least 15 sec after a previous reward. Both 5-HT and fluoxetine reduced errors 35% and PCPA increased errors 41%. This suggests that raphé projections to the amygdala and the release of serotonin play a role in an animal's ability to actively withhold the impulse to respond. Additional neurotransmitter systems are being investigated prior to histological verification of cannulae placement.

This work was supported in part by ARO contracts DAAG 2980K0015 and DAAL 0388K0043.

152.8

SEROTONERGIC MODULATION OF SEXUAL BEHAVIOUR IN MALE RATS. B.Olivier, J.Mos* and P.Bevan. Dept. of Pharmacology, Duphar B.V., P.O.Box 2, 1380 AA Weesp, Holland.

Recent evidence indicates a differential contribution of the brain serotonergic system to the modulation of sexual behaviour in male rats; 5-HT_{1A} receptors and 5-HT_{1B} receptors exert opposite effects upon activation by specific serotonergic agonists. Treatment with 5-HT_{1A}-agonists like 8-OH-DPAT, buspirone, ipsapirone and Cle-PAT may stimulate sexual behaviour, dependent on the behavioural status and sexual experience of the animals. In sexually experienced males, we were unable to stimulate sexual behaviour with 8-OH-DPAT, whereas in sexually sluggish or naive rats, 8-OH-DPAT strongly stimulates sexual behaviour, measured by a decrease in the number of mounts and intromissions preceding ejaculation, a decreased ejaculation latency and an enhanced number of animals reaching ejaculation. Buspirone, ipsapirone and Cle-PAT showed a similar, although less strong effect in naive rats. After ejaculation, male rats produce 22 kHz ultrasonics, the postejaculatory song (PES). 8-OH-DPAT influenced PES dramatically. In most rats no PES occurred and many males displayed a pre-ejaculatory sound in the frequency range of 30-40 kHz.

Drugs (agonists) with high affinity for the 5-HT_{1B}-receptor like TFMPP and RU 24969 (both also having high affinity for 5-HT_{1A}) inhibit male sexual behaviour. These drugs enhance the latency to reach ejaculation and increase the number of mounts and intromissions to reach ejaculation. Apparently, the 5-HT_{1B} effects overrule the possible stimulatory 5-HT_{1A} effects of both drugs. Interaction studies with 5-HT_{1A} and 5-HT_{1B} ligands (including antagonists) should unravel whether both receptors play a dualistic role in the modulation of male sexual behaviour on a receptor or a functional level.

152.9

SELECTIVE 5-HT RECEPTOR AGONISTS HAVE AN INHIBITORY CONTROL ON AGGRESSION. K. A. CULHANE and K. M. KANTAK. Lab of Behav. Neurosci., Dept. Psychol., Boston Univ., Boston, MA 02215.

Decreases in serotonin (5-HT) have facilitory influences on most forms of aggression. Thus, an inhibitory control by 5-HT on aggression is suggested. In order to elucidate the differential roles of 5-HT receptor subtypes in mediating aggression, the effects of 5-HT_{1a}, 5-HT_{1b}, and 5-HT_2 agonists on resident-intruder offensive aggression were measured. The 5-HT_{1a} agonist PAPP and the 5-HT_{1b} agonist mCPP significantly reduced aggressive attacks; the 5-HT_2 agonist DOB also reduced aggression. Further analysis revealed a dose response function for the effect of each agonist on lowering aggression. It is unclear whether the 5-HT_{1a} and 5-HT_{1b} agonists are reducing aggression via their actions at inhibitory presynaptic 5-HT receptors or at postsynaptic sites. The results for DOB suggest that 5-HT_2 stimulation is inhibitory for aggression, and further, suggest that the inhibitory effects of mianserin, a non-selective 5-HT_2 antagonist, on aggression may be more related to its effects on α_2 receptors.

152.10

MEDIAN RAPHE RADIOFREQUENCY LESIONS, BUT NOT DORSAL RAPHE LESIONS OR PARA-CHLORO-AMPHETAMINE, INCREASE ACOUSTIC STARTLE RESPONSES IN RATS. J.H. Kehne*, V.L. Taylor, C.K. Black, P.J. Robinson*, and C.J. Schmidt, Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215.

It has been suggested that various substituted amphetamines, including para-chloroamphetamine (PCA), have selective neurotoxic effects on serotonergic neurons originating in the dorsal raphe (DR) while having little effect on the median raphe (MR) serotonergic pathway (Soc. Neurosci. Abst., 13: 907, 1987). One goal of the present study was to investigate a possible functional correlate of serotonergic destruction. Combined lesions of the DR and MR nuclei have previously been shown to increase acoustic startle responses (Physiol.Beh.,12:425, 1974). To separate the relative contributions of these two nuclei, rats were administered multistage radiofrequency lesions of the MR or DR, tested behaviorally a week later, and sacrificed for subsequent brainstem histological reconstruction and forebrain neurochemical analysis. Relative to the appropriate controls, MR lesions produced elevated acoustic startle responses, whereas DR lesions or PCA treatment (20 mg/kg; 1 wk before) were without effect. These data (1) suggest that the MR tonically inhibits acoustic startle responses; and (2) are consistent with the notion that PCA neurotoxicity does not alter MR function.

152.11

FURTHER EVIDENCE THAT SEROTONIN PRODUCES NEOCORTICAL LOW VOLTAGE FAST ACTIVITY, HIPPOCAMPAL RHYTHMICAL SLOW ACTIVITY AND CONTRIBUTES TO INTELLIGENT BEHAVIOR. C.H. Vanderwolf, G.B. Baker, L.-W.S. Leung and B. Robertson*. Dept. of Psychology, Univ. of Western Ontario, London, Ont., Canada, N6A 5C2 (CHV & BR); Dept. of Psychiatry, Univ. of Alberta, Edmonton, Alta., Canada (GBB); Dept. of Clin. Neurological Sci., Univ. of Western Ontario, London, Ont., Canada (L-WSL).

Anesthetized rats, pretreated with desmethylimipramine, received injections of 5,7-dihydroxytryptamine (0.5-1.0 µl, 25 µg/µl) or vehicle at 4 sites in the midbrain (in cell groups B7, B8 and B9). After recovery, polygraphic recording plus power spectral analysis revealed severe attenuation or total loss of atropine-resistant neocortical low voltage fast activity and atropine-resistant hippocampal rhythmical slow activity. Polygraphic patterns characteristic of normal quiet sleep and active sleep and a circadian rhythm of motor activity were present but there were severe abnormalities in a variety of behavioral tests (open field, swim-to-platform, grooming, social behavior, shock avoidance). Post-mortem histological and HPLC analysis revealed a selective 90-95% depletion of neocortical and hippocampal serotonin in the experimental rats.

Supported by grants from NSERC, MRC and the Alberta Heritage Fund for Medical Research.

152.12

ESTROUS CYCLE CHANGES IN 5-HT_{1A} BINDING SITES AND THE 5-HT SYNDROME. D.Croissant*, R. Richards*, L. Addison*, and L. Uphouse. (SPON: John Hines), Texas Woman's University, Denton Texas, 76204.

The role of serotonin during female reproduction is well recognized but poorly understood. Recent studies suggest that some of the contradictory findings may stem from the presence of multiple serotonin receptors which have quite different effects on female reproduction. In the following studies, emphasis was placed on the 5-HT_{1A} binding site, which recently has been suggested to play an inhibitory role in female sexual receptivity. Female proestrous rats were tested for sexual behavior with a sexually experienced male. After a minimum of 10 mounts, the female was injected with 0 to 0.25 mg/kg of the 5-HT_{1A} agonist, 8-OH-DPAT. A dose dependent reduction of sexual behavior occurred. When present, inhibition usually occurred 5 to15 minutes following injection. Previous studies have shown that 5-HT_1 binding sites and 5-HT levels increase on the night of proestrus. It was suggested that an upregulation of 5-HT_1 sites might result from a relative reduction in the synaptic availability of serotonin. To determine if this upregulation occurred for 5-HT_{1A} sites, binding sites were examined with ^{3}H-8-OH-DPAT plus or minus unlabelled serotonin. The binding showed a complex profile indicative of a possible change in K_d for binding In a test of functional variations in 5-HT_{1A} sites, diestrous, proestrous and estrous females were injected with varying concentrations of 8-OH-DPAT and were monitored for the "serotonin syndrome". Sensitivity to 8-OH-DPAT was lower on proestrus than on other stages of the estrous cycle. Collectively, these observations suggest that for those stages of the estrous cycle where sexual behavior is facilitated, the female's sensitivity to activation of the inhibitory 5-HT_{1A} site is low. Alternatively, sensitivity to 5-HT_{1A} agonists is high on the stages of the cycle when sexual behavior is inhibited.

(Supported by NIH ESO-3351)

152.13

EFFECTS OF LY237733, A SELECTIVE 5-HT_2 RECEPTOR ANTAGONIST, ON COPULATORY BEHAVIOR OF MALE RATS. M. M. Foreman, R. L. Love* and J. L. Hall*. Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285.

Previously we have reported that DOI, a selective 5-HT_2 receptor agonist, suppressed sexual behavior and LY53857, a selective 5-HT_2 antagonist, amplified sexual behavior in male rats (Foreman et al., Neurosci. Soc. Abstr. 116.8, 1987). LY237733, a selective 5-HT_2 receptor antagonist activity with a greater potency and duration of action than LY53857, was evaluated for effects on male rat sexual behavior. Male, Sprague-Dawley rats were selected for stable sexual performance. The performance divisions included rats that did not mate, rats that were unable to ejaculate and rats that had full sexual capacity. These rats were given 0.001-100 µg/kg LY237733 s.c. 30 min prior to testing. Over half of the non-maters and non-ejaculators showed mating behavior and ejaculatory responses, respectively, with 0.1 µg/kg LY237733. This dose was also the minimum effective dose to induce significant reductions in ejaculatory latency and significant increases in copulatory rate and copulatory efficiency in the "normal" rats. LY237733 is 1000X more potent than LY53857 in activating and amplifying male rat sexual behavior. These studies add further supportive evidence to the proposal that 5-HT_2 receptors have an inhibitory role in modulating sexual behavior in the male rat.

152.14

BEHAVIORAL EFFECTS OF D,L-5-HYDROXYTRYPTOPHAN (5-HTP) ADMINISTRATION TO RATS WORKING ON AVOIDANCE AND APPROACH SCHEDULES. J.N. Hingtgen, M.L. Brust*, D.A. Grossman* and M.H. Aprison. Inst. Psychiat. Res. and Program in Medical Neurobiol., Depts. of Psychiatry and Biochem., Indiana U. Sch. Med., Indianapolis, IN 46223.

The hypersensitive postsynaptic serotonin receptor theory of depression (Aprison et al., In: Neuropharm. and Behavior, Plenum, 1978; Aprison and Hingtgen, In: Serotonin, Plenum, 1981) was developed from an animal model in which 5-HTP induced suppression of food-reinforced operant behavior was correlated with CNS serotonergic mechanisms. Previous studies from our group have not observed changes in avoidance responding after 5-HTP, in spite of the elevated 5-HT levels in key forebrain areas. Since the avoidance rats had not been also trained on approach schedules, in the present study rats were trained on both a milk-reinforced VI schedule and a Sidman avoidance schedule (RS 20; SS 10). Injections as high as 75 mg/kg of 5-HTP did not produce disruptions in avoidance rats, but did produce the typical period of depression in the same rats working on VI approach schedules. Other rats, trained on a milk-reinforced VI alternating-lever schedule, showed significant reductions in accuracy, as well as response rates, following 5-HTP. These data are discussed in terms of the 5-HTP model of depression. (Supported in part by Indiana Dept. Mental Health.)

152.15

STRAIN SPECIFIC REDUCTION IN QUIPAZINE RESPONSIVENESS FOLLOWING ISOLATION IN MICE. **K. Ornstein and A. Malnoë* (SPON:ENA). Nestlé Research Centre, Nestec Ltd., Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland.**

Head twitch response induced by the serotonin (5-HT) agonist quipazine and its modification by isolation or exposure to a L-tryptophan (TRP) supplemented diet was compared in DBA/2 and C57BL/6 mice. In C57 mice, central 5-HT turnover, as judged by the 5-HIAA/5-HT ratio is faster than in DBA mice. Concomitant to the higher 5-HT turnover rates, C57 mice showed a weaker head twitch response following s.c. administration of a 2.5 mg/kg dose of quipazine [38±2 (n=6) vs 62±4 (n=6)]. Individual housing for a 6 week period decreased quipazine-induced head twitch response in both mouse strains, with a more pronounced decrease in DBA mice [C57:-26 %; DBA:-47 %]. Following exposure to a diet supplemented with 4 % TRP to a final concentration of 4.3 %, group housed DBA mice showed reduced head twitches following application of quipazine (-40 % as compared to control mice fed a diet containing 0.3 % TRP), while no reduction in the response to quipazine occurred in C57 mice. Furthermore, the reduction in quipazine induced head twitches observed following TRP supplementation no longer occurred in isolated DBA mice. These results suggest that the reduction in quipazine response observed following isolation or TRP exposure rely on a common neural substrate.

152.16

EFFECTS OF ACUTE SEROTONERGIC MODULATION ON BEHAVIOR IN CHAIR-RESTRAINED RHESUS MONKEYS. J.H. Krystal, C. Heninger, A. Smith*, G.R. Heninger. Abraham Ribicoff Research Facilities, Connecticut Mental Health Center, Yale Univ. Sch. of Med., New Haven, CT 06508.

Serotonergic (5-HT) systems have been implicated in anxiety, depression, schizophrenia, sleep, and appetite regulation, but the absence of selective 5-HT receptor ligands for human use has limited the study of serotonergic influences on behavior. In this study, a spectrum of 5-HT ligands are employed to evaluate 5-HT influences on behavior in the chair-restrained non-human primate.

Method: 2 sets of 4 chair-restrained male monkeys received intravenous infusions of serotonergic agonists +/- antagonists including : 5-HT_{1A} agonists: buspirone, gepirone, ipsaperone, 8-OHDPAT, or MCPP; 5-HT_2 agonist: mescaline; 5-HT_{1A} antagonist, metergoline; and 5-HT_2 antagonists: ritanserin or ketanserin. Dopaminergic antagonists SCH-23390 (D_1) and haloperidol (D_2) were employed as comparison drugs. Behavior was recorded on videotapes which were rated by a blind observer. Plasma sampled via venous catheters was analyzed for endocrine indices and are reported in a companion abstract (G.R. Heninger, et al. this volume).

Results: The behavioral effects of 5-HT_{1A} and 5-HT_2 agonists could be distinguished in the monkeys. 5-HT_{1A} agonists produced sedation, decreased goal-directed behavior (chair grasps and picks), and decreased chewing; effects which were insensitive to reversal by 5-HT_2 antagonists. 5-HT_2 agonists increased chewing slightly and had mild sedative effects, both of which were antagonized by 5-HT_2 antagonists. Non-selective 5-HT agonists or partial agonists could be distinguished by their behavioral effects and partial antagonism by selective agents. The behavioral profilesof the 5-HT drugs generally paralleled the prolactin responses which they provoked. However, buspirone dose-dependently elicited catelepsy and marked increases in plasma prolactin consistent with its anti-dopaminergic actions.

152.17

INHIBITION OF DOM-INDUCED ALTERATIONS IN LOCOMOTOR BEHAVIOR BY THE 5-HT2 ANTAGONIST KETANSERIN. L.L. Wing and M.A. Geyer. Dept. of Psychiatry, UCSD, La Jolla, CA 92093.

Previous studies of rat locomotor and investigatory behaviors identified a behavioral profile characteristic of 5-HT2 agonists such as the hallucinogens LSD or DOM. Specifically, when rats are tested in a novel environment, 5-HT2 agonists decrease the initial levels of crossovers and holepokes and reduce the time spent in the center of the test chamber.

In the present study, male Sprague-Dawley rats were injected with 0.16-2.5 mg/kg of the 5-HT2 antagonist ketanserin 30 min prior to a 60 min test. No siginificant behavioral effects were found. Other animals were then tested after s.c. injections (at 30 min and 10 min) of either saline/saline, saline/DOM (0.5 mg/kg), ketanserin (1.0 mg/kg)/saline, or ketanserin/DOM. While having no effect by itself, the ketanserin pretreatment blocked the DOM-induced decreases in crossovers, holepokes, and time spent in the center. Similar results were also obtained with the selective 5-HT2 antagonist ritanserin. These findings indicate that these behavioral effects of DOM are mediated by agonist actions at 5-HT2 receptors.

152.18

EFFECTS OF PHENCYCLIDINE ON PATTERNS OF LOCOMOTION AND INVESTIGATORY RESPONDING. V.L. Masten* and M.A. Geyer (SPON: P. Tueting). Dept. of Psychiatry, UCSD Sch. of Med., La Jolla, CA 92093.

A Behavior Pattern Monitor (BPM) was used to assess the effects of phencyclidine (PCP) on investigatory responding and on the perseverative quality of hyperactivity produced by various doses of PCP in rats. The BPM provided both quantitative measures of crossovers, rearing, and holepokes, and qualitative measures of spatial patterns of locomotion. Male Sprague Dawley rats weighing 250-300 g were obtained from Charles River Lab. Each animal was tested for 60 min at 10 min after an s.c. injection of saline, 0.25, 0.5, 2.5, or 5.0 mg/kg PCP. PCP produced a dose-dependent increase in locomotion; however, the perseverative quality of the locomotor patterns markedly changed as a function of dose (ie, low dose randomization and high dose perseveration), as reflected by a decrease or increase in the coefficient of variation (CV) of area transitions, respectively. During the initial 30 min in the BPM, the CV of region transitions was increased in a dose dependent manner. Rearing was reduced by the 2 highest doses. Holepoking was also altered in an inverted U-shaped manner, with the highest dose reducing this measure of investigatory responding. Additionally, low doses of PCP produced a preferential enhancement of perseverative (repeated) holepokes. These results are discussed in relation to the effects of other psychoactive drugs in this paradigm.

152.19

SITE SPECIFICITY IN MAHPOA HEAT GAIN PATHWAYS OF KETAMINE ANESTHETIZED RATS. C.W. Simpson* and G.E. Resch* (SPON: Fred Samson) Sch. Basic Life Sci., Univ. Missouri, Kansas City, MO 64108. Medial anterior hypothalamic preoptic area (MAHPOA) sites have been identified with heat gain control in our previous work. Female Sprague Dawley rats were prepared as before. Acetylcholine (ACh), Nicotine (Nic.), Serotonin (5-HT), and Prostaglandin (PGE2) were injected in lul volumes to elicit a criterion response of 0.5°C. increase in colonic temperature in 30 min. Sites were screened with PGE2 or other agonists in 0.2 mm increments below the guide tube until a criterion response was observed. An example will illustrate some of the diverse subtypes of heat gain sites demonstrated in these mapping experiments. The first injection of PGE2 elicited a rise in Tc of 0.6°C. in 30 min. at a site 0.2 mm below the guide tube. Injections of PGE2 at the same dose at 0.4 and 0.6 mm below the guide tube however, failed to elicit criterion responses. An injection of PGE2, 0.8 mm below the guide tube elicited a 0.6°C rise in colonic temperature. In addition to the anatomical diversity, we found unique neurochemical diversity within the anatomically defined microsites for heat gain. Different sites responded to 4,3,2 or 1 agonist. The combination of anatomical and neurochemical data and the strong criterion evidence of effective sites suggests a disperse population of differentially neurochemically coded microsites. These sites are functionally important in the heat gain pathway in Ketamine anesthetized rats. Supported by AFOSR 87-0297.

152.20

A KETAMINE ANESTHETIZED ANIMAL MODEL TO STUDY HEAT GAIN REGULATION. G.E. Resch* and C.W. Simpson* (SPON: Don R. Justesen). Sch. Basic Life Sci. and Sch. Med., Univ. Missouri, Kansas City, MO 64108. Medial anterior hypothalamic/preoptic area (MAHPOA) heat gain control sites previously reported in awake animals show response to microinjection of 100 ng to 50 ug of various agonists. In this report data from Ketamine (50 mg/kg, IP) anesthetized female Sprague Dawley rats (200 - 250 gm) demonstrated a rise in colonic temperature (Tc) elicited by 1 ag to 100 ng of the agonists prostaglandin E2 (PGE2), acetylcholine (ACh), nicotine (Nic), or serotonin (5-HT). Doses of each agonist were test until a minimum effective dose was found. Rises in Tc were recorded after PGE2 at 1 ag, ACh at 1 fg, Nic at 1 pg, and 5-HT at 1 fg. Over a wide dose range the Tc response did not show dose dependency but rather an "off-on" character. Starting Tc values, which varied from 38 to 34 C due to ketamine, showed no effect on the magnitude of elicited rise in Tc ($F = 1.65$; $P>0.202$). The just effective dose of antagonists were found for SC 19220, atropine, hexamethonium, and methylergonovine maleate. Specific blockade of Tc response was demonstrated against their respective agonists. Duration of blockade (up to 150 min) did not reverse while the rat remained anesthetized, unlike in the awake rat. The data indicate that ketamine anesthetized rats show heat gain responses to microinjection of four specific agonists into the same MAHPOA heat gain site at doses lower than previously reported. Implications for ketamine separation of heat loss and heat gain controls will be discussed. The ketamine anesthetized rat model may open new possibilities for the study of central organization of heat gain controls. (Supported by AFOSR-87-0297).

INVERTEBRATE SENSORY SYSTEMS I

153.1

NEUROPHARMACOLOGY OF THE PHOTORECEPTIVE PATHWAY IN CORALS. R. S. Jakubczak*, C.H.Keith , and J. W. Porter*. Dept. Zoology, University of Georgia, Athens, GA 30602.

In addition to the familiar contractile response of coral polyps on tactile stimulation, they show a slow contractile response in response to light. The nature of this photoreceptive pathway - neuronal or nonneuronal in nature, and the mediators involved - is completely unknown.

We have employed a schedule of impulse blockers and neurotransmitter agonists and antagonists to characterize this pathway in the scleractinian coral Meandrina meandrites. Low levels of tetrodotoxin inhibit photocontraction without affecting contraction in response to touch, suggesting that the photoresponse is neuronally mediated, and is distinct from the tactile contractile pathway. General cholinergic agonists, nicotine, and muscarinic antagonists cause contraction in a light-independent fashion. Muscarinic agonists and GABA inhibit light-triggered contraction. Thus, the photocontractile response appears to involve two pathways: a nictoinic cholinergic pathway that stimulates contraction, and a muscarinic cholinergic/GABAergic pathway that inhibits contraction.

This work represents the first evidence for a neural component in coral photosensation, and the first characterization of synapse nature in the Scleractinia.

153.2

DESCRIPTIVE CATALOG OF DIMMING CELLS IN HEMIGRAPSUS NUDUS. E.Pak*. D.D. Rafuse* (SPON: B.J. Vasquez). Dept. of Physiol Loma Linda Univ., Loma Linda, CA 92350.

Cells that respond specifically to a decrease in light intensity (dimming cells) form a distinct population of visual neurons in the intertidal crab Hemigrapsus nudus. Pin electrode recordings from the optic nerve tract established cells responsive to decremental changes in relative light levels (40-0.08 lux). Dimming cell responses are well documented in other species. Study of observed activity in this dimming cell population (n=30) identified several subgroups. A description of these subgroups identified whole eye receptive fields; responses to jittery movement; abrupt light On/Off; tactile mechanoreceptive sensitivity; and pacemaker activity. Some dimming cells also showed increased sensitivity to a specific lux level of light intensity. Depending on the extent of this enhanced range, they were described as Brief Range (BR) or Extended Range (ER) responses. From these observations, dimming cells in H.nudus may be considered multimodal. Dimming cells may detect movement by changes in total light via changes in overall illuminance. Yamaguchi and Ohtsuka's model of reciprocal inhibition in dimming and sustaining fibers (J.Fac.Sci.Hokk.Univ.Ser.VI Zool. 19:15-30, 1973) may help explain the dimming cell characteristics of H.nudus.

153.3

SEX-SPECIFIC CONNECTIVITY IN THE FIRST OPTIC NEUROPILE OF THE MALE HOUSEFLY. A. Fröhlich, J.L. Chiasson and C.F. Winchcombe. Mt. St. Vincent Univ., Halifax, NS, Canada, B3H 2J6.

The frontal-dorsal region of the compound eye of the male housefly, Musca domestica has been related to sex-specific behaviour such as the chasing of female flies. Here a male-specific class of photoreceptor R7 ends and synapses in the first optic neuropile (as do R1-R6) instead of terminating (together with R8) in the second optic neuropile like its female counterpart. The connectivity of this male-specific R7 has been shown to be different (Hardie, 1983) from that of R1-R6 in the female. We have largely confirmed and extended Hardie's results. An R7 terminal was reconstructed. The number of R7 synapses was determined to be about 600. 186 R7 synapses of 2 terminals were analyzed: 92% had 2 (dyads), 8% had 3 (triads) postsynaptic elements. One of these was always monopolar cell L3, the other always the same central monopolar (L1 or L2). 35 R1-R6 synapses of 3 terminals were analyzed at the level of the R7 terminal. In contrast to their female counterparts with four postsynaptic elements, most synapses were dyads, one element being L3, the other being either L1 or L2. In a few cases glial elements were postsynaptic, but amacrine cells were never found to be postsynaptic as they are in the female.

Thus the major differences between male and female connectivity emerging so far from our studies are the enormously increased visual input to L3 and a possibly decreased input to either L1 or L2 and to amacrine cells.

153.4

FAST OSCILLATORY RESPONSES IN PERIPHERAL VISUAL NEURONES OF DIPTERA. D. Moore and S.R. Shaw, Life Sciences Ctr., Dalhousie University, Halifax, N.S., Canada B3H 4J1.

The responses of the postsynaptic lamina (L) monopolar cells in the dipteran visual system are much more transient than those of the photoreceptors (R cells) which drive them directly. R cells are also directly presynaptic to amacrine (Am) neurones. The transience could be due in part to direct L→R or Am→R feedback, the two local reciprocal synaptic circuits present in advanced flies. Negative feedback circuits oscillate unless critically damped, and we find pronounced light-on (ON) oscillations in some L cell responses. Electrical measurements suggest that the active oscillatory site is the presynaptic R terminal, in agreement with recent optical evidence (van Hateren, J. Comp. Physiol. 161: 849, 1987).

We have also investigated a primitive dipteran family (Stratiomyidae) in which a direct, local Am→R feedback loop is absent, finding much more pronounced and often prolonged oscillatory responses both at ON and OFF, at a significantly lower tuning frequency. The first few cycles of OFF oscillation often trigger action potentials, not usually seen in the L responses of more advanced groups unless the neurones are hyperpolarized extrinsically (Guy and Srinivasan, J. Comp. Physiol. 162: 317, 1988). The OFF oscillation has a lower characteristic frequency than that at ON, suggesting an origin in a different underlying mechanism. [Supported by NSERC, Canada].

153.5

BOTH INSECTS AND SPIDERS PROCESS WIDE-FIELD MOTION AND SMALL-FIELD FORM IN SEPARATE PATHWAYS. Wulfila Gronenberg* and Nicholas J. Strausfeld [SPON: J.B. Angevine]. ARL Div. Neurobiology., Univ. of Arizona, Tucson, AZ 85721.

In insects, parallel relays from the compound eye diverge to two subsystems, one supplying wide-field motion sensitive neurons, the other supplying assemblies of small-field neurons associated with form and color vision. The first system is conserved amongst a variety of insect orders, involving a modest population of small-field retinotopic neurons that segregate out to a specialized neuropil (the lobula plate or its homologue) containing wide-field directional-motion-sensitive neurons. The second system is associated with complex stratified neuropil (the medulla and lobula), containing discrete aggregates of retinotopic units, many expressing great morphological complexity. In spiders, which have single-lens eyes, specific eyes (anterior lateral, posterior lateral, posterior median) are employed for wide-field motion perception, whereas the dichromatic tiered retina of the anterior median eyes are devoted to small-field form detection [e.g., salticids; Land, M.F. J. Exp. Biol. 51, 443-493 (1969)]. The lateral and posterior median eyes supply a panoramic array of small-field retinotopic inputs to the dendrites of giant wide-field neurons of the so-called "mushroom body." In contrast, the anterior median eyes, specialized for form and color perception, are associated with the highly complex stratified and columnar neuropil of the "central body." The separation of visual pathways into distinct form and motion neuropils in arthropods suggests analogies at the level of cellular organization with the segregation of form- and motion-processing pathways in the mammalian visual system. **Supported by NIH Grant No. R01 EYO7151-01**

153.6

THE LAMINA CARTRIDGE IN THE OPTIC LOBE OF DROSOPHILA. I.A. Meinertzhagen and S.D. O'Neil*, Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

The anatomy and electrophysiology of the optic lobe in adult Drosophila has been little studied, despite a clear need to analyse the departures from wild type found in many visual mutants. We have examined the synaptic connections and their quantitative characteristics in the wild-type lamina cartridge of D. melanogaster, from a series of 600 consecutive transverse EM sections. Synapses are most readily itemised by comparison with those in the housefly, Musca, whose cells are larger (by 67% in receptor terminals) and allow examination of the effects of scaling cell size. By contrast, synaptic size differs little between the two.

The cells in the Drosophila cartridge have corresponding positions to and are presumed homologues of those in Musca, but their positional organisation is less orderly. Their interconnections differ from those in Musca as follows: L3 contributes at R1-6 afferent tetrads in the proximal lamina; L2 forms feedback synapses upon R1-6 but with L4, not T1, the second element of a postsynaptic dyad; L3 receives α input along with β at dyads; L5 receives rare inputs from C2; T1 receives α input at modified gnarls but there are no reciprocal T1 inputs upon α; α processes form a second feedback upon R1-6. Our observations on the wild type provide the first basis for the analysis in Drosophila of connectivity mutants that we hope will reveal the nature of the genetic control of selectivity during synaptogenesis.

Supported by grants A 0065 (NSERC) and EY03592 (NIH).

153.7

LIGHT-ADAPTATION OF VISUAL OBJECT SIZE SELECTIVITY IN IDENTIFIED INTERNEURONS OF DRAGONFLY. R.B.Pinter,R.M.Olberg,E.Warrant*,Univ.Wash.,Seattle;Union College,Schenectady;Australian Nat'l Univ.,Canberra.

In dragonfly ventral nerve cord eight large descending visual interneurons,identified by dye injection,have highly selective response to small visual objects moved in their visual field.The change in object size selectivity as a function of mean background luminance was measured intracellularly.The preferred object size consistently decreased with increase of mean luminance,from 10 deg.visual angle at low levels (.11cd/ft^2)to 1 deg.at outdoor midday sunlit levels (680cd/ft^2).Latency of response and incremental sensitivity also decreased with increase of mean luminance.The time course of shift of size preference on luminance decrease was less than 30 sec.,sometimes longer.We have measured photoreceptor receptive field width as function of similar mean luminance decrease(and increase)and found changes in width are insufficient to account for adaptation of size preference, which must therefore arise from changes in neural circuitry, such as efficacy of lateral inhibition. A deterministic nonlinear differential equation set describing nonlinear lateral inhibition is shown to be a sufficient model for size preference dependence on mean luminance. Supported in part by NSF grant BNS-8510188.

Olberg,R.M.(1986) Identified target-selective visual interneurons descending from the dragonfly brain.J.Comp.Physiol. A 159:827-840.

153.8

ULTRASTRUCTURE OF RETINAL DEGENERATION IN THE COMPOUND EYE AND OCELLI OF Drosophila. W. S. Stark and R. Sapp*. Div. Biol. Sci., Univ. of Missouri, Columbia, MO 65211.

Since Harris & Stark introduced rdgA & rdgB (J.Gen. Physiol.69,261-291,1977), Drosophila is a model of retinal degeneration. EM observations of receptors and their synapses underly this review of degeneration in white eyed flies from degeneration & excitation mutants vs aging in cyclic room light. rdgB has light induced degeneration of R1-6 but not R7/8 in the compound eye and not in ocelli. Higher order neurons are spared. rdgB's demise is blocked by ora (outer rhabdomeres absent, an allele of R1-6's opsin gene) and norpA (no receptor potential) but not trp (transient receptor potential or Acph- (Acid phosphatase null). The Acph- result was confirmed by microspectrophotometry (MSP). $rdgA^{BS12}$ has widespread degeneration of compound eye receptor somata. Some terminals and synapses survive; some ocellar receptors lose rhabdomeres and degenerate. $rdgA^{PC47}$ has a more gradual demise, preferentially in R1-6. Acph- does not slow rdgA's demise, confirmed by MSP. trp has degeneration in the compound eye but not in ocelli. ora remains intact. norpA has diminution of rhabdomeres, but only the scattered cell death of white eyed otherwise nonmutant flies reared for 3 weeks in cyclic room light (Stark et al., J. Neurogenet. in press). norpA loses rhabdomeres in the ocelli, but cells, feed forward and feed back synapses remain.

153.9

PHOTORECEPTOR SCREENING PIGMENT IN *LIMULUS* LATERAL EYE: DISTRIBUTION, MOVEMENT, AND CONTROL MECHANISMS. C.K. Kier* and S.C. Chamberlain (SPON: A. Ecker). Dept. of Bioengineering and Institute for Sensory Research, Syracuse University, Syracuse, New York 13244.

The radial and longitudinal distribution of retinular pigment in the lateral eye of *Limulus* was quantified with specialized point counting stereology under a variety of experimental conditions. Pigment position was characterized by the center and width of the radial distribution.

Under diurnal lighting, intact animals show movement of pigment granules from the periphery of the retinular cell at night towards the junction of the arhabdomeral and rhabdomeral segments of the retinular cell in the day. In total darkness, intact animals exhibit the same circadian rhythm. Animals with cut optic nerves (and thereby no circadian efferent input), show little pigment movement in diurnal lighting. In none of these cases did pigment granules move into the R-segment.

When dark-adapted animals are suddenly exposed to increments of light, pigment enters the R-segment. This phenomenon is not specifically associated with membrane shedding, but occurs whenever, and as often, as such exposures occur.

We investigated the effects of length of light increment (15 to 240 min) during the day (1600) and at night (0300). In both cases, pigment enters the R-segment, but the response at night is sluggish. Four hours of light exposure at night, however, moves the pigment to a daytime position.

Circadian efferent input, not diurnal lighting, appears to be the major determinant of screening pigment position. A second, faster mechanism is overlaid to provide protection during sudden light increments and appears to be optimized for daytime performance. Supported by NIH grants.

153.10

A LATERAL LINE ANALOGUE IN CEPHALOPODS. B.U. Budelmann* and H. Bleckmann* (SPON: J.I. Kitay). Marine Biomedical Inst., Univ. Texas Med. Branch, Galveston, TX 77550 and Div. Biol., Univ. Bielefeld, D-4800 Bielefeld, FRG.

For more than 75 years it has been a subject of discussion whether cephalopods, like aquatic amphibians and fish, are able to sense local water movements and thus have a system similar to the lateral line.

Naef (Fauna Flora Golfo Napoli, 35:1, 1928) was the first who mentioned 4-5 pairs of regularly arranged lines of epidermal cells that run in an anterior-posterior direction on their head and arms of embryonic cephalopods. Later, Sundermann (Cell Tissue Res., 232:669, 1983) described these cells as being ciliated, probably sensory, cells. We have now been able to demonstrate that these lines of cells serve to detect small water movements and thus are a further example of convergent evolution between a sophisticated cephalopod and vertebrate sensory system.

In the cuttlefish Sepia and the squid Lolliguncula, local water displacements, generated by a vibrating sphere, cause receptor potentials that are local to the lines and have many features known from the lateral line microphonic potentials. The minimal threshold of the lines on the head is 0.2 µm peak-to-peak water displacement. Besides physiological data, details on the gross-morphology of the lines and the ultrastructure of the hair cells will be presented.

Support: NSF, NIH, UTMB Annual Fund and German DFG grants.

153.11

HAIR CELL MORPHOLOGY IN THE STATOCYST OF THE SQUID *ALLOTEUTHIS SUBULATA*. R. Williamson*. (SPON: G.E. Meredith). The Marine Laboratory, Citadel Hill, Plymouth PL1 2PB, England.

The sensory epithelium of the octopus vestibular system is unusual in that it contains both primary and secondary hair cells (B.U. Budelmann et al., Phil.Trans.R.Soc.Lond.B.315:305, 1987). To ascertain whether both types of hair cells also occur in decapod cephalopods and to visualise the morphologies of the cells, hair cells in the squid statocyst were intracellularly injected with the dye Lucifer Yellow.

It was found that the hair cells on the dorsal side of the statocyst crista are primary hair cells with axons passing into the statocyst nerve. However, fills of ventrally located hair cells showed that many had short processes extending from their bases and some had long processes extending along, and parallel to, the length of the crista section. It was not possible to trace these processes into the crista nerve, either because they do not enter the nerve or because dye filling is incomplete.

As a test of whether these cells have an axon in the crista nerve, the nerve was backfilled with HRP from the brain cavity. This procedure clearly stained the primary hair cells on the dorsal side of the crista, the afferent neurons beneath the crista, and the afferent neurons on the ventral side of the crista section. However the hair cells on the ventral side of the crista were not stained.

Thus the squid does appear to have both primary and secondary hair cells in the statocyst crista but the latter, in contrast to descriptions of vertebrate hair cells, can have long processes extending along the crista section.

Supported by The Wellcome Trust.

153.12

SENSORY ORGANS OF THE THORACIC LEGS OF THE MOTH MANDUCA SEXTA. L. Griffin-Austin*, K.S. Kent, and R.B. Levine (SPON: T. Tobin). ARL Div. Neurobiol., Univ. Ariz., Tucson, AZ 85721

We have used cobalt filling, anti-HRP, and SEM to characterize sensory organs of the larval and adult thoracic legs and to examine the transition between stages. Externally, larval legs contain singly innervated mechanosensory hairs and few recessed dome-shaped sensilla (probably campaniform sensilla). Internally, they contain sensory organs (probably stretch receptors) consisting of clusters of small cells with dendrites extending into strands that attach at more distal sites within the leg. During prepupal stages, sensory neurons innervating external sensilla retract from the sensilla as the underlying epidermis retracts from the larval cuticle. For several days the sensory neurons remain associated with their sensilla via greatly elongated dendrites. As late as 2 days following pupation, some larval sensory neurons are still present within the new pupal leg suggesting that they may persist to serve as guides for the new adult sensory axons. All segments of the adult leg (except the claw) contain singly innervated flat hairs beneath the scales. Singly innervated dome-shaped sensilla located on the femur, tibia, and tarsus are probably campaniform sensilla. The coxa and trochanter contain hair-plates consisting of clusters of singly innervated sensilla. The tibia and tarsus contain multiply-innervated stout hairs which curve outward, extending beyond the scales. These sensilla contain marked surface indentations and dendrites extending within the lumen (suggesting an olfactory function). Singly innervated spines cover the tarsus, and several characteristic sensilla are present at the distal tip of the tarsus, including multiply innervated blunt hairs. The blunt hairs have small terminal indentations suggestive of a gustatory function. Internally, chordotonal organs are obvious within the femur and tibia. (NIH #NS28422 and NSF #8607066).

153.13

SEGMENTAL HOMOLOGY OF AUDITORY AND LEG SENSORY SYSTEMS IN LOCUSTS. H. Reichert and T. Meier*. Department of Zoology, University of Geneva, CH-1211 Geneva, Switzerland.

The locust ear is a peripheral sensory structure located on the first abdominal segment of the animal. It is composed of numerous auditory sensory neurons and an associated tympanal membrane. We use cellular, developmental and immunological methods to show that this sense organ derives in the embryo from a transiently expressed first abdominal proleg (pleuropod) that is lost after hatching. The sensory cells of the auditory system are organized and develop in this proleg in the same manner as do the chordotonal organs, groups of proprioceptive neurons, found in each of the true thoracic legs. Axonal pathfinding processes and axonal projections of the auditory sensilla into the central nervous system are segmentally equivalent to those made by the leg proprioceptors. However, the auditory sensory cells are established closer to the body wall and can thus survive proleg loss. The fact that the peripheral auditory system of locusts is segmentally homologous to leg proprioceptors suggests that parts of the central nervous organization of the auditory system, such as the G-neuron network, may also be an evolutionary elaboration of the circuitry associated with the sensorimotor control of an arthropod leg.

Supported by the Schweizerische Nationalfonds.

153.14

ENCODING PROPERTIES OF MECHANORECEPTIVE NEURONS ON THE FLY WING. M.H. Dickinson*(SPON:C.M. Lent). Dept. of Zoology, U of WA, Seattle, WA 98195.

Insect wings are flexible structures, subject to both active and passive bending during flight. Wings are equipped with a set of campaniform sensilla that act as strain gauges to monitor these deformations. White noise analysis has been used to examine the linear and non-linear characteristics of encoding in these mechanoreceptive neurons.

The encoding characteristics of these sensilla are accurately modeled as a linear filter followed by a zero memory rectifier. The linear filter is composed of the series of coupling processes that link wing strain to the transduction current. The sharp rectification is most-likely the result of the encoding process when the transduction potential is transformed into a train of action potentials.

The sharp rectification in these neurons might act to reduce timing errors in control circuits used during flight. When stimulated with pure sinusoidal inputs at wingbeat frequency, these neurons are strongly phase locked. The linear component of the encoding establishes the rough phase delay, while the rectification sharpens the response to a more precise delay value.

153.15

PHYSIOLOGICAL RESPONSES OF MECHANOSENSORY AFFERENT NEURONS INNERVATING THE LOBSTER SWIMMERET. K.A. Killian and C.H. Page. Dept. Biol. Sci., Rutgers Univ., Piscataway, N.J. 08855.

Lobsters have four pairs of abdominal swimmerets. These biramous appendages consist of two paddle-like rami attached to a stalk-like coxa. Mechanical stimulation of the swimmerets modulates both postural and swimmeret motor activity produced within the abdominal nerve cord (Cattaert,D. and Clarac, F., J.Comp.Physiol.160:55,1987; Kotak,V.C. and Page, C.H., J.Comp.Physiol.158:225,1986; 161:695,1987). To better understand these reflex responses, the properties of several mechanosensitive swimmeret afferents have been physiologically and morphologically investigated. These afferents include large and small feathered hair sensilla fringing the rami and subcuticular hypodermal mechanoreceptors. Two populations of large feathered hairs have been found on the swimmeret rami: 1) proximal feathered hairs, localized to the basal 2/3 of each ramus, and innervated by a single bipolar mechanosensory neuron responding phasically, with a single spike, to hair deflection and 2) distal feathered hairs located on the distal 1/3 of the rami. These hairs are not innervated by a mechanosensory neuron but are mechanically coupled to nearby hypodermal mechanoreceptors. Afferents innervating small feathered hairs respond to nearfield water vibrations and tactile stimulation.

153.16

DIVERSE MODULATORY EFFECTS OF PROCTOLIN, FRMRAMIDE, OCTOPAMINE AND 5-HT ON CRAYFISH MECHANORECEPTORS. V.M. Pasztor and D.L. Macmillan Biology Dept., McGill Univ., Canada and Zoology Dept., Univ. of Melbourne, Australia.

Specific elements of signals in primary afferents of a crustacean mechanoreceptor, monitoring ventilatory movements in *Homarus*, can be reversibly modified by neuromodulators (Pasztor and Bush, Neurosci. Abst. *13*, 1987). Responses from two of the three oval organ afferents are enhanced by proctolin and reduced by octopamine and 5-HT, whereas those from the other unit are unaffected. The present study compares three mechanoreceptors with very different response characteristics in the crayfish, *Cherax destructor*, to determine whether a relationship exists between receptor type and sensitivity to modulators.

Three mechanoreceptors were studied: 1. The abdominal muscle receptor organ (MRO) which has two spiking neurons, one with a phasic and one with a tonic response (Wiersma *et al.*, 1953). 2. The swimmeret non-spiking stretch receptor (NSSR) which has two similar units lacking impulse generation and using analog signals (Heitler, 1982). 3. The oval organ of the scaphognathite which has 3 units conducting both analog and digital signals, whose fibers range identifiably from the sparsely-spiking to the full-spiking condition (Bush & Pasztor, 1982).

The three mechanoreceptors and their individual sensory afferents have diverse responses to the modulators proctolin, FRMF-amide, Octopamine and 5-HT.

Supported by NSERC (Canada) and ARGS (Australia).

153.17

CRUSTACEAN PROPRIOCEPTION: SINGLE CELL ANALYSIS OF CHORDOTONAL ORGANS. Robin L. Cooper and H. Bernard Hartman. Dept. of Physiology, Health Sciences Center and Dept. of Biological Sciences, Texas Tech University, Lubbock, TX 79430, and Oregon Institute of Marine Biology, Charleston, OR 97420, U.S.A.

Joint receptors of Crustacea consist of elastic strands into which are inserted the dendrites of paired bipolar sensory cells. Recordings made from axons teased from nerves indicate that these cells signal movement, direction of movement and static position. Analysis of individual cells from the PD organ revealed an orderly arrangement of these neurons along the elastic strands according to function. We sought to determine if the chordotonal organs at the other joints are similarly arrayed. Extracellular recordings made from cell bodies and axons of the CP1 and MC1 as well as the CP2 and MC2 organs revealed cell position and size determines cell function. In each case, the most distal cells (10-20μm) are tonic and signal static positions. Proximal cells are phasic, responding to dynamic position and direction of displacement of the joint. Large proximal cells (30-40μm) fire upon strand relaxation while smaller proximal cells (20-30μm), most commonly seen in MC1 and CP1, respond to stretch of the strand. Cells sensitive to stretch of the elastic strands are more numerous than has been previously reported, but are still outnumbered by their counterparts. Supported by NSF grant BNS-8700506 to H.B.H.

153.18

TENSION RECEPTORS ON THE OPENER, PRODUCTOR, AND REDUCTOR TENDONS OF THE DUNGENESS CRAB CANCER MAGISTER. H. Bernard Hartman and Robin L. Cooper (Spon: R. Nathan). Dept. of Biol. Sci., Texas Tech Univ. and Dept. of Physiol., Health Sciences Center, Lubbock, TX 79430, and Oregon Inst. of Marine Biology, Charleston, OR 97420.

Tension receptors in arthropods were first described by Macmillan and Dando (Mar. Behav. Physiol. 1: 185, 1972) on the tendons of the powerful extensor and flexor muscles of the meropodite of walking legs. Hartman (J. Comp. Physiol. A 157: 355, 1985) examined the closer muscle and with rigorous physiological criteria proved that neurons there also respond specifically to muscle tension. To determine if tension receptors are to be found on the other tendons of the limbs, we examined the opener, productor, and reductor muscles of chelipeds and walking legs. The opener muscle exerts little force. Located along the lower margin at the distal end of the tendon are four sensory cells with the anatomical and physiological properties described for tension receptors. Upon emerging from the muscle, axons from these neurons immediately join the opener motor nerve. Receptors of typical anatomical profile and meeting the physiological criteria for tension reception are found on the productor and reductor tendons. Axons from these receptors form a nerve which exits near the CP1 organ and merges with its nerve; those from the reductor join the CP2 organ joint receptor nerve. Supported by NSF grant BNS-8700506 to H.B.H.

INVERTEBRATE SENSORY SYSTEMS II

154.1

PERSISTENCE OF THE EFFECTS OF BRIEF PERTURBATIONS IN NEURONAL OSCILLATORS. M. Holmgren*+, O. Diez-Martínez*+ç and R. Budelli*+ (SPON: E.J. Muñoz-Martínez). + Depto. de Ciencias Fisiológicas, Instituto de Ciencias, Universidad Autónoma de Puebla; ç Departamento de Fisiología, Facultad de Medicina, UNAM.

Abdominal slowly adapting stretch receptors (SASR) of crayfish were submitted to single pulse-like lengthenings (i.e. tugs). Neuronal discharges of SASR were recorded extracellularly. A large number of tugs were applied irregularly to the receptor muscle, at long intervals, and independently of SASR spikes. The perturbed interval was shortened with respect to the expected or natural interval (N); the shortening was a function of the phase. Weak stimuli affected the perturbed interval but the following ones were identical to N. Intermediate stimuli lengthened the first post-perturbed interspike interval (PPII), rarely affecting subsequent ones. Strong stimuli significantly prolonged the first PPII and sometimes the second and third. Thus, effects upon PPII depend on the stimulus parameters. We developed a computer program based on the model of Perkel et al. (Science, 145: 61, 1964) that, by varying the time constant of the threshold, reproduced our results and those of Buño and Fuentes (Brain Res., 303: 101, 1984) who reported phase compensation employing thoracic SASR. Discrepancies between results in abdominal and thoracic receptors can be explained on the basis of a greater sensitivity to velocity of the abdominal receptors.

154.2

TIMING OF ACTION POTENTIALS IN GIANT INTERNEURONS OF THE COCKROACH. J. Westin and R.E. Ritzmann. Department of Biology, Case Western Reserve University, Cleveland, OH 44106

The giant interneurons (GI's) of the cockroach are involved in triggering escape from wind. They are excited by sensory neurons, which, in turn, are excited by deflection of wind sensitive hairs on the cerci. The GI's are arranged in two groups in each connective of the nerve cord, with the larger ventral GI's having a shorter latency than the smaller dorsal GI's (Westin, et al, 1977).

It has been shown that the ventral GI's excite several populations of thoracic interneurons (TI's). PSP's produced by two GI's at the same time can summate dramatically (Ritzmann et al, 1988). We have now undertaken an investigation of the relative timing of action potentials (AP's) in the different GI's. We would like to determine the relative temporal properties of the various individual GI's and whether AP's of GI's within the ventral group with similar directional properties (eg. GI2 left and GI2 right), and which excite the same TI's, are timed to arrive in the thorax at the same time. If this is the case, we will explore the mechanism used for insuring synchronous arrival of action potentials.

To accomplish these goals we have recorded intracellularly from pairs of GIs in preparations set up in an apparatus that delivers reproducible wind stimuli from various different directions. Preliminary results show that, as expected, there is no relationship between the timing of action potentials in a ventral and a dorsal GI, even when their responses overlap. However, a reproducible relationship between the timing of individual ventral GIs does exist. For some pairs of ventral GI's, at least the first two to three AP's are synchronized.

Supported by NIH grant NS 17411.

154.3

MORPHOLOGICAL ANALYSIS OF CONNECTIVITY BETWEEN GIANT INTERNEURONS AND THORACIC INTERNEURONS. J.L. Casagrand and R.E. Ritzmann, Dept. of Biology, Case Western Reserve Univ., Cleveland, OH 44106.

The nervous systems of insects are functionally organized. Sensory neurons which receive input from particular regions project to predictable locations in the central nervous system. The giant interneuron system of the cockroach, Periplaneta americana, consists of two populations of identified giant interneurons, dorsal (dGIs) and ventral (vGIs), which mediate wind-evoked escape behavior. The GIs make a unique pattern of connections onto a population of identified thoracic interneurons (TIs). All of the TIs characterized as receiving vGI input have ventrally located midline (VM) branches in the general area of vGI axons. Some of the TIs have bilateral VM branches, while others have unilateral VM branches. The physiological data suggest that these VM branches are the site of connection with vGIs. We are examining overlap directly at the light microscopic level by filling GIs and TIs with different fluorescent dyes (Texas Red and Lucifer Yellow) in the same preparation and seeing whether the cells overlap, and if so, on which branch overlap occurs.

Preliminary experiments in which GI axons in one connective were lesioned early in development also support the idea that the VM branches receive GI input. In three cells which normally have symmetric VM branches, loss of all GI input on one side resulted in a reduction in the VM branch on the deprived side as compared to the nondeprived side. More lateral branches, which are believed to receive proprioceptive input from the legs, were not affected, nor were any branches from cells which do not receive GI input. Thus, selective effects were observed on those branches believed to receive GI input.

Supported by NRSA Training Grant HD 07104 to J.L.C. and NIH grant NS 17411 to R.E.R.

154.4

INPUT-OUTPUT RELATIONSHIPS OF SENSORY AFFERENT NEURONS IN THE CRICKET CERCAL SENSORY SYSTEM. M.A. Landolfa*, G.A. Jacobs and J.P. Miller. Grad. Group in Neurobiology, U.C. Berkeley, Berkeley, CA 94720.

The cercal sensory system of the cricket extracts and encodes relevant sensory information from the animal's air current environment. The inputs to this system consist of about 2000 afferent neurons, each of which innervates a single mechanosensory hair on the cerci. The input-output relationships of single sensory neurons were characterized by recording from an afferent axon while presenting appropriate stimuli to the sensory hairs. 1)Afferents are directionally sensitive. Graphs of afferent response amplitude versus wind stimulus direction are approximately sinusoidal, with distinct preferred and anti-preferred directions. 2)Afferents are velocity sensitive. Each afferent encodes wind velocity over a range of about 1.5 log units. 3)Different afferents have different velocity thresholds. The overlap of these different sensitivity curves insures that the system as a whole can encode wind velocities that span several log units. 4)The nature of the afferent response to deflection of its sensory hair indicates that the parameter transduced by the afferent is not hair displacement, but change in hair displacement. Thus, a significant portion of the processing which occurs within the cercal sensory system is accomplished at the level of the sensory afferents. Supported by: NSF BNS 85-19416.

154.5

DETERMINATION OF I/O RELATIONSHIPS OF CRICKET CERCAL SENSORY INTERNEURONS USING A MULTI-CHANNEL SPIKE DISCRIMINATOR. S.N. Gozani*, J.P. Miller and G.A. Jacobs. (SPON: E.R. Lewis) Departments of Computer Science and Zoology, UC Berkeley, Ca. 94720.

In the cricket cercal sensory system, information about the direction and velocity of wind stimuli is encoded by the relative firing rates of at least ten pairs of identified sensory interneurons. A full analysis of the input/output properties of this system requires that the activity of these output neurons be monitored simultaneously. We have implemented a computer based system capable of extracting the firing patterns of individual neurons from multi-unit recordings. Extracellular electrodes were arrayed along the abdominal nerve cord. Wind stimuli of varying directions, velocities and frequencies were presented to the animal. The responses of the cells were analyzed by spike discrimination software based upon an algorithm originally described by Roberts and Hartline (1975). The algorithm employs multiple linear filters, and is capable of discriminating spikes that are coincident in time. The number of spikes that could be discriminated was roughly equal to the number of independent recording electrodes. Using this tool we have characterized the output of the cercal sensory system in terms of the simultaneous activity patterns of ten identified interneurons. Supported by NSF BNS 85-19416.

154.6

ELECTRO-ANATOMICAL PARAMETERS OF SENSORY INTERNEURONS IN THE CRICKET CERCAL SENSORY SYSTEM. R. Nevin*, J.W. Tromp*, J.P. Miller and G.A. Jacobs. (SPON: D.A.Weisblat) Zoology Dept., UC Berkeley, Ca. 94720

Primary sensory interneurons in the cricket cercal sensory system extract information about the direction and velocity of wind stimuli from the afferent map of "wind space" within the terminal abdominal ganglion. The morphology of these 1st order interneurons determines their functional properties to a great extent. We have characterized the major structural features of these interneurons and determined the extent to which variability in their structure may affect their function. The structural features we have examined are: 1) location of dendrites within the afferent map, 2) total length of specific dendrites, 3) number and order of the branches, and 4) daughter-to-parent branch diameter ratios. Anatomical reconstructions were carried out using a computer controlled microscope integrated with a video frame grabber and graphics system, and software developed in the lab. This system allows 3-D reconstructions of neurons accurate to the limit of resolution of light microscopy. Based on this morphological analysis we have modeled the electrical properties of the neurons using SPICE. As a control to these modeling calculations, the effects of variations in structural features that are unresolvable at the light microscope level were also investigated. Supported by NSF BNS 85-19416

154.7

SENSORY ADAPTATION OF NEURONS INNERVATING THORACIC BRISTLES OF Drosophila melanogaster. G. Corfas* and Y. Dudai (SPON: I. Ginzburg). Dept. of Neurobiology, Weizmann Institute, Rehovot, Israel.

The macrochaetae that cover the thorax of *Drosophila* are each innervated by single sensory neurons that respond to deflection of the hairs. Stimulation ultimately elicits a behavioral response wherein the fly cleans the area covered by the bristles. This cleaning reflex undergoes habituation, which is abnormally brief in the memory mutant *rutabaga* (Corfas and Dudai, *Soc. Neurosci. Abs.* 13:388, 1987). To analyse the physiological basis of this behavior and its modification by experience, we have recorded with extracellular electrodes the receptor potential and the action potentials generated in the sensory neurons upon deflection of the hairs. Upon sustained deflection, and depending on the direction of the deflection, there is a negative receptor potential and a burst of superimposed positive action potentials that can reach a firing frequency higher than 250 Hz. The cells are rapidly adapting and fire for less than one second. The sensory neurons can also respond to brief (450 msec) transient deflections. Upon repetitive presentation of these brief stimuli, bursting activity diminishes and finally vanishes in a frequency dependent manner. At 0.2 Hz the cells continue to fire without substantial decrement for more than 100 stimuli. At frequencies higher than 0.5 Hz the response vanishes faster. The response recovers after 1-2 minutes of rest. The temporal characteristics of adaptation and the effect of memory mutations on this phenomenon are being investigated. (Supported by the Israel-US Binational Agricultural Research and Development Fund (BARD) and the Israel-US Binational Science Foundation, Jeursalem).

154.8

THE ROLE OF SCORPION TRICHOBOTHRIA IN WIND AND PREY DETECTION. E. J. Teufel*, S. C. Folta* and T. M. Root. Dept. of Biology, Middlebury College, Middlebury, VT 05753.

Trichobothria are fine sensory hairs on the pedipalps (claws) of scorpions. Some studies have suggested that they function in menotactic orientation or in prey detection, but experimental evidence to support these ideas is lacking. In response to weak air currents these hairs oscillate strongly in a single plane, and both the location of all 94 hairs and their planes of oscillation are consistent in every animal. Extracellular recordings from single trichobothria in response to direct mechanical stimulation showed phasic bursts of spikes from multiple receptor cells, but with no apparent directional differences. Similar recordings from freely-walking animals in response to both natural and simulated flying prey, however, showed directional attack behavior correlated with neural responses in the hairs. The attack behavior was lost when the hairs were removed. The findings suggest that trichobothria could mediate both wind orientation and prey detection by providing the animal with a precise sensory map of changes in air flow.

154.9

PHYSIOLOGY AND MORPHOLOGY OF HIGHER-ORDER NEURONS IN OLFACTORY PATHWAYS OF THE BRAIN OF THE MALE MOTH MANDUCA SEXTA. R Kanzaki, EA Arbas, NJ Strausfeld, JG Hildebrand. ARL Div. of Neurobiol., Univ. Ariz., Tucson AZ 85721.

In our ongoing studies of olfaction in Manduca, we have used intracellular recording and staining and reconstruction from serial sections to characterize the responses and structure of brain neurons that link the antennal lobes (ALs) with the protocerebrum (PC) (n=27), neurons local to the PC (n>40), and neurons that descend from the brain in the ventral nerve cord (n>10). Neuronal arborizations in the ALs were restricted either to the male-specific macroglomerular complex (MGC) or to ordinary glomeruli (Gs). Neurons innervating the Gs branched in a single G (uni-glomerular), many or all Gs of one AL (multi-glomerular), or in one case, single Gs in both ALs (bilateral uniglomerular). Bombykal or crude sex pheromon extracts excited all MGC neurons, although some were inhibited by other odors. G neurons were excited or inhibited by non-pheromonal odors. Some also showed mechanosensory responses. Both MGC and G neurons typically showed prominent branches in the calyces of the ipsilateral mushroom body and in the lateral protocerebrum with some differences in termination sites of the two classes noted. Neurons local to the PC and descending neurons showed diverse responses to the odors tested. Examples will be discussed. Many neurons of the latter two classes were multimodal, also showing responses to visual stimuli.
[Supported by NIH grant NS 23405.]

154.10

IMMUNOCYTOCHEMICAL IDENTIFICATION OF OLFACTORY RECEPTOR NEURONS FROM MALE *MANDUCA SEXTA* PUPAE IN VITRO M. Stengl, J.G. Hildebrand, ARL Div. of Neurobiol., Univ. of Ariz., Tucson, Arizona 85721

Only *Manduca* males are able to detect the females' sex pheromones with sex-specific antennal sensory hairs, the long trichoid sensilla. The olfactory receptor neurons (ORNs) innervating olfactory sensilla are not easily accessible for electrophysiological recordings in situ. In order to study the development of excitable membrane properties and the olfactory transduction processes in the ORNs, we have developed a primary cell culture system of ORNs from *Manduca sexta* pupae.

Because conventional morphological criteria could not be used to distinguish neuronal from non-neuronal cells, the ORNs were identified immunocytochemically with the help of two antibodies that are known to stain ORNs in vivo. These were: the OSA-Ab (olfactory specific antibody) and the MOSA-Ab (male olfactory specific antibody) [*J. Neurosci* 8:296-307; 308-315 (1988)]. Both antibodies, which specifically recognize ORNs in intact *Manduca* at defined times in development, labeled cells at specific times in antennal-cell cultures, in good correlation with the in situ findings. These immunocytochemical results make anatomical identification of cultured ORNs possible by assigning certain morphological characteristics to OSA- and MOSA-antigenic cells. Patch-clamp recordings will be used next to determine the physiological properties of the ORNs at different stages of development in vitro.
[Supported by NIH Grant AI-23253 and Monsanto Company.]

154.11

VOLTAGE-GATED CURRENTS IN INSECT CENTRAL OLFACTORY NEURONS IN PRIMARY CULTURE. J.H. Hayashi and J.G. Hildebrand ARL Div. of Neurobiol., Univ. Ariz., Tucson, AZ 85721. Studies of the antennal lobe (AL) of the moth Manduca sexta have revealed much about physiological mechanisms underlying central processing of olfactory information. Attempts to study membrane currents using voltage-clamp methodology, however, have been frustrated by an inability to achieve space clamp owing to the extensive arborizations of the neurons. Thus we have dissociated cells from pupal ALs and grown them as solitary cells with limited arbors. We find at least five different types of neuron-like cells based on their morphology in culture and immunoreactivity to GABA antisera. In current-clamp recordings, some of the cells in culture generate action potentials as do neurons in intact ALs. One morphologically distinct cell type responds to a depolarizing current pulse in a manner that resembles an AL interneuron in situ: it displays a burst strikes of variable height that outlasts the stimulus. We examined cells grown in culture for 3-21 days with the whole-cell patch-clamp technique. Cells of a particular morphological class have similar, characteristic voltage-gated membrane currents. Of particular interest are cells that display a fast inward Na^+ current that apparently gives rise to the action potential. These cells also have a delayed rectifier K^+ current, which does not inactivate within the 50 msec pulse. In other cells we found an "A" type K^+ current that activates rapidly (t_{peak}<10 msec), inactivates slowly ($t_{0.5}$>50 msec), and is abolished at holding potentials positive to -40 mV. (Supported by NIH AI-23253, Monsanto Co., and an NIH postdocoral fellowship.)

154.12

RESPONSES OF OLFACTORY INTERNEURONS IN THE BRAIN OF LARVAL *MANDUCA SEXTA*. H. Itagaki and J.G. Hildebrand. ARL, Div. Neurobiol., Univ. Arizona, Tucson, AZ 85721.

Behavioral experiments have shown that caterpillars use odor cues for the initial location and identification of foodplants (e.g. Dethier, 1937, 1941; Hanson and Dethier 1973). Caterpillars possess a relatively small number of olfactory receptor neurons (32 cells in the 2 antennae) with which to identify possible foodplants (Schoonhoven and Dethier, 1966). To investigate how so few receptors can mediate the complex task of foodplant choice in lepidopterous larvae, we study central olfactory mechanisms in tobacco hornworms (larval *Manduca sexta*).

Using standard intracellular recording techniques, we have recorded from over 1700 neurons in larval *Manduca* brains while stimulating the antennae with foodplant odors (tomato, tobacco) and with an important constituent of the volatiles given off by leaves (E-2-hexenal). We recorded excitatory responses in about 75% of the responding neurons, inhibitory responses in 21%, and mixed responses in 4%. We have found no evidence of specific "labelled lines" in the neurons: most olfactory interneurons are broadly tuned in their response spectra. Dose-response curves with E-2-hexenal show that different interneurons have different responses to a single dose of odorant: a finding consistent with mediation of plant recognition being by the "across-fiber" firing pattern of the neurons. (Supported by USDA grant 87-CRCR-1-2362, NIH Postdoctoral Fellowship 1-F32-NS07990, and the Monsanto Company.)

154.13

PROJECTION NEURONS IN THE GYNANDROMORPHIC ANTENNAL LOBES OF FEMALE *MANDUCA SEXTA* WITH GRAFTED MALE ANTENNAE. T.A. Christensen and J.G. Hildebrand. ARL Div. of Neurobiol., Univ. Arizona, Tucson, AZ 85721.

Previous studies using trans-sexually grafted antennae have shown that pheromone-sensitive sensory axons in the antennal nerve (AN) can induce (or permit) the formation of the male-specific macroglomerular complex (MGC) in the antennal lobe (AL) of female moths (Schneiderman et al., *Nature 298*:844; 1982). In normal males, the MGC is the area of neuropil where axons from pheromone receptors on the ipsilateral antenna terminate. Local and projection neurons that process pheromonal information also arborize in the MGC. In female gynandromorphs with grafted male antennae, at least some local neurons were found to be similar to those in normal male ALs, but the projection neurons that convey olfactory information to higher-order centers in the protocerebrum were not examined.

Out of 87 gynandromorphs examined, 36 had an AN innervating the AL. We obtained stable intracellular recordings from 18 neurons. Nine cells were excited by electrical stimulation of the AN or pheromonal stimulation of the antenna. One such neuron had dendritic arbors in the induced MGC and sent an axon through the inner antenno-cerebral tract to the protocerebrum, as do comparable neurons in normal male ALs. The other 9 cells responded to pheromone either with a mixed response (4 cells) or with complete inhibition (5 cells). One of the inhibited cells was identified as a projection neuron with dendrites confined to an "ordinary" (non-sexually dimorphic) glomerulus. Such cells in normal males have never been shown to respond to sex pheromones. Cobalt staining of trichoid sensilla on the grafted antenna revealed that some pheromone-receptor axons terminate in areas other than the MGC in the gynandromorphic AL.
[Supported by NIH grant AI-23253].

154.14

CODING IN OLFACTION: EFFECT OF STIMULUS CONCENTRATION ON QUALITY CODING BY CELL POPULATIONS IN SPINY LOBSTERS. M.-N. Girardot and C.D. Derby. Dept of Biology, Georgia State University, Atlanta, GA 30303

The primary olfactory tract lobster identifies chemicals by the absolute response magnitude (ARM) and the relative response magnitude (across-neuron pattern, ANP) of cell population; the former seems to encode concentration, the latter, type. The present study is aimed toward evaluating these differential effects of concentration and type, and the effects of concentration on quality coding, using a discrimination index (D.I.) for each stimulus, which takes into account the Euclidean distances within-type (between the 3 concentrations) and between-type (between the 3 concentrations of a type and all other stimuli). The distances were derived from the stimulus coordinates resulting from multidimensional scaling applied to extracellular responses of neurons isolated from the antennular nerve to stimulation of the antennule with either four types of natural extracts, or four types of artificial mixtures based on the chemical composition of the extracts. All stimuli were applied at .005, .05 and .5 mM. The results confirm that stimulus concentration is encoded predominantly by ARMs, and slightly (but only for the artificial mixtures) by ANPs, and that stimulus type is encoded essentially by ANPs. They further demonstrate that the stimuli could not be identified by their overall population responses because of stimulus concentration. For the natural extracts, concentration affects quality coding only by its effect on ARM: removing this effect improved mean D.I. from -.11 to .49 ($0<D.I.<1$, $D.I.\leq 0$ = no discrimination). For the artificial mixtures, the effect of concentration was divided between ARM and ANP: sequentially removing these two effects improved mean D.I. from -.10 to .23 to .37. The results have important implications for choice of parameter in the field of chemical quality coding.
Supported by NINCDS grant NS22225 and a grant from the Whitehall Foundation.

154.15

PROJECTION PATTERN OF OLFACTORY NEURONS IN CRAYFISH BRAIN. DeF. Mellon and S. Munger. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

In the crayfish, Procambarus clarkii, the primary olfactory receptor organs are blunt antennular hairs called aesthetascs. Within each aesthetasc are branched distal dendrites from about 175 functionally heterogeneous primary olfactory neurons in a ganglionic cluster. In older Procambarus there are more than 150 aesthetascs; thus the major olfactory field comprises about 30,000 elements on each side. Axons of the primary olfactory neurons run within the antennular nerve to the brain and enter the ipsilateral olfactory lobe. Each olfactory lobe is comprised of local, afferent, and efferent fiber tracts, and regions of dense neuropile organized into more than 100 columnar arrays, or glomeruli. We used axoplasmic transport of tritiated leucine to examine the afferent projection to the olfactory lobe. If each sensory neuron penetrates only one glomerulus, several patterns are possible: 1) sensory neurons from any one aesthetasc all may project to the same glomerulus; 2) functionally identical neurons in every aesthetasc may project to the same glomerulus; 3) there may be random projection. By restricting uptake and transport of tritiated leucine to just a few aesthetascs we could test the validity of possibility #1, since the number of labeled glomeruli should then equal the number of exposed aesthetascs. Instead, we found uniform labeling of every glomerulus in the ipsilateral olfactory lobe, although the density of label was considerably less on the experimental side compared to the maximally exposed control side. Our data eliminate possibility #1, suggesting multiglomerular targeting by neurons in each sensory ganglion. Supported by the Whitehall Foundation.

154.16

IDENTIFIED DESCENDING INTERNEURONS IN THE MALE GYPSY MOTH RESPOND TO COMBINATIONS OF SEX-PHEROMONE, VISUAL AND WIND STIMULI. M.A. Willis* and R.M. Olberg. *Dept. of Entomology, University of Massachusetts, Amherst MA 01003 and Dept. of Biological Sciences, Union College, Schenectady NY 12308.

Like many insects, gypsy moths use an airborne pheromone for mate location. In response to female pheromone, the male flies a zigzagging course upwind. This anemotactic behavior is visually guided, the moth detecting his drift relative to the environment to determine wind direction and velocity.

In an effort to understand the neural basis for this complex orientation behavior, we studied the responses of large descending interneurons to pheromone, visual pattern movements, and wind. We presented varying levels of these three stimuli alone and in combinations designed to simulate conditions experienced in flight.

About 80% of axons penetrated with microelectrodes showed responses to the female sex-pheromone, and of these, most also responded to other modality stimuli. After recording sensory responses we injected each neuron with Lucifer Yellow. Pheromone-sensitive descending neurons originate in the protocerebrum and run dorsally in the VNC, passing through the prothoracic ganglia via the median dorsal tract and extending to the pterothoracic ganglia.

Of particular interest is an interneuron whose visual responses are gated by pheromone. This cell showed no obvious response to either onset or offset of pheromone or to wide-field visual pattern movement. When the same visual stimuli were presented with pheromone in the airstream the neuron became strongly directionally selective, with leftward and forward movements inhibiting it and backward and rightward movements exciting it. Such pheromone-modulated visual responses are what we might expect from the neural elements involved in steering the complex upwind flight path of these insects.

154.17

INPUT-OUTPUT ORGANIZATION OF THE SNAIL PROCEREBRUM. R. Chase and B. Tolloczko*. Dept. of Biology, McGill Univ., Montreal, Quebec, H3A 1B1.

The procerebrum is a distinctive region occupying one-third the total volume of the brain in all terrestrial molluscan species. Hänstrom, in 1925, described a massive afferent input via the tentacular (olfactory) nerve and an intrinsic population of small neurons having no axons. However, a neural pathway has not yet been identified leading from the procerebrum to any other part of the CNS. We have investigated the functional connections of the procerebrum by a series of labelling experiments employing the anterograde and retrograde movement of hexamminecobalt. We have identified a population of about 20 neurons in Achatina fulica which send lengthy dendrites into the procerebral neuropile from the pedal ganglion. These are small cells, arranged in 2 groups at a posterior, medial position on the dorsal surface of each pedal ganglion. With the EM, we see labelled postsynaptic processes in the procerebrum after application of hexamminecobalt to the cerebral stump of the cerebro-pedal connective. The procerebral neuropile contains a finely textured intermingling of processes derived from the tentacular nerve (input), the procerebral neurons (local interneurons) and the pedal neurons (output).

TRANSMITTERS IN INVERTEBRATES II

155.1

RESPONSES OF THE SPINE LIGAMENT OF THE SEA URCHIN EUCIDARIS TRIBULOIDES TO CHOLINERGIC DRUGS. M. Morales*, J. del Castillo* & D.S. Smith*+. (spon. by C. Zuazaga), Inst. of Neurobiol. UPR-MSC, 201 Blvd. del Valle, San Juan, Puerto Rico, 00901 and + Dept. of Zoology, U. of Oxford, Oxford OX1 3PS, England.

The spine-test articulation of the sea urchin is surrounded by a collagenous ligament whose consistency can change reversibly from compliant to stiff. Acetylcholine (ACh) induces stiffness at concentrations as low as 10^{-7}M (Takahashi, K., J. Fac.Sci.U.Tokyo, 11:121-130,1967) suggesting the presence of ACh receptors. To characterize them we have examined the effects of several compounds: carbamylcholine, nicotine, methacholine, tetramethylammonium, choline and d-tubocurarine induced stiffness, while dimethylphenylpiperazine, a potent agonist, and decamethonium relaxed it. Muscarine and atropine had weak and inconsistent effects thus discarding the presence of muscarinic receptors.

It seems improbable that the observed responses are mediated by conventional nicotinic receptors. However, all the drugs tested are likely to establish ionic bonds with the acidic sites of the proteoglycans. The response (relaxation or stiffening) resulting from this interaction may reflect the overall physicochemical properties of the compounds bound to the matrix rather than a chemical complementarity.

(Supported by NIH grants NS-07464 and NS-14938).

155.2

LOCALIZATION OF DROSOPHILA CHOLINERGIC NEURONS WITH IN SITU HYBRIDIZATION. R.P.Barber,* M.Lee,* H.Sugihara,* P.M. Salvaterra, and J.E.Vaughn. Div. of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

An antisense cRNA probe complementary to Drosophila ChAT mRNA has been localized within the cortical rind and primary sensory neural regions of Drosophila using *in situ* hybridization methods. Substantial concentrations of the probe were observed in cellular regions of the cerebral and optic lobe cortices. Hybridized probe within the monopolar cell layer of the lamina appeared to be homogeneously distributed. This contrasted to that in other cortical regions where the probe concentration in some areas was very heavy, whereas in others it ranged from moderate to none. No significant amount of probe was found in cell body sparse neuropil regions. There were substantial amounts of hybridized probe in the antennae and significant amounts in the compound eye. The sense (control) probe, a sequence of RNA not complementary to ChAT mRNA, did not hybridize in significant amounts in either cortical or neuropil regions. A quantitative analysis revealed that the highest concentration of probe was in the laminae and cerebral cortical regions. About half of that concentration was observed in the second and third order optic lobe cortex and in the antennae. The probe was less concentrated in the compound eye than in the antennae, possibly reflecting the reduced density of retinule cell packing. Supported by NIH grant NS18858.

155.3

CHOLINERGIC MODULATION OF AN IDENTIFIED MOTONEURON AND ITS AFFERENT INPUT IN THE NICOTINE-RESISTANT INSECT MANDUCA SEXTA. B.A. Trimmer and J.C. Weeks. Dept. of Entomological Sciences, Univ. of California, Berkeley, CA 94720.

The tobacco-feeding insect Manduca sexta, like other insects, uses acetylcholine (ACh) as its primary sensory neurotransmitter. Thus, unitary EPSPs evoked in the motoneuron PPR by direct afferent input from mechanosensory hairs can be blocked with cholinergic antagonists. Our studies suggest that nicotinic ACh receptors in M. sexta may be adapted to have a low affinity for nicotine itself. In addition, muscarinic agents evoke responses in PPR that are distinct from those caused by nicotinic agents. These effects include agonist-induced suppression of the afferent EPSPs, resting potential oscillations, and a decrease in the spike threshold. Local application of muscarine by iontophoresis suggests that the change in spike threshold is mediated by receptors on PPR itself. Muscarinic antagonists can block these effects and also potentiate the unitary EPSP. These results are consistent with the presence of modulatory postsynaptic muscarinic receptors and another group of inhibitory receptors that act presynaptically to regulate ACh release from the afferents. We are presently using physiological and biochemical methods to determine the location and actions of these putative muscarinic acetylcholine receptors. Supported by a research grant from the Whitehall Foundation.

155.4

IMMUNOCYTOCHEMICAL LOCALIZATION OF HISTAMINE IN THE PHOTORECEPTORS AND SEGMENTAL GANGLIA OF THE BARNACLE, *BALANUS NUBILUS*. J.C. Callaway, A.E. Stuart, and J.S. Edwards. Dept. Zoology, Univ. of Washington., Seattle, WA 98195 and Dept. Physiol., Univ. of North Carolina, Chapel Hill, NC 27599.

Recent biochemical (M.S. Elias and P.D. Evans, *J. Neurochem.*, **41**:562-568, 1983) and physiological (R.C. Hardie, *J. Comp. Physiol.*, **161**:201-213, 1987) studies designate histamine as an insect photoreceptor (PR) neurotransmitter. The barnacle also appears to use histamine as a PR transmitter. Histamine is synthesized from histidine in PRs and also mimics the action of transmitter on second-order visual cells (A.E. Stuart and J.C. Callaway, *Invest. Opthal. Vis. Sci.*, **29**:223, 1988).

To determine whether histamine is localized to the PRs we used an antiserum provided by P. Panula against a histamine-protein conjugate (P. Panula et al., *J. Histochem. Cytochem.*, **36**:259-269, 1988). The antiserum labeled each of the barnacle PRs. The terminals were intensely labeled. Though label could be detected in the PR axons and cell bodies, it was sparse and appeared in small punctate granules rather than spread evenly through the cytoplasm. Incubation of PRs with 100μM histidine 18 hrs. prior to fixation significantly increased the signal in the cell bodies, but it was still punctate.

In the segmental ganglia of the barnacle only eight neurons were labeled. Label was found in the cell bodies, neurites, and terminals and was not obviously punctate. These cell bodies were located in only the most posterior segments of the fused ventral ganglion but had had an extensive network of processes and endings in every segment of this ganglion. None of these cells sent processes to to supraesophageal ganglion which contains the PR terminals. Supported by NIH grants NS07778 to JSE and EY03347 to AES.

155.5

HISTAMINE IMMUNOREACTIVITY IN THE COCKROACH BRAIN AND VISUAL SYSTEM. U. Pirvola *and P. Panula. (Spon. L. Kivipelto). Department of Anatomy, University of Helsinki, Finland.

The distribution of histamine immunoreactivity (HA-ir) in carbodiimide-fixed brain of the cockroach, Blaberus craniifer, was revealed immunocytochemically with an antiserum against HA. The results showed a widespread distribution of HA-ir somata and fibers in the brain, particularly in the visual system. The most intense immunolabelling was seen in the retinal photoreceptors and in the lamina, where short visual fibers terminate. HA-ir long visual fibers as well as reactive monopolar neurons traversed the outer chiasma and terminated in the distal medulla. HA-ir inside photoreceptors was revealed also in samples prepared for electronmicroscopy. HA-ir neurons and tracts of immunolabelled fibers were seen as well in other parts of the optic lobes and in the protocerebrum. Central parts of the brain, including the central body, contained strongly fluorescent varicose processes. In addition, substantial HA-ir was seen in the antennal lobes, but not in the antennal nerves. These results support strongly the hypothesis of HA acting as a neurotransmitter in the insect central nervous system and particularly in the photoreceptors.

155.6

GABA-IMMUNOREACTIVITY IN THE BRAIN OF LARVAL AND PUPAL *MANDUCA SEXTA*. U. Homberg and J.G. Hildebrand ARL Div. of Neurobiol., Univ. of Ariz., Tucson, Arizona 85721

The sphinx moth *Manduca sexta* is a holometabolous insect with 5 larval instars and adult development of about 3 weeks (18 pupal stages P0-P17). During metamorphosis, its nervous system undergoes dramatic changes: some neurons die, many neurons are remodeled, and many new neurons are born [*TINS* 9:315 (1986)].

We have investigated the distribution of GABA-immunoreactivity (GI) in the larval brain of *Manduca* and changes in GI during metamorphosis. GI in the adult brain has been studied previously [*Cell Tiss Res 248*:1 (1987)].

In the larva, about 300 cells per brain hemisphere exhibit GI. All brain neuropils are innervated by immunoreactive fibers. The staining pattern and the number of immunoreactive cells change little during the 5 larval instars. Shortly before pupal ecdysis, GI starts to decrease and later disappears in many neurons in the central brain, while it first appears in the optic lobes and in a thin layer of fibers in the central body. GI in the ventro-lateral protocerebrum and in the circumoesophageal connectives remains unchanged. Starting at pupal stage P5, the adult pattern of GI slowly emerges. In lamina monopolar cells, mushroom-body Kenyon-cells, and some cells in the pars intercerebralis, GI is expressed in the pupa but disappears before adult ecdysis.

The changes in GI partly correlate with peaks in ecdysteroid-titers. The results suggest that many larval GABA-immunoreactive cells do not express GI during morphological reorganization or die, while others express GI only during a certain period of pupal life. [Supported by NIH Grant AI-23253 and Monsanto Company.]

155.7

THE GABA RESPONSE OF THE ISOLATED HEART OF LIMULUS POLYPHEMUS EXHIBITS NOVEL PHARMACOLOGY. J.A. Benson, R & D Plant Protection, Agricultural Division, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland.

GABA (gamma aminobutyric acid) causes a dose-dependent decrease in the beat frequency of the isolated heart of Limulus. The GABA receptor or receptors mediating this response are potently activated by muscimol and isoguvacine, $GABA_A$ agonists. Baclofen, the diagnostic $GABA_B$ agonist, is without effect. However, bicuculline, the diagnostic $GABA_A$ antagonist does not block the GABA-induced inhibition at concentrations up to 10^{-4} M and the response is affected only weakly or not at all by pitrazepine, a more potent $GABA_A$ antagonist. Picrotoxin, which blocks $GABA_A$ Cl^- channels, reduced the GABA response weakly or not at all (up to 10^{-4} M). Taurine and glycine are inactive. The response is not modulated by 10 min. preapplication of the benzodiazepine flunitrazepam (10^{-6}, 10^{-5} M) nor by sodium pentobarbital (10^{-6} - 10^{-4} M). Thus, although exhibiting $GABA_A$ agonist characteristics, the Limulus heart GABA response differs in pharmacological profile from both of the vertebrate GABA receptor types, and does not seem to be functionally associated with either benzodiazepine or barbiturate modulatory receptors.

155.8

A MAP OF THE GABAergic NEURONS IN SEGMENTAL GANGLIA AND THE STOMATOGASTRIC SYSTEM OF THE CRAYFISH. B. Mulloney and W.M. Hall*. Dept. of Zoology, Univ. California, Davis CA 95616.

Gamma-aminobutyric acid (GABA) is known to be a transmitter at inhibitory peripheral synapses in crustaceans (Otsuka, et al. 1966). We have mapped the locations of all the nerve cell bodies that show GABA-like immunoreactivity in the segmental ganglia and stomatogastric system of the crayfish, *Pacifastacus leniusculus*, using a polyclonal antibody specific for GABA fixed *in situ* (Hoskins, et al. *Cell Tis. Res.* 244: 243-252 1986).

To localize GABA reliably, we fixed the nervous system by perfusion under pressure. Primary fixation with 4% formaldehyde-0.1% glutaraldehyde in PBS at pH 7.4 for 15 min was followed by a secondary fixation with picric acid-formaldehyde for 6 hr. To label GABAergic neurons in whole-mounts, ganglia were desheathed, washed in PBS, dehydrated to 70% EtOH overnight, rehydrated and soaked in a blocking solution with 0.03% Triton X-100. Ganglia were incubated with primary antibody (1:750) for 24 hours, washed repeatedly with blocking solution, and incubated overnight with an FITC-conjugated secondary antibody, before being dehydrated, cleared and photographed.

These methods label known GABAergic motor neurons in each abdominal ganglion, e.g. FI and fi, but fail to label other motor or sensory neurons that run in the same peripheral nerves. Therefore, interneurons and other motor neurons labeled by these procedures are probably also GABAergic.

In ganglia that innervate swimmerets, each nerve to a swimmeret contained three labeled axons, one projecting to return-stroke muscles and two to power-stroke muscles. Other labeled neurons in the OG, CGs, SOG and terminal abdominal ganglion will also be described.

Supported by NSF Grant BNS 84-06931 and NIH grant NS21194.

155.9

EVIDENCE THAT GABA IS A NEUROTRANSMITTER IN THE CRAYFISH MEDULLA. C.L. Pfeiffer, L. Wang-Bennett, and R.M. Glantz, Department of Biology, Rice University, Houston, TX. 77001.

There is growing evidence that Gaba is a neurotransmitter in arthropod optic lobes. We examined the distribution of Gaba in the eyestalk of the crayfish with an antisera to a Gaba-glutaryl-protein conjugate. Tissues were fixed in a modified Bouin's fixative. Optic lobes and cryostat sections were incubated in primary antibody at 1:1000. Reactive sites were visualized by the PAP procedure. Controls included preabsorption with immunogen and tests for nonspecific secondary antibody binding. Gaba-like reactivity was observed in photoreceptor R8; in the outer and inner lamina cell body layers and in a thin layer of lamina plexiform. Reactivity was also strong in 3 layers of each medullary neuropile (ME, MI), the medullary cell body region and in localized regions of the medulla terminalis.

Pharmacological actions of Gaba were examined in sustaining fibers (SF), tangential (Tan 1) and medullary amacrine cells (MA) in the ME. SFs are tonic "on" interneurons, synaptically depolarized by light and subject to surround inhibition by MA. The MAs act presynaptic to the SFs. Gaba and muscimol mimic the action of MA on SF light response. MA neurites also overlie Tan 1 dendrites. Tan 1 exhibits an Ach mediated, chloride dependent hyperpolarization to light and depolarize to light off. Gaba and muscimol mimic the light off response. Thus, Ach and Gaba have antagonistic effects on medullary interneurons. These results support the hypothesis that Gaba is an important neurotransmitter in the ME. Supported by NSF Grant BNS87-11141.

155.10

EFFECTS OF ACH AND GABA ON CONTRACTIONS OF THE OPENED FILARIID, DIPETALONEMA VITEAE. D. Christ, M.J. Goebel* and H.J. Saz*. South Bend Center for Medical Education, Indiana University School of Medicine and Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556.

ACh and GABA are putative neuromuscular transmitters in nematodes, however they are relatively inactive on intact D. viteae. The cuticle may block the penetration of these substances; thus ACh and GABA were tested on adult, female D. viteae which were isolated from hamsters, slit open longitudinally, and mounted in an isolated tissue bath containing warmed, aerated physiological solution. ACh (10^{-7} M) induced rapid, reversible increases in the spontaneous isotonic contractions. At higher concentrations contractures were produced. Nicotine (10^{-6} M) and pilocarpine (10^{-6} M) were also effective agonists, however their potencies were similar to the potencies on intact D. viteae. The cholinoceptor antagonists, atropine, d-tubocurarine and hexamethonium, were relatively inactive in reducing either spontaneous activity or the effect of ACh. GABA (10^{-7} M) depressed the spontaneous activity. This effect was rapid in onset and readily reversible. Muscimol (10^{-5} M) had a similar action, however the potency of muscimol was the same as in the intact D. viteae. The GABA antagonists, picrotoxin and penicillin G, did not affect the action of GABA. These results indicate that ACh and GABA do not readily penetrate the body wall of D. viteae. Also, the filariid receptors have unique pharmacological properties. (Supported in part by NIH Grant AI 09483)

155.11

THE PRESENCE OF DOPAMINE IN NERVE RICH TISSUE OF THE HYDROZOAN *Polyorchis penicillatus*. J.M. Chung* and A.N. Spencer, Dept. Zoology, Univ. of Alberta, Edmonton, AB, CANADA T6G 2E9.

Several lines of morphological and physiological evidence support the existence of chemical transmission in the Cnidaria. However, there are no conclusive data on the nature of these neurotransmitters. Some histochemical and chromatographic studies implicate the biogenic amines in several cnidarian species while other studies deny the presence of known catecholamines. In the present study, we have quantitatively demonstrated the presence of dopamine (DA) using HPLC with electrochemical detection (LCED) and confirmed its presence qualitatively using gas chromatography/mass spectrometry (GC/MS) in the hydrozoan *Polyorchis penicillatus*.

Selected tissues were dissected from live animals starved for a minimum of 2 days and treated with activated alumina to extract catecholamines. The alumina extracts gave complex chromatograms with peaks having retention times similar to those of norepinephrine (NE), epinephrine (E), dihydroxyphenylalanine (DOPA), and DA. We therefore used two sets of LCED conditions (LCED-1 and -2), in which the columns and mobile phases were different. The chromatograms using LCED-1 showed peaks which retention times corresponding to NE, E, and DA while the chromatograms using LCED-2 showed peaks corresponding to DOPA and DA. GC/MS confirmed the presence of DA. Alumina extracts were dried, derivatized using trifluoroacetic anhydride and ethyl acetate, and then introduced to the GC/MS. Negative ion chemical ionization mass spectra and selective ion current chromatograms demonstrated the presence of DA and not NE, E, DOPA in tissue samples of *Polyorchis penicillatus*.

Three types of tissue were analyzed: nerve-rich tissue from the margin, endoderm-rich tissue from the radial canals, and mesoglea. Nerve-rich tissues contain DA at the highest concentration (120±30 fmol/mg wet wt.) followed by endoderm-rich tissues and mesoglea (30±5 fmol/mg wet wt. and 10±1.5 fmol/mg wet wt., respectively). Any substances existing as a neurotransmitter can be expected to be found in relatively higher concentrations in nerve-rich tissues than tissues having few or no neurons. Thus, these results suggest tentatively the biological role of DA as a putative neurotransmitter in the hydrozoan *Polyorchis penicillatus*.

(Supported by NSERC grant A0419 to A.N. Spencer)

155.12

OCTOPAMINE IMMUNOREACTIVE NERVE TERMINALS ARE FOUND ON A SINGLE IDENTIFIED MUSCLE FIBER OF THE *DROSOPHILA* LARVAL BODYWALL. M.E. Halpern, M.S. Anderson, J. Johansen and H. Keshishian. Dept. of Biology, Yale University, New Haven, CT 06511.

Previously we described the segment and muscle fiber specific distribution of proctolin nerve terminals on the bodywall musculature of *Drosophila* larvae. Using antisera against the neurotransmitter octopamine, we have discovered that octopamine immunoreactive nerve endings are located on a single muscle type, the pleural external longitudinal fiber #12. Although muscle #12 homologs are found in the metathorax and in all the abdominal segments, the stained endings are principally located on this muscle in abdominal segments 2 through 5. Fewer than 5% of the muscles examined in the first abdominal segment exhibited octopamine immunoreactive nerve endings, and they were never found on the #12 homologs in the metathorax or in the abdominal segments 6 and 7. As was shown with proctolin, the pattern of octopamine immunoreactive innervation can be altered in larvae that are haploinsufficient for Bithorax complex function, resulting in an increase of A1 endings and endings on an inappropriate target, muscle #13. Since the mature distribution pattern is present in first instar larvae, we are examining when it is established in embryogenesis in both normal and mutant embryos.

Double labeling experiments indicate that the octopamine immunoreactive terminals also contain glutamate, and that they comprise a subset of the total innervating processes found on muscle #12. This muscle fiber also receives proctolin innervation and we are determining whether this peptide also is present in the octopamine immunoreactive motor endings. Neurochemical techniques are underway to demonstrate whether the immunoreactive material is authentic octopamine. and physiological approaches will enable us to dissect the functions of the segmental, muscle fiber, and nerve terminal neurochemical specializations. (The octopamine antisera were generously provided by Dr. E. Kravitz.)

155.13

OCTOPAMINE AND FORMAMIDINES INCREASE PROTEIN SYNTHESIS IN Drosophila NERVOUS SYSTEM. M. Ofarim* and Y. Dudai (SPON: V. Teichberg). Department of Neurobiology, Weizmann Inst., Rehovot 76100, Israel.

Low concentrations of formamidines selectively interfere with behavior and development in insects and acarides. In Drosophila, formamidines disrupt sensory and motor functions and reduce learning ability; this is at least partially due to stimulation of the cAMP cascade via octopamine receptors (Dudai et al., J. Comp. Physiol. 161: 739, 1987). Since cAMP, in addition to activating protein kinase, may also regulate gene expression, we have tested whether formamidines affect protein synthesis in Drosophila nervous system. Larval brains were incubated with [^{35}S]methionine in the absence and in the presence of chlordimeform, N,N-dimethyl-N2-(2,4-dimethylphenyl)-formamidine-HCl, and octopamine. Following incubation, the brains were homogenized and subjected to SDS-gel electrophoresis. Increased radiolabeling was detected in brains incubated with octopamine and formamidines, especially in polypeptides of MW of ca. 76 and 53 kDa. The results support a potential role for octopamine in modulating protein synthesis in the nervous system, and suggest that whereas the acute effects of formamidines on behavior probably result from interference with ongoing aminergic transmission, their effects on development may result from cAMP-mediated alteration in gene expression. (Supported by the US-Israel Agricultural Research and Development Fund (BARD)).

155.14

Modulation of two portions of the cockroach escape circuit by biogenic amines. R. S. Goldstein, & J.M. Camhi, Dept. Zoology, Hebrew University, Jerusalem, Israel

We have studied the effects of serotonin (5-HT), dopamine (DA) and octopamine (OA) on the neural circuit underlying cockroach escape behavior. The release of one or more of these neuromodulators may be responsible for the natural variability of the behavioral response to wind stimuli (J.M. Camhi, this volume).

The first synapse in the escape circuit is in the terminal abdominal ganglion (A6), from afferents of wind-receptive hair cells on the cerci (paired abdominal appendages), onto interganglionic giant interneurons (GI's). 5-HT and DA perfused locally over A6 had little or no effect on the wind-evoked response recorded from the axons of these interneurons. However, OA dramatically enhanced the sensitivity and duration of the wind-evoked response of GI's of both the dorsal and ventral groups, and the effect of OA was reversed by rinsing with normal saline.

The next synapses of the circuit are located in the thoracic ganglia, where GI's activate motorneurons innervating the legs, via thoracic interneurons. We studied the effects of these amines on the response of metathoracic (T3) leg motoneurons to activation of the abdominal GI's. In contrast to their lack of effect in A6, both 5-HT and DA had profound effects on the circuit in T3. 5-HT consistently depressed, and DA enhanced the magnitude of the motor response to GI activation when perfused over the metathoracic ganglion. 5-HT also increased the rate of habituation of the motor response. OA's effects were less clear.

We are now beginning to study the cellular basis of these central aminergic effects, as well as examine behaviorally their modulation of cockroach running and flying escape.

155.15

SELECTIVE DEPLETION OF DOPAMINE, OCTOPAMINE AND 5-HYDROXYTYPTAMINE IN THE NERVOUS TISSUE OF THE COCKROACH (***PERIPLANETA AMERICANA***). B.D.Sloley* and S.Orikasa* (SPON: J.S. Richardson). Neuropsychiatric Research Unit, Dept. of Psychiatry, Univ. of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0.

High performance liquid chromatography with electrochemical detection (HPLC/ED) was used to measure the concentrations of 3,4-dihydroxyphenylethylamine (dopamine, DA), 5-hydroxytryptamine (5-HT), p-hydroxyphenylethanolamine (octopamine), α-methyl-p-tyrosine and tryptophan in the cerebral ganglia of cockroaches (*Periplaneta americana*) after peripheral administration of α-methyl-p-tyrosine or α-methyltryptophan. In addition, the levels of DA, 5-HT, octopamine, α-methyl-p-tyrosine and tryptophan were determined after injection of α-methyly-p-tyrosine, 6-hydroxydopamine or 5,7-dihydroxytryptamine directly into the cerebral ganglia by means of microinjection needles. Peripheral administration of α-methyl-p-tyrosine 400-1600 µg/insect) caused a reduction in dopamine and 5-HT concentrations in cockroach cerebral ganglia although the reduction in DA concentrations was more pronounced. Peripheral injections of α-methyl-p-tyrosine also reduced octopamine levels in the cerebral ganglia. Peripheral injection of α-methyltryptophan (400-1600 µg/insect) caused a marked reduction in 5-HT and tryptophan concentrations in cockroach cerebral ganglia without altering dopamine or octopamine concentrations. Central injections of α-methyl-p-tyrosine (80 µg/insect) reduced DA concentrations in the cerebral ganglia. However, neither 6-hydroxydopamine (20 µg/insect) nor 5,7-dihydroxytryptamine (20 µg/insect) caused reductions in amine levels when applied near or directly into the cerebral ganglia. The results suggest that specific lesions of aminergic neurons in insects by either 6-hydroxydopamine or 5,7-dihydroxytryptamine are impractical. The specific, long lasting depletion of 5-HT by α-methyltryptophan suggests that this chemical may be useful in elucidating the functions of 5-HT in insects. Research supported by the Saskatchewan Health Research Board.

155.16

RESERPINE DEPLETES SEROTONIN FROM THE CNS OF THE LEECH (HIRUDO MEDICINALIS) AND DRASTICALLY ALTERS BEHAVIOR. B. A. O'Gara and W. O. Friesen, Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Serotonin is known to have an important role in the control of swimming and feeding in the leech (Willard, J. Neurosci. 1:936-944, 1981; Lent, Brain Res. Bull. 14:643-655, 1985). As a first step in characterizing the effects of serotonin on the neural elements involved in swimming activity, we have determined the extent and time course of reserpine-induced depletion of serotonin from the nervous system. Injection of reserpine into the crop (100 µg/leech) causes depletion of serotonin (determined with HPLC-EC) from about 20 pmoles/ganglion in normal leeches to undetectable levels (< 0.1 pmoles/ganglion) by 3 - 5 days post-injection. Recovery of serotonin levels occurs slowly, taking weeks to months.

Reserpine-injected leeches show grossly abnormal behavior. The animals are lethargic and are less responsive to stimuli than normal. With sufficient prodding, the animals will swim, but the swimming movements appear abnormally stiff. However, after continued swimming, the movements become normal in appearance. When handled, the leeched show these abnormal behaviors: dorsal-ventral flattening, increased body wall tonus, erected annuli, reduced sucker attachment, reduced exploratory movements of the head, and occasional swimming movements. Biting behavior is eliminated by reserpine with a time course similar to serotonin depletion. However, biting reappeared when serotonin levels were still very low. Supported by NIH grants NS08263 (B.A.O.) and NS21778 (W.O.F.)

155.17

SEROTONIN MODIFIES SYNAPTIC TRANSMISSION IN NEURONAL CIRCUITS OF THE LEECH (HIRUDO MEDICINALIS). W. O. Friesen, A. K. Cometa and H. Hashemzadeh, Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

The ventral nerve cord obtained from untreated leeches exhibits episodes of swimming activity subsequent to appropriate electrical stimulation. Treatment of leeches with reserpine depletes serotonin from the nervous system and engenders a reduction in the activity levels of these animals (see abstract by O'Gara and Friesen, this volume). We found that nerve cords obtained from treated animals rarely exhibit swimming activity, even when subjected to strong electrical stimulation. Following bath application of 50 μM serotonin these previously quiescent nerve cords express swimming activity both spontaneously and following electrical stimulation.

To determine the mechanisms by which serotonin restores swimming activity in these preparations, we searched for serotonin-induced alterations in the synaptic interactions between neurons in the neuronal circuits that generate leech swimming activity. We tested synaptic transmission between swim-related neurons prior to and then following bath application of 50 μM serotonin in isolated leech nerve cords obtained from animals treated previously with reserpine. Inhibitory synaptic interactions between motor neurons exhibited synaptic fatigue following, but not prior to the application of serotonin. Similarly, synaptic fatigue in the inhibitory synapse between oscillator cell 115 and DI-102 increased substantially with the application of serotonin. Recovery from synaptic fatigue occurs more rapidly in the presence of serotonin. Such modifications can facilitate oscillations in neuronal networks with reciprocal inhibition. Supported by NSF grant BNS84-14988.

155.18

MODULATION OF THE ELECTROTONIC STRUCTURE OF APLYSIA NEURONS BY SUBTHRESHOLD VOLTAGE SHIFTS AND SEROTONIN. M. Spira,* I. Segev,* R. Werman* and Y. Yarom* (SPON: M.J. Gutnick), Dept. Neurobiology, Hebrew Univ., Jerusalem 91 904, Israel.

Aplysia metacerebral neurons have an inherent capability to dynamically alter their input-output characteristics. We have studied this capability in isolated, cultured adult neurons by analyzing their cable properties (R_N, τ_0 and L) and correlated them with a compartmental model based on their structure, reconstructed from light-microscopy photographs. The membranes of these neurons exhibit marked nonlinear behavior at sub-threshold potentials, -30 to -70 mV. High conductance states were observed at -30 to -40 mV and at -60 to -70 mV. As a result, L (estimated from transient analysis) increases more than three-fold over a potential range of 20 mV. A close correlation between τ_0, L and R_N was always observed. Since the membrane time constant τ_0 was in the range of hundreds of msec, a short L (0.2 - 0.3) could, for the first time, be resolved using the peeling technique. Under such circumstances, the decay of a voltage response to a short current pulse injected into the soma was characterized by two clearly separable time constants of decay. This behavior, as well as the increase in L seen in the high conductance state, was predicted from the compartmental model. This serotinergic cell is also remarkably sensitive to 5-*HT*, which increased R_N significantly (1 μM). This increase in R_N, however, was always restricted to the voltage range where R_N usually peaked, indicating a voltage dependence of the 5-*HT* response. A corresponding decrease in L and an increase in τ_0 were observed physiologically and predicted theoretically. Our study provides, for the first time, a demonstration that the electrotonic structure of a neuron and, thus, its integrative capabilities can be dramatically altered by subthreshold potentials and by neuromodulators.

155.19

THE RELEASE OF SEROTONIN FROM THE PERIPHERAL NERVOUS SYSTEM OF THE BLOOD-FEEDING INSECT, RHODNIUS PROLIXUS STÅL, IN RESPONSE TO FEEDING. T. Orchard, A.B. Lange and F.M. Barrett*, Dept. of Zoology, Univ. of Toronto, Toronto, Ontario, Canada, M5S 1A1

A peripheral role for serotonin in the blood-feeding bug Rhodnius prolixus has been implied by the presence of a plexus of serotonin-like immunoreactive processes lying on several of the abdominal nerves (Flanagan, T.R.J., Brain Res. 306:235, 1984). We have now examined the serotoninergic system in Rhodnius in more detail and show here, using HPLC, that serotonin is contained within these neurohaemal areas and that it can be released in a calcium-dependent manner. Serotonin-like immunoreactive processes also extend along the full length of the abdominal nerves and form an extensive plexus covering the epidermis. The immunoreactivity over the abdominal integument is barely visible in fifth instars 15 min after the onset of gorging. Furthermore haemolymph serotonin concentration is elevated 16-17 fold within 5 min of the onset of gorging on blood or an artificial diet. The stimulus for the release of serotonin is not associated with merely the onset of feeding since the serotonin levels do not continue to rise in insects allowed to gorge for only one minute. These findings indicate that serotonin is associated with feeding in Rhodnius and we are examining the action of the neurotoxin 5',7'-dihydroxytryptamine upon serotonin content and feeding activity.

155.20

BILATERALLY HOMOLOGOUS CELLS IN LYMNAEA CONTAIN DIFFERENT NEUROTRANSMITTERS. BJ Chiasson*, RP Croll, PC Kind*, JD Boyd*, RL Cowling*, MM Meriam*, DN Chilibeck*, LC Boghiar*. Dept Psychol, Dalhousie Univ, Halifax, NS, Canada B3H 4J1

RPeD1 and LPeD1 are two large central neurons located in symmetric positions in the right and left pedal ganglia, respectively, of Lymnaea. In addition to size and position, these cells are distinct from most pedal cells since they have no processes in any pedal roots. (Slade et al, 1981, J Exp Biol). Dye fills reveal basic symmetry between neurites of LPeD1 and RPeD1 (see Haydon & Winlow 1981, J Exp Biol); both cells project to ipsilateral pleural and parietal ganglia and into the visceral ganglion. Few other pedal cells are revealed by backfills of parietal and visceral nerves. In spite of their similarities the two cells differ in one important regard. Previous work has shown that RPeD1 contains dopamine whereas LPeD1 contains serotonin (Cottrell et al, 1979, Neurosci). We further show that RPeD1 contains no detectable serotonin and that LPeD1 contain little or no dopamine. As a likely consequence of the biochemical differences, the functional output of the cells is quite different. RPeD1 elicits discrete psp's in several parietal and visceral cells (Winlow et al, 1981, J Exp Biol), whereas our initial studies reveal no corresponding cells post-synaptic to LPeD1. Since morphological similarities suggest bilateral homology, very specific cues might dictate different transmitter phenotypes during what is probably the otherwise similar development of these cells.

BIOLOGICAL RHYTHMS: CELLULAR MECHANISMS

156.1

EXTRACELLULAR CALCIUM AND CALCIUM CHANNEL DEPENDENCY OF PHASE SHIFTS OF THE BULLA OCULAR CIRCADIAN PACEMAKER. S.B.S. Khalsa and G.D. Block. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

The in vitro ocular circadian pacemaker of Bulla is phase-shifted by light and other depolarizing treatments of the putative pacemaker cells (the basal retinal neurons). McMahon & Block (J. Comp. Physiol. 161:335, 1987) observed that light-induced phase delays were blocked in low Ca^{2+} EGTA artificial seawater (ASW) suggesting that extracellular Ca^{2+} is essential for phase-shifting.

We now report that light-induced phase advances and both delays and advances to depolarizing high K^+ ASW are also blocked in low Ca^{2+} EGTA ASW, verifying that extracellular Ca^{2+} is a general requirement for light and depolarization-induced phase-shifting.

Light-induced phase delays were also blocked in ASW without EGTA at Ca^{2+} concentrations of 400 uM or lower; concentrations of 1 mM and 3.5 mM yielded reduced phase shifts relative to those in normal ASW ($[Ca^{2+}]$=10 mM). These data suggest that it is not the EGTA which blocks phase shifts and that the threshold Ca^{2+} concentration for phase-shifting in this study is at least 400 uM.

The calcium channel blocker Ni^{2+} (5 mM NiCl) was found to be effective in blocking light-induced phase delays and advances. Phase delays to high K^+ pulses were also blocked by Ni^{2+}, however the concentration required was higher (50 mM NiCl) and the treatment produced long term changes in the waveforms of the circadian activity record. Other blockers were toxic, produced their own shifts or were ineffective. It is likely that calcium channels mediate light and depolarization-induced phase shifting. Support: NS09621, NS15264

156.2

LOW CALCIUM ATTENUATES PERIOD LENGTHENING IN CONSTANT LIGHT OF BULLA OCULAR RHYTHM: G.D. Block and L.B. Latham. Department of Biology, Univ. of Virginia, Charlottesville, VA 22901.

The Bulla ocular pacemaker can be phase shifted by depolarizing agents but only if extracellular calcium is present (McMahon & Block, J. Comp. Physiol. **161** 1987). When calcium levels are reduced below 1 mM or when Ni^+ is added to the bath, phase shifts are blocked (Khalsa & Block, this volume).

In addition to light's phase shifting action, constant illumination (L:L) affects the free-running period. In Bulla the ocular free-running period is lengthened from 23.5 to 24.4 hr in bright L:L (McMahon & Block, J. Comp. Physiol. **161** 1987). The relationship between the phase shifting action of light pulses and the effects of constant light on free-running period has been formally addressed (Daan & Pittendrigh, J. Comp. Physiol. **106** 1976) but it is not known whether the same cellular mechanisms subserve both phenomena.

We now report that reducing the extracellular calcium level to 1 mM attenuates the period lengthening observed in L:L. We find that in normal seawater the Bulla ocular rhythm lengthens to 24.3 hr (0.13, N=5) in continuous dim light (22 lux). When calcium is reduced to 1 mM the free-running period in L:L is indistinguishable from D:D (23.7 hr, 0.16, N=5). Eyes run in 1 mM calcium seawater continue to show elevated impulse rates in L:L suggesting that the lack of period lengthening in not due to a reduced sensory response. These data indicate that similar to phase shifting, the period lengthening effects of light are calcium dependent and support the hypothesis of a common mechanism for both processes. NS15264, NS00714.

156.3

EFFECTS OF LIGHT ON CIRCADIAN MELATONIN RELEASE FROM CHICK PINEAL CELLS: CALCIUM-DEPENDENT AND -INDEPENDENT MECHANISMS. L.M. Robertson and J.S. Takahashi. Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

Chick pineal cells express a circadian oscillation of melatonin release that persists in constant conditions. The cells are photoreceptive and exposure to light has 2 major effects: 1) entrainment of the circadian oscillator and 2) acute inhibition of melatonin production. We explored the effects of calcium (Ca) on both the acute and phase-shifting effects of light on dissociated chick pineal cells. During the subjective night of the 5th day in culture cells were exposed to treatments which decreased extracellular or increased intracellular Ca. Low Ca treatment resulted in a dose-dependent decrease in melatonin release. Treatment with the Ca ionophore A23187 or the Ca channel agonist BAY K8644 blocked the light-induced reduction in melatonin release. We used a flow-through culture system to determine whether variations in Ca affect the phase-shifting effects of light. Cultures exposed to a light pulse beginning at circadian time (CT) 20 were advanced relative to dark controls. Treatment with low Ca, A23187 or BAY K8644 had no effect on either the phase of the rhythm in constant darkness or the phase-shift induced by light. Thus, the phase-shifting effects of light do not appear to involve Ca pathways. In contrast, the acute effects of light can be mimicked by low Ca treatment and blocked by treatments which increase intracellular Ca.

156.4

CALMODULIN REGULATES MELATONIN PRODUCTION IN CHICK PINEAL CELL CULTURES. S. S. Nikaido and J. S. Takahashi. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Dissociated chick pineal cells express a circadian rhythm of melatonin release. Melatonin production in chick pineal cells is regulated by both cAMP and Ca^{2+}. Melatonin levels can be elevated with cAMP analogs, forskolin and 3-isobutyl-1-methylxanthine. Melatonin levels can be depressed by lowering Ca^{2+} levels with EGTA and with Ca^{2+} channel blockers. Furthermore, melatonin levels can be increased with the dihydropyridine Ca^{2+} channel agonist, Bay K8644.

The effect of Ca^{2+} on melatonin production appears to be mediated by calmodulin. Calmodulin antagonists, calmidazolium, W7 and W13, inhibit melatonin production during the night in a dose-dependent manner. Inactive naphthalenesulfonamides, W5 and W12, have no effect on melatonin production up to 10^{-4} M. In addition, calmidazolium blocks the increase in melatonin seen with Bay K8644, yet only partially blocks the increase in melatonin seen with 8-Br cAMP and forskolin.

These results suggest that melatonin is regulated by Ca^{2+} through a calmodulin-dependent process and that part of the cAMP regulation of melatonin also occurs through a calmodulin-dependent process.

(Supported by NIMH R37 MH39592, Searle Scholars 85-H-107, NSF DCB-8451642 and NIMH F31 MH09572.)

156.5

THE SCN CIRCADIAN CLOCK IS RESET IN VITRO BY NIGHTTIME APPLICATIONS OF A cGMP ANALOG. R. A. Prosser and M. U. Gillette. Neural and Behavioral Biol. Program and Dept. of Physiol. and Biophysics, Univ. of Illinois, Urbana IL 61801.

Recent investigations on the biochemical substrates of the mammalian circadian clock in the suprachiasmatic nuclei (SCN) have found that daytime but not nighttime applications of cAMP analogs in vitro reset the SCN clock. In contrast, pulses of high K^+ are effective only during the night. Here we show that, like high K^+, pulses of a cGMP analog reset the SCN clock.

Rat SCN were isolated in a brain slice preparation and maintained in perifusion culture. Electrical activity recorded extracellularly from a population of single SCN cells normally shows a 24 hr rhythm that peaks at circadian time (CT) 7.0. 1 hr pulses of the cGMP analog Br-cGMP ($5x10^{-4}$M) between CT 16 and 18 significantly advanced the time of peak electrical activity (6.58±.37 hr advance; N=3) while 1 hr treatment between CT 7 and 10.5 had no effect on the rhythm (0.42±.74 hr advance; N=3). Thus, cGMP appears to affect the SCN clock at times similar to those when high K^+ is effective, but when cAMP is ineffective. These results suggest that these cyclic nucleotides may affect the SCN circadian clock in an antagonistic relationship.

156.6

CIRCADIAN PACEMAKER PROPERTIES ARE RETAINED BY ISOLATED SUPRACHIASMATIC NUCLEI IN VITRO. M.U. Gillette and R.A. Prosser. Neural & Behavioral Biology Prog. and Dept. of Physiol. & Biophys., Univ. of Illinois, Urbana, IL 61801.

Our previous studies on rat hypothalamic brain slices demonstrated a circadian rhythm of SCN neuronal firing rate that can be reset in vitro. In this study we determined whether the circadian pacemaking and resetting properties attributed to the SCN in vitro are endogenous, or whether there is contribution by peripheral regions in the slice. 500 µm coronal slices were surgically reduced on three sides to within 50 µm of the SCN; the chiasmatic border was not disturbed. The circadian pattern of neuronal activity was then measured in both controls and SCN exposed to $5x10^{-4}$ M 8-benzylamino cAMP (BAcAMP) for 1 hr at circadian time 7.

We found that 500 µm slices (N=4) reduced to SCN and optic chiasm maintained circadian rhythms of single unit firing rate. The amplitude and phase were unchanged from SCN rhythms in much larger hypothalamic slices including periventricular, paraventricular and supraoptic nuclei. BAcAMP induced phase-advances of 4.92±.44 hr (N=3). We conclude that these pacemaker properties are endogenous to the SCN.

156.7

PHASE-SHIFTING RESPONSES OF SUPRACHIASMATIC EXPLANTS TO IONIC MANIPULATION *IN VITRO*. D.J. Earnest, S.M. DiGiorgio and C.D. Sladek. Dept. of Neurobiology/Anatomy, Univ. of Rochester Med. Ctr., Rochester, NY 14642

Consistent with the well-established role of the suprachiasmatic nucleus (SCN) as a circadian pacemaker, we have demonstrated that vasopressin (VP) is released in a circadian fashion for multiple cycles from perifused SCN explants. To characterize the phase-shifting effect of KCl on the circadian pattern of VP release from SCN explants and better understand the formal properties of circadian rhythms generated by the SCN *in vitro*, the present study entailed systematic analysis of this circadian effect of KCl with regard to its dependence on the time of administration.

SCN explants were dissected from the hypothalami of male rats housed under LD 12:12. Individual explants were placed in a perifusion culture system under conditions of constant darkness and serial samples of the perifusate were collected at 1 or 2 hr intervals for 4 days. On day 2 in culture, test explants were exposed to medium containing KCl (peak conc. = 41mM) for 45 min at various circadian times. Control explants were exposed to NaCl-supplemented medium that provided an osmotic challenge equivalent to that generated by KCl treatment. VP levels in the medium were measured by RIA and the VP patterns expressed by control and test explants were assessed for evidence of phase shifts in comparison with untreated explants.

Concomitant with an acute stimulation of VP release, KCl exposure yielded advances, delays, or no change in the phase of the VP rhythm. This effect of KCl pulses was dependent on the time during the circadian cycle at which the pulse was administered. KCl treatment consistently induced phase delays of the rhythm, when administered near the middle of the subjective day, but elicited phase advances when this perturbation occurred either late in the subjective day or early in the subjective night. KCl pulses delivered late in the subjective night and osmotic challenges associated with NaCl treatment had no effect on the phase of the VP rhythm. These findings indicate that the cellular mechanisms underlying the phase-shifting effect of KCl on SCN neurons may be important in the neural regulation of circadian rhythms. Moreover, the phase-dependency of the circadian effect of KCl on SCN explants serves to underscore the similarities in the properties of SCN-generated rhythms *in vitro* and *in vivo*, and suggests that this system may provide a basis for examining the roles of various transmitters in regulating the circadian clock located in the SCN. Supported by NS-24661(D.E.).

156.8

THE SINGLE UNIT RESPONSE OF NEURONS WITHIN THE SUPRACHIASMATIC NUCLEUS TO LOW CHLORIDE PERFUSATE IS PHASE-DEPENDENT. Liou S.Y.*, and H.E. Albers (SPON: K. Wallen). Lab. of Neuroendocrinol. and Behav., Depts of Biol. and Psychol., Georgia State Univ., Atlanta, GA 30303.

SCN neurons maintain a circadian rhythm of spontaneous activity in isolated brain explants. The present study examined whether chloride ions are involved in maintaining circadian rhythms of spontaneous neural activity within the SCN. Extracellular single unit recordings were made from spontaneously firing cells in hamster hypothalamic slice preparations. Coronal brain slices containing the SCN were cut during the light phase of the LD cycle (CT 12h corresponding to the time of light off). Exposure of the slice to low Cl- medium (80% of NaCl was replaced with equimolar Na isethionate) during the late light period or early dark period (CT 7-15h) abolished spontaneous activity of most cells (12/14). However the neurons discharged in response to advancing the electrode and the inhibition was reversed when chloride was replaced. During the dark phase (CT 12-22h) exposure to the low Cl- medium excitated most spontaneously firing cells (9/11) and initiated activity in some silent cells (5). The present results indicate that chloride channels may be involved in the circadian variation of neural firing within the SCN.

(Supported by ONR-N00014-87-K-0172)

156.9

SEROTONIN BLOCKS LIGHT-INDUCED PHASE DELAYS BUT NOT ADVANCES IN APLYSIA. C.S. COLWELL* (SPON:D.Hudson). Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

The phase shifts induced by 6 hour treatments of light (200 lx), 5-HT (10^{-5}) and light in combination with 5-HT were determined. These treatments were given at two time points: one during Ct 6-12 when light causes a phase delay of 1.37 hr ($^{+}$ 0.31, 95% Conf. Int., n=6) and one during Ct 18-24 when light causes a phase advance of 2.64 hr ($^{+}$ 0.51, n=7). Serotonin produces an advance of 1.66 hr ($^{+}$ 0.63, n=6) at Ct 6-12 and a 1.5 hr ($^{+}$ 0.63, n=6) delay at Ct 18-24. Serotonin plus light at Ct 6-12 results in no phase shift (0.08 hr, $^{+}$ 0.74, n=6) while at Ct 18-24 light plus serotonin results in a 3.84 hr ($^{+}$ 0.66, n=8) phase advance. This phase advance is not distinquishable from a phase shift produced by light alone. These results suggest that the phase shifts produced by light and 5-Ht are non-additive and implies that the light and 5-HT phase shifting pathways converge and interact. Studing the points of convergence may provide new insights into the functional role of 5-HT in this system as well as help to identify meaningful components of the oscillatory mechanism.

156.10

LIGHT INDUCED PHASE SHIFTS OF THE BULLA EYE IN THE PRESENCE OF PROTEIN SYNTHESIS INHIBITORS. B.L. Bogart and G.D. Block Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Our laboratory is currently involved in an effort to identify the cellular events underlying pacemaker entrainment in the basal retinal neurons of the marine snail Bulla gouldiana. Recent studies have implicated depolarization and a calcium flux in the phase shifting pathway (McMahon and Block, J. Comp. Physiol. 161:1987).

Protein synthesis inhibitors have been shown to phase shift the circadian pacemakers of numerous systems, suggesting a role for protein synthesis in oscillator function. More specifically, Eskin and coworkers (PNAS 81: 1984) have demonstrated that protein synthesis is required for phase advancing the Aplysia ocular pacemaker by pulses of 5-HT. In the current study, we have attempted to determine whether light induced phase shifts are likewise blocked by simultaneous exposure of the retina to protein synthesis inhibitors.

We initially determined that 3 hr pulses of anisomycin ($7.5x10^{-7}$ M) at both CT 13-16 and CT 21-24 generated phase delays (-168 min $^{+}$56; 95% C.I., N=5), indicating the inhibitor was affecting the pacemaker. For the light phase shifting experiments, both the control and experimental eye received the 3 hr anisomycin pulse at either CT 13-16 or CT 21-24, while the experimental eye also received a concurrent light pulse. We report that light still causes phase delays at CT 13-16 despite the presence of the protein synthesis inhibitor (-75min $^{+}$24, N=10). The phase advances to light normally seen at CT 21-24, were highly variable in the presence of anisomycin and not statistically significant (21 min $^{+}$37, N=9).NS15264

156.11

INHIBITORS OF PROTEIN SYNTHESIS PHASESHIFT THE CIRCADIAN CLOCK IN CHICK PINEAL CELLS. N. Murakami* and J. S. Takahashi. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208

Dissociated chick pineal cells contain circadian oscillators that regulate the rhythmic release of melatonin. To determine whether protein synthesis is required for the generation of circadian oscillations, dispersed chick pineal cells were perfused in flow-through microcarrier culture in constant darkness and treated with 10^{-6}M anisomycin, an inhibitor of 80s ribosomal protein synthesis, for 6 hrs at various circadian times. Phase shifts were analyzed by comparison with controls as described by Robertson and Takahashi (J. Neurosci. 8:22, 1988). Single pulses of anisomycin shifted the phase of the circadian oscillation in a phase dependent manner. Phase delays were observed for drug pulses beginning late in the subjective night and early in the subjective day (CT 18-3). Conversely, phase advances were observed for drug pulses in the late subjective day (CT 6, 9). No phase shift was observed for pulses at CT 12. The magnitude of phase delay observed for anisomycin pulses at CT 3 was dose-dependent and was correlated with the magnitude of inhibition of protein synthesis measured by ^{35}S-methionine incorporation.

These results suggest that there is a phase-dependent requirement for protein synthesis in the circadian clock of chick pineal cells and that protein synthesis may be a component of the circadian pacemaking mechanism.

156.12

LIGHT AND SEROTONIN: EFFECTS ON PROTEINS BY TWO MODULATORS OF THE CIRCADIAN RHYTHM IN THE APLYSIA EYE. R. E. Zwartjes*, S. J. Yeung*, and A. Eskin. (SPON: G. F. Gwilliam). Biol. Dept., Univ. of Houston, Houston, TX 77004.

Light and serotonin (5-HT) both regulate the circadian rhythm in the eye of Aplysia californica. Since the two have interacting effects on the rhythm, we compared the effects of both on protein synthesis and phosphorylation. Both light and 5-HT affected the incorporation of ^{3}H-leucine into a 34 kilodalton (kDa) ^{32}P protein, and both increased incorporation of ^{32}P into a 57kDa protein. Light affected incorporation of ^{3}H-leucine into four additional proteins (46kDa, 66kDa, 30kDa and 52kDa), and affected incorporation of ^{32}P into two additional proteins (50kDa and 30kDa). The effects of light and 5-HT on the incorporation of ^{3}H-leucine into the 34kDa protein were phase dependent. However, at the phase when 5-HT produces an advance and light a delay, both increased incorporation into the 34kDa protein. These results indicate that the phase shifting effects of light and 5-HT cannot simply be explained by changes in the 34kDa and 57kDa proteins, but the convergence of information from light and 5-HT on these two proteins suggests an important role for each in circadian timing.

156.13

CIRCADIAN RHYTHMICITY IN BULLA EYES: REGULATION BY PROTEIN PHOSPHORYLATION. M.H. Roberts, V. Bedian* and Y. Chen*, Dept. of Biology, Clarkson Univ., Potsdam, NY 13676

We have investigated the involvement of protein phosphorylation in the regulation of circadian rhythmicity for three reasons: 1) Phosphorylation of ion channels is a common mechanism for changes in neuronal excitability. 2) Zwartjes and Eskin have shown that phase shifts of the Aplysia eye rhythm by 5-HT may involve the phosphorylation of specific proteins. 3) Gillette et al. have found daily changes in kinase activity in the rodent circadian clock.

In our studies we find that: 1) labeling Bulla eyes with 32P from CT 23-1 or from CT 11-13, reveals consistent differences in the phosphorylation of a number of proteins. 2) The circadian rhythm is not affected by PK-C activation, since the phorbol ester TPA is ineffective in phase shifting the eye rhythm when applied from CT 23-1 or CT 11-13. 3) Finally, phosphorylation by PK-A may be involved in the regulation of circadian rhythmicity since an inhibitor of PK-A, H-8, can affect the phase of the eye rhythm. Initial studies indicate that application of 50uM H-8 from CT 12-15 results in a small phase delay (-0.41 $\pm$ 0.23 SEM, n=6), while application from CT 23-1 generates a small phase advance (0.5 hrs, 1.0 hrs, n=2).

The correlation of phosphorylation changes with the effects of kinase inhibitors, suggests a mechanistic relationship between cyclic nucleotide dependent protein phosphorylation and the generation of circadian rhythmicity

156.14

PHOSPHORYLATION OF THE 122 KD PROTEIN IN VIVO IN LIMULUS LATERAL EYES BY EFFERENT STIMULATION. S.C. Edwards[1], A.C. Wishart[1]*, G.H. Renninger[2], E.M. Wiebel[1]*, B-A. Battelle[1]. [1]The Whitney Laboratory, Univ. of Florida, St. Augustine, FL 32086, and [2]Biophysics Group, U. Guelph, Guelph, ON.

Limulus eyes are innervated by efferent fibers that are driven by a circadian clock. Activation of these fibers during the animal's subjective night produce anatomical and physiological changes resulting in increased light response of both the lateral and ventral eyes. We have shown that octopamine, the putative neurotransmitter of the efferent fibers stimulates the cAMP-dependent phosphorylation of a 122 kD protein in vitro in slices of the lateral eye and in ventral eye photoreceptor cell body-rich fractions.

Here we show that the 122 kD protein is phosphorylated in vivo in response to efferent stimulation. Using a "back phosphorylation" technique (Valtorta et al., Anal. Biochem. 158:130-137 (1986)) we found that the 122 kD protein becomes phosphorylated in vivo at night in response to activation of efferent fibers by the circadian clock, as well as during the day when efferent fibers were electrically stimulated.

The distribution of the 122 kD is restricted to those tissues in Limulus involved in vision. Since it can become phosphorylated in response to both efferent activity as well as light, we postulate that it plays an important role in the photoresponse and its modulation. (Supported by the NSF, the Whitehall and Grass Foundations).

156.15

AUTORADIOGRAPHIC CHARACTERIZATION OF 2-^{125}I-MELATONIN BINDING SITES IN RODENT AND HUMAN BRAIN. D.R. Weaver, S.A. Rivkees, S.M. Reppert & E.G. Stopa. Children's Service, Massachusetts General Hospital & Neuropathology Division, Tufts University Schoool of Medicine, Boston, MA 02114.

The availability of a biologically active melatonin analog, 2-[125-I]-melatonin (IMEL), has allowed the autoradiographic localization of putative melatonin receptors in brain (Brain Res 435:359, 1987; FEBS Lett 228:123, 1988). In rats and Djungarian hamsters, we observed a remarkably discrete pattern of specific (e.g., displaced by 1 uM melatonin) binding. The suprachiasmatic nuclei (SCN) and the external zone of the median eminence were consistently and most intensely labelled. In human hypothalamus, only the SCN contained specific IMEL binding sites. In all species, specific binding of IMEL (100 pM) was prevented by inclusion of melatonin or 6-chloro-melatonin (1 uM) in the incubation solution. IMEL binding was only slightly reduced by inclusion of serotonin or norepinephrine (1 uM). Experiments in which the melatonin concentration was varied from 10 pM to 1 uM revealed monophasic competition curves, with IC50 values for melatonin below 1 nM. The results suggest that IMEL binding sites in the central nervous system represent high affinity, saturable melatonin receptors. These putative melatonin receptors in the hypothalamus may mediate the effects of melatonin on circadian rhythms and reproductive function in a variety of mammalian species.

156.16

BINDING CHARACTERISTICS OF 2-[125I]-IODOMELATONIN TO DJUNGARIAN HAMSTER BRAIN MEMBRANES. M.J. Duncan, J.S. Takahashi, M.L. Dubocovich. Dept. Pharmacol., Northwestern Univ. Med. School, Chicago, Il 60611; Dept. Neurobiol. and Physiol., Northwestern Univ., Evanston, Il 60208.

2-[125I]-Iodomelatonin selectively labels high affinity melatonin binding sites in Syrian hamster brain (Endocr. 122, 1988). We now report the binding characteristics of 2-[125I]-iodomelatonin to membranes from Djungarian hamster brains. Specific binding of 2-[125I]-iodomelatonin, as defined by 10 uM 6-Cl-melatonin, was 90% of total binding. In time course studies, specific binding reached a plateau within 10 min, was stable for at least 60 min and was completely reversible. Competition studies showed that the relative order of potency (IC50, nM) of several drugs is: 2-I-melatonin (3.2) > 6-Cl-melatonin (5) > melatonin (30) > 5-OCH3-tryptophol (300) > N-acetyl-tryptamine (1,000) > serotonin = methysergide (5,000). Scatchard analysis using 2-[125I]-iodomelatonin (0.05-11.00 nM) demonstrated that specific binding was saturable. Injections of melatonin (15 ug/day) for 7 weeks affected neither the affinity nor the maximal number of binding sites (Kd = 2.95 $\pm$ 0.68, Bmax = 268.9 $\pm$ 41.9 for melatonin-injected animals vs. Kd = 3.32 $\pm$ 1.58, Bmax = 251.7 $\pm$ 26.2 for vehicle-injected animals). These binding and pharmacological characteristics of 2-[125I]-iodomelatonin to Djungarian hamster brain resemble those demonstrated in Syrian hamster brain. (Supported by DK 38607 and 5T32NS07140.)

156.17

EARTH-STRENGTH MAGNETIC FIELDS SELECTIVELY ALTER ACTIVITY OF THE PINEAL GLAND AND HIPPOCAMPUS. A. Chicz-DeMet, E. DeMet, H. Wu*, R. Coopersmith, M. Leon. Depts. of Psychiatry and Psychobiology, Univ. of Calif., Irvine, CA. 92717.

The effects of low intensity magnetic fields on pineal function and regional neuronal activity were studied. Neuronal activity was measured by 14C-2-deoxy- glucose (14C-2DG) autoradiography in the presence and absence of an earth strength magnetic field. The horizontal component of the earth's magnetic field was compensated in one of paired animals using an air core Helmholtz coil. The experiments were conducted under red light between 2400-0300 hrs. Parallel 30% decreases were found in 14C-2DG uptake and N-acetyl transferase activity of the pineal glands of magnetically compensated rats. No significant differences were found in other brain regions with the exception of an increase in the hippocampus. A highly significant inverse relationship between this region and the pineal was amplified by the treatment. The latter probably reflects an indirect action of melatonin decreases on adrenal function. Therefore, the results suggest that low intensity magnetic fields may have a selective action on the activity of the pineal gland which can indirectly alter hippocampal function.

156.18

CIRCANNUAL VARIATIONS OF SEROTONIN UPTAKE AND 3H-IMIPRAMINE BINDING IN HUMAN PLATELETS. D. Marazziti, M. Falcone*, G.M. Pacifici*, G.F. Placidi*, P. Castrogiovanni*. Depts. of Psychiatry and General Pathology, Medical School, University of Pisa, I-56100 Pisa, Italy. (SPON:European Neuroscience Association).

The similarities between blood platelets and presynaptic serotonin (5HT) neurons offer an interesting research tool to explore 5HT system. The binding sites for 3H-imipramine (3H-IMI) seem to be allosterically coupled to 5HT utake sites both in platelets and in neurons. Rhythmicity is postulated to be an attribute of several neurotransmitters and neuroreceptors. We thus measured IMI binding parameters and 5HT uptake in platelets of 8 healthy drug-free volunteers (age between 21 and 43 years). The results showed that the maximum binding capacity (Bmax) of 3H-IMI binding followed seasonal variations, with highest values in the spring, and lowest in autumn. A similar trend was observed also for the maximum velocity (Vmax) of 5HT uptake. It would be interesting to investigate whether or not psychiatric illnesses influence such seasonal variations.

156.19

RETINAL CHANGES IN DEPRESSION. J. Seggie, M. Steiner, J. Martin, S. Fawcett, M. Fairman and J. Simpson, Departments of Biomedical Sciences, Psychiatry, Medicine and Surgery, McMaster University and Clinical Studies Program, St. Joseph's Hospital, Hamilton, Ontario L8N 3Z5.

Altered perception of light and hence the use of light and dark cues for entrainment of circadian rhythms has been suggested in depressive disorders. We assessed retinal function in control subjects (N=3) and acutely depressed inpatients (N=6) following a 10 day drug washout and after clinical improvement. During the depressive episode patients evidenced increased amplitude of response on the electro-retinogram (ERG) to 7 stimuli ($0.05<p<.10$). Following clinical improvement (14 days after the last ECT) non medicated patients evidenced decreased amplitude of response to all 7 stimuli of the ERG. Changes reached signficance ($p<.05$) for the blue/dark adapted, red/dark adapted, red-yellow light adapted and blue-green/ light adapted responses. Visual Evoked Potentials and Electrooculograms did not differ between controls and patients in either the ill or well condition. Changes in sensitivity to light in depression appear to be specific to retinal photoreceptors and not post-retinal elements or visual pathways.

Supported by the Canadian Psychiatric Research Foundation and St. Joseph's Hospital Foundation. J. Seggie is an Ontario Mental Health Foundation Associate.

157.1

THE EFFECT OF ACTIVATION TASKS ON PARKINSONIAN RIGIDITY.
E.S. Dannenbaum*, P.L. Weiss, R.E. Kearney, S. Gauthier. School of Physical and Occupational Therapy, Biomedical Engineering Unit, Center for Studies in Aging, McGill University, Montreal, Canada, H3G 1Y5.

Clinically it can be seen that the level of parkinsonian rigidity increases when going from a relaxed state to conditions of greater stress. The objective of this study was to assess quantitatively the effect of different levels of activity on parkinsonian rigidity and to investigate the peripheral mechanisms involved. Subjects lay supine while their left ankles were slowly driven (0.05 rad/s) through most of its range of motion. Joint position, torque and surface EMGs from tibialus anterior and triceps surae were recorded. Recordings were done under four conditions; 1) relaxed, 2) while executing preferred cadence flexion/extension of the elbow, 3) while performing fast elbow/flexion extension and 4) while engaged in a maximal pincer grasp.

In 10 stage III parkinsonian patients the work required to drive the foot through its range of motion increased when an activation task was performed. Frequently the most dramatic increase occurred when the maximal pincer grasp task was performed during the displacement. Activation had little or no effect in the age matched controls. These findings demonstrate the importance of activation in the evaluation and detection of parkinsonian rigidity.

157.2

ON THE MECHANISM OF PRODUCING MULTIPLE CYCLES OF EMG ACTIVITY IN PARKINSON'S DISEASE.
N. Teasdale*, G.E. Stelmach, and J.G. Phillips*. Motor Behavior Laboratory, University of Wisconsin-Madison, WI 53706.

In normal individuals, simple arm movements are performed with a single biphasic or triphasic pattern of EMG. For bradykinetic patients, additional cylces of alternating bursts in the agonists and antagonists are necessary to produce those simple arm movements. An EMG saturation of the agonist muscle and an underestimation of necessary EMG activity have been suggested to explain the increased EMG cycles. According to these hypotheses, movements of shorter durations and movements of longer amplitudes should be characterized by an increased number of EMG cycles. However, movement amplitude and movement time were confounded in those experiments. The aim of this experiment was to determine whether the increased EMG cycles are associated with the production of faster or slower movements. Patients and age-matched control subjects were asked to produce movements that were percentages of their maximum speed. Although slower, the patients were able to vary their movement speed. The increased EMG cycles in patients were associated with the production of slower movements. We suggest that the problem of additional EMG cycles in bradykinetic patients is due to irregular and low motor units firing frequencies as well as pauses and delays in recruitment.

157.3

THE STATISTICAL RELATION BETWEEN PARKINSONIAN RESTING TREMOR IN OROFACIAL AND LIMB MUSCLES.
C.J. Hunker & J.H. Abbs, (SPON: F. SIEGEL) Dept. of Rehab. Med. & Speech Motor Control Labs., Waisman Center, University of Wisconsin, Madison, WI 53706-2280.

The neural substrate of Parkinsonian (PD) resting tremor remains controversial. From neurosurgical intervention and single unit studies, this tremor appears due either to (1) rhythmical activity within the cortex or within the ventrolateral region of the thalamus, or (2) oscillatory neural networks between the thalamus and the cortex. In contrast, aberrant segmental reflexes or longer latency peripheral loops have also been postulated as responsible for resting tremor. Hunker & Abbs (1983) observed coincident tremor frequencies in the lip, jaw, tongue, and index finger, supporting the notion that resting tremor is based exclusively on peripheral pathways.

To extend this finding, the statistical relation between simultaneously recorded resting tremor epochs from limb and orofacial sites was examined in predominantly tremorous PD subjects, including power spectra and cross-correlations. The frequency of PD resting tremor was remarkably uniform at orofacial and finger sites despite their substantial differences in biomechanics and peripheral neurophysiology. Cross-correlations of 1 second segments yielded coefficients greater than 0.8. However, in the analyses of samples of longer duration, the cross-correlation coefficients rarely exceeded spurious levels. A single central pacemaker is perhaps too simple. The CNS may have multiple somatotopically-organized pacemakers, each activated in the same general manner by a common source, but separately driving lower motoneurons to generate resting tremor; this would give rise to partially correlated tremor in diverse muscle groups, but would also permit variations in these relations. Supported by NIH grants NS-13274 & HD-03352.

157.4

NEUROPSYCHOLOGICAL AND ELECTROPHYSIOLOGICAL EFFECTS OF BRAIN AUTOGRAFT OF ADRENAL MEDULLARY TISSUE TO THE CAUDATE NUCLEUS FOR THE TREATMENT OF PARKINSON'S DISEASE.
F. Ostrosky-Solís*, S. Meneses*, L. Quintanar*, M. Pérez*, D. Zarabozo*, I. Madrazo and R. Drucker-Colín. (SPON: A. Tay). Depto. de Psicofisiología, Fac. de Psicología, Fac. de Medicina, Instituto de Fisiología Celular, UNAM, México.

We studied the pre and post-operative auditory event related potentials (ERP) and the neuropsychological profiles of ten patients who received an autograft of adrenal medullary tissue to the caudate nucleus for the treatment of Parkinson's Disease (PD). The pre-operative neuropsychological evaluations revealed specific cognitive deficits which varied in degree. The patients showed frontal lobe-type deficits with alterations in behavioral programming leading to difficulties in the organization of motor sequences and alternating programs. They also showed memory disorders and visuospatial and visuoperceptual deficiencies. The post-operative evaluation suggested amelioration of most frontal lobe-type symptoms, and of the visuospatial deficits. Post-operatively, the ERPs elicited in a target detection stimulus paradigm showed significant changes in the amplitude of the N2-P2 EP components without significant changes in the earlier N1-P2 components. The neuropsychological and electrophysiological post-operative changes suggest that there are no negative effects in the cognitive area as a result of the surgery and that the graft has positive effects on specific cognitive symptoms.

157.5

DIFFERENTIAL REGULATION OF STRIATAL DA D1 AND D2 RECEPTOR SYSTEMS IN PARKINSON'S DISEASE (PD) AND EFFECTS OF ADRENAL MEDULLARY TRANSPLANT
J. Costello, H.I. Hurtig*, J.O. Trojanowski, J. Sladek and J.N. Joyce. Dept. Psychiatry and Pharmacology, Univ. Pennsylvania School of Medicine, Philadelphia, PA

Coronal slabs that included striatum were removed at autopsy from 3 control, 2 PD, 2 PD with dementia and 2 PD with Alzheimer's disease cases. In addition, coronal slabs from the left and right (transplant side) hemispheres of a PD case having received an adrenal medulla transplant into the dorsal striatum 4 months prior to death were removed for analysis. Coronal sections (25 um) were processed for autoradiographic analysis of D1 ([^{3}H]SCH 23390) and D2 ([^{3}H]spiroperidol) receptors and DA uptake sites ([^{3}H]mazindol) located on DA terminals. All cases of PD showed profound loss (>92%) of [^{3}H]mazindol labeling in the dorsal putamen (PUT) and caudate nucleus (CN) but showed significant preser-vation in the NAS and medial CN. The density of DA D2 receptors was reduced in the PD cases, by 22-44%. In contrast, the density of D1 receptors was increased significantly, 48% in dorsal PUT, 30% in dorsal CN and 12-13% more ventrally. Immunohistochemical results indicated that the cells in the adrenal medulla transplant could be identified as adrenal chromafin cells but without neurites or processes. The striatum of the unoperated (left) side of the transplant case showed DA receptor density similar to the other PD cases, but with somewhat higher D2 receptor density. The striatum of the transplant side showed normalization of D1 receptor density, particularly in the region of the highest surviving density of DA terminals (visualized with [^{3}H]mazindol binding). **Supported in part by a grant from American Federation for Aging Research to JNJ.**

157.6

ADRENAL AUTOTRANSPLANT IN BEHAVIORALLY TRAINED, MPTP-TREATED MONKEYS.
B. Brooks-Eidelberg, E. Eidelberg, J. Story, R. Barret-Tuck, F. Boop, and S. Murk. Dept. of Physiol., Univ. of TX Hlth Sci. Ctr, San Antonio, TX 78284.

Adolescent cynomolgus monkeys were trained to bar press rapidly with either hand as an objective measure of motor function. After stable baseline press rates were achieved, the monkeys underwent MPTP intoxification by: 1) unilateral internal carotid artery injection via transfemoral catheterization, or 2) systemic injection of the femoral vein (dose 0.5 mg/kg). These treatments resulted, respectively, in: 1) unilateral, or "Hemi-Parkinsonian" symptoms, or 2) bilateral bradykinesia, rigidity, and tremor. In hemiparkinson animals, pressing in the ipsilateral hand was unaffected by MPTP-injection, while pressing with the contralateral hand ceased. Oral L-Dopa-Carbidopa resulted in transient improvement in both bilateral and unilateral preparations. Autologous adrenal medulla to caudate transplantation was performed, and was followed within 1-3 weeks by some degree of statistically significant improvement in behavioral measures, which persisted until sacrifice several weeks post-transplant. Histological examination revealed typical chromaffin cells with dense core granules in the head of the caudate nucleus, and degeneration of pars compacta neurons in the substantia nigra. Finally, one animal received transplant into the frontal cortex anterior to the caudate. This individual showed no behavioral improvement in the 12-week post-transplant period.

157.7

CHARACTERIZATION OF THE EFFECTS OF MPTP HEMIPARKINSONISM ON THE KINEMATICS OF A VISUALLY GUIDED ARM MOVEMENT IN THE RHESUS MONKEY. R.G. Parker*, L. Gao*, S.-K. Park, S.J. Haines*, D.A. Turner, S.N. Chou* and T.J. Ebner. Depts. Neurosurgery and Physiology, Neuroscience Grad. Prog., Univ. of MN, Mpls., MN 55455.

This study quantifies and analyzes the kinematic properties of a visually guided, multi-joint arm movement in a MPTP treated hemiparkinsonian monkey. A Rhesus monkey was trained to perform a visually guided arm movement throughout 2-dimensional space. The task required the positioning of a cursor by a manipulandum into video displayed target boxes while kinematic parameters of arm movement and muscle EMG activity were recorded. After 8 months MPTP was administered via unilateral intra-carotid injection and the animal extensively retested. Trajectory profiles were markedly abnormal following MPTP injection, characterized by a striking decomposition of the movement during deceleration. Tangential velocity and time to peak velocity were significantly increased ($p<.01$, 2-tailed, T test) for small and medium movements to most targets while larger movements were absent. Oral L-dopa increased velocity and made larger movements possible. Bradykinesia and akinesia were quantitatively demonstrated. The results suggest the nigrostriatal pathway is crucial to the organization of smooth, continuous multi-joint movements. Supported by the Minnesota Medical Foundation, the Russell Sabor Foundation and Mr. Hal Seth.

157.8

BILATERAL AND HEMI-PARKINSONIAN MONKEYS SPONTANEOUSLY RECOVER FROM THE EFFECTS OF MPTP. J.C.Brewer*,J.S.Schneider, T.Hawk*, J.M.Hoffman*, J.R.Barrio*, S.C.Huang*, A.Luxen,* N. Satyamurthy*, J.C. Mazziotta, and M.E. Phelps. (SPON: T.J.Bak) UCLA Sch. Med., Los Angeles, CA 90024-1769.

Nemestrina monkeys were trained to perform a bar press (BP) task or a reaction time (CRT) task which required a bar press and response to 1 of 3 lights. Two monkeys (one for each task), trained to work with both hands, were given unilateral intra-carotid (i.c.) MPTP injections. Performance on the CRT task ceased with the "affected" limb for 4 wks while the "unaffected" limb performed accurately but with increased movement time (MT). When the "affected" limb was again used, reaction time (RT) and MT were increased and accuracy decreased. Task accuracy, MT and RT have shown improvement over a 3-month period. The other monkey had decreased BP rate in both limbs acutely following i.c. MPTP, which has continued to recover over an 18-week period. 6-[F-18] fluoro-L-dopa (FD) PET scans during recovery periods showed negligible FD activity on the "affected" side and a normal-appearing caudate on the other side. Similar effects have been observed in bilateral parkinsonian monkeys in terms of behavioral recovery and abnormal FD PET findings. These data suggest that i.c. MPTP may acutely affect bilateral striatal functioning and that the hemi-parkinsonian disorder has a similar propensity for recovery as the bilateral syndrome induced by i.v. MPTP. Furthermore, recovery of function may not be entirely linked to striatal dopamine levels.

157.9

EFFECTS OF MPTP ON HABIT FORMATION AND MOTOR FUNCTION IN RHESUS MONKEYS. R.Q. Wan*, T.G. Aigner, and M. Mishkin (SPON:R. Schneider). Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

To test the hypothesis that the formation of visual discrimination habits in monkeys depends on cortico-striatal interactions, we administered MPTP, a neurotoxin known to damage the nigrostriatal dopaminergic system. Two monkeys received a total dose of 4.8 mg/kg in 14 doses (0.1 - 0.5 mg/kg, i.v.) with long interdose intervals (up to 30 days). Between doses, the animals were trained in object discrimination learning with 24-hr ITIs, a measure of habit formation, as well as on a simple motor task. Discrimination learning rates were slowed after each dose increment, but deficits were most evident when the cumulative dose exceeded 3.8 mg/kg. Motor task impairments were minor until the cumulative dose exceeded 4.3 mg/kg, and did not correlate with learning rates. Both impairments resolved within 2 weeks after the last dose of MPTP. Two additional monkeys were given 4 doses of 0.5 mg/kg MPTP (3 doses at 1-week intervals and 1 dose 40 days later). After the third dose, both animals showed severe motor impairment that resolved within 2 weeks, but only 1 showed a slower learning rate. The impairments were similar, though less severe, after the last dose of MPTP. The results suggest that MPTP impairs learning as well as motor abilities and that the magnitude of the impairment depends strongly on the dosing regimen.

157.10

IMPAIRED DETOUR REACHING IN RHESUS MONKEYS AFTER MPTP LESIONS. J.A. Saint-Cyr, R.Q. Wan*, D. Doudet, and T.G. Aigner. Lab of Neuropsychology and Clinical Brain Imaging Section, NIMH, Bethesda, MD, 20892.

Young adult monkeys were injected with MPTP under 2 regimens. Two animals had a low cumulative dose with short interinjection intervals (3 x 0.5 mg/kg, 1 week apart; 1 x 0.5 mg/kg 40 days later); 2 others had a high cumulative dose with long interinjection intervals (14 x 0.1-0.5 mg/kg, up to 30 days apart, total dose 4.8 mg/kg). Above a threshold that varied among animals, transient impairment followed by recovery was observed in 3 of the 4 animals on a discrimination learning task and in all 4 animals on a motor task. Eighteen months after the last injection of MPTP, all animals were significantly impaired on a detour reaching task compared to normal controls. At that time, PET scanning revealed a substantial decrease in 6-F-DOPA accumulation in the basal ganglia in the lesioned monkeys compared to controls. Presumably the recovery from the learning and motor impairments was due to utilization of ancillary neural circuits. The deficit in detour reaching may reflect a generalized inefficiency in acquiring novel adaptive strategies or may be a specific deficit uniquely dependent on striatal-complex-loop circuits intimately linked to premotor and prefrontal cortical regions.

157.11

CHRONIC TREATMENT WITH SKF 38393 DOES NOT MODIFY THE BEHAVIORAL AND BIOCHEMICAL RESPONSE TO BROMOCRIPTINE IN PARKINSONIAN MPTP-TREATED MONKEYS. C. Rouillard[1], T. Di Paolo[1]*, P.J. Bédard[2] and R. Boucher[2] [1]School of Pharmacy, Laval Univ. and Dept. of Molecular Endocrinology, Laval University Medical Center, Quebec, G1V 4G2; [2]Dept. of Anatomy, Fac. Med., Laval Univ., Québec, G1K 7P4.

The neurotoxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) causes destruction of nigrostriatal dopamine (DA) neurons and symptoms resembling Parkinson's disease in humans and subhuman primates. We have recently reported different effects of chronic treatments with various DA agonists in MPTP-treated monkeys (Brain Res., 379: 294, 1987; Soc. Neur. Abs., 17: 567, 1987). D_2 receptors were increased by 30% in the striatum of MPTP-treated animals. Chronic treatment with L-DOPA, Bromocriptine (BRC) or SKF 38393 (SKF) reversed the effects of denervation. No dyskinesia was seen in monkeys on BRC or SKF as opposed to the L-DOPA treated animals. However, SKF alone was ineffective in relieving the parkinsonian symptoms. In the present study, we have investigated the behavioral and biochemical effects of chronic treatment with BRC and BRC + SKF (5 mg/kg). No dyskinesia was seen in either group and there was no difference in motility. Monkeys were sacrificed three days after the last dose. [^{3}H]-spiperone binding studies in caudate and putamen showed no significant difference among Bmax and K_D values of the two groups. We also examined the density of D_1 receptors by using two concentrations of [^{3}H]-SCH 23390 (0.2 and 0.3 nM). At these concentrations, both groups had similar specific binding to D_1 receptors in the caudate and putamen. These results demonstrate that chronic treatment with SKF 38393 does not modify the behavioral and biochemical response to Bromocriptine in an animal model of Parkinson's disease. Supported by the MRC and the Parkinson Foundation of Canada.

157.12

DIFFERENT METABOLIC RESPONSES TO APOMORPHINE AND L-DOPA IN MPTP-INDUCED HEMIPARKINSONIAN MONKEYS. E. Palombo[1]*, K.S. Bankiewicz[2], J.J. Viola[1,3], A.M. Crane[1]*, I.J. Kopin[2], L. Sokoloff[1], and L.J. Porrino[1,3]. [1]NIMH; [2]NINCDS; [3]HHMI, Bethesda, MD 20892.

MPTP-induced hemiparkinsonism (HP) in monkeys is accompanied by selective metabolic changes in the striatum (↑), external pallidum (↑), and subthalamus (↓), as shown by the 2-[^{14}C]deoxyglucose method. Acute L-DOPA (200 mg P.O. with 20 mg carbidopa; n=4) reverses hemiparkinsonian signs and normalizes the metabolic activity in the striatum and subthalamus, but not in the external pallidum. It also produces metabolic activation in the substantia nigra reticulata (SNpr) and internal pallidum and depression in the lateral habenula ipsilateral to the lesion. In contrast to L-DOPA, apomorphine (0.5 mg/kg I.M.; n=2) reduces the metabolic hyperactivity of the external pallidum; induces metabolic depression in the subthalamus to levels lower than in untreated animals; produces bilateral metabolic depression in the lateral habenula; and causes a patchy increase in metabolism in the striatum ipsilateral to the lesion. Similarly to L-DOPA, apomorphine induces metabolic activation in the SNpr. These results show that L-DOPA and apomorphine act differently in MPTP-induced HP, with both agents having effects beyond mere reversal of the metabolic disturbances associated with the untreated syndrome.

157.13

TRANSMITTER HYBRIDISATION AND HISTOCHEMISTRY:APPLICATION TO MOVEMENT DISORDERS

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To investigate the biochemical organisation of the "Parkinsonian" basal ganglia we used in situ hybridisation with cDNA/cRNA probes. Hybridising fresh MPTP treated monkey striatum with a 35S cDNA probe we have shown an increase in enkephalin gene expression in the denervated striatum, with the enkephalin positive cells being homogenously distributed. Recent histochemical studies have shown that this mRNA increase is translated into an increase in enkephalin-LI in the denervated pallidum (GPe) of 6-OHDA lesioned rats. cRNA probes have been used to monitor the extent of nigral cell death in the 6-OHDA rat and MPTP treated monkey. The recent finding of a calcium binding protein (CaBP) as a matrix marker has led to the study into its sites of localisation. Using a CaBP radiolabelled probe we have shown specific localisation of this protein mRNA in the substantia nigra pars compacta cells. A double staining (AChE and CaBP) immunohistochemical technique shows that these cells are localised in the dorsal tier of the pars compacta. These cells correspond to the dopaminergic innervation of the striatal "matrix".

Supported by the Parkinson's Disease Society (UK)

157.14

ALTERATIONS IN THE DISTRIBUTION OF DOPAMINE D1 AND D2 RECEPTORS IN MPTP-INDUCED HEMIPARKINSONIAN MONKEYS.

JJ Viola[1,2], LJ Porring[2,3], KS Bankiewicz[3], AM Crane[2*], J Jehle[2*], IJ Kopin[3]. [1]HHMI, [2]NIMH, [3]NINCDS Bethesda MD

The distribution of dopamine D1 and D2 receptors in striatum and substantia nigra (SN) of MPTP-induced hemiparkinsonian monkeys was determined autoradiographically by means of in vitro binding with 3H-SCH 23390 for D1 receptors and 3H-spiperone for D2 receptors. D1 receptor density was greatest in the medial striatum and medial SN reticulata. On the MPTP lesioned side D1 receptor density was moderately increased in the dorsomedial and ventral caudate. D2 receptor density in anterior striatum was greatest in the lateral caudate and lateral putamen in agreement with previous findings (Joyce et al,'87). D2 receptor density in posterior putamen was also greatest laterally. Within the SN the highest D2 receptor density was located medially. On the MPTP lesioned side D2 receptor density was markedly increased in the striatum, particularly in the lateral anterior striatum. D2 receptor density was decreased in the lesioned SN compacta due to the loss of dopaminergic cells. In conclusion, first, there are opposite medial to lateral gradients of D1 and D2 receptors in the striatum. Second, in MPTP-induced hemiparkinsonism both D1 and D2 receptor density is increased in striatum ipsilateral to the lesion. These increases are primarily in regions of highest respective receptor density.

BEHAVIORAL ASPECTS OF AGING

158.1

DEFICITS IN MEMORY FOR PASSIVE AVOIDANCE OCCUR IN AGED C57BL/6NNia AND YOUNGER AUTOIMMUNE MICE.

M.J. Forster, M.J. Flores*, and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

We previously reported that autoimmune NZB/BINJ mice exhibited accelerated, age-dependent declines in their ability to learn an active avoidance response (Forster, M.J. et al., *Beh. Neural. Biol.*, 49:139, 1988). In order to further clarify the possible relationship between autoimmunity and decline of learning or memory, the current studies examined retention for passive avoidance learning in separate age groups of non-autoimmune C57BL/6NNia (1.5, 4, 7, 13, or 27 months) and autoimmune NZB/BINJ mice (1.5, 4, or 7 months). Following a single light/dark, step-through passive avoidance trial, separate groups from each age received retention tests after delays of either 1, 24 ,or 168 h. One- and 24-h retention was nearly equivalent for all ages of C57BL/6NNia mice, although 1.5- and 27-month-olds showed deficient performance at 168 h relative to the middle age groups. Retention performance of the two strains at 1.5 months was nearly equivalent, but retention performance after 24- and 168-h delays became poorer with advancing age for NZB/BINJ mice, whereas improvement in retention performance was evident for C57BL/6NNia mice during the same period (1.5 to 7 mo). These findings indicate that age-related increases in the rapidity of "forgetting" for passive avoidance learning occur at earlier ages in autoimmune NZB/BINJ mice than in C57BL/6NNia mice. [Supported by NIH grant AG06182 (M.J.F.)]

158.2

OXOTREMORINE-INDUCED HYPOTHERMIA: ABSENCE OF AGE-RELATED DIFFERENCES IN C57BL/6NNIa AND AUTOIMMUNE NZB/BINJ MICE.

D. Benjamin*, M.J. Forster, H.Lal, and K.C. Retz.Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

Mature autoimmune NZB/BINJ mice exhibit an attenuated hypothermic response to oxotremorine when compared with age-matched C57BL6NNia mice (Retz, K.C. et al., *Neuropharmacol.*, 26: 445, 1987). Other investigations have suggested accelerated, age-related changes in behavioral capacities (Forster et al., *Beh. Neural. Biol.*, 49:139,1988) and pharmacological responsiveness (Forster, M.J. et al., *Life Sci.*, 38:1433, 1987) in NZB/BINJ mice. Therefore, the current studies investigated the possibility that the attenuated oxotremorine-hypothermia response of mature NZB/BINJ mice reflected the end point of an age-related decline. Male NZB/BINJ (2, 6, or 10 mo.) and C57BL/6NNia mice (9, 13, or 28 mo) received oxotremorine (0.01 to 0.64 mg/kg, i.p.) in a counterbalanced repeated measures design with a 2-3 day washout elapsing between each drug presentation. Colonic temperature was measured 15 minutes prior to injection and at regular intervals up to three hours thereafter. Each of the strains showed an age-related decline in basal colonic temperature, between 13 and 28 mo for C57BL6NNia and between 2 and 10 mo in NZB/BINJ. However, neither strain showed substantial age-related differences in peak temperature drop or total degrees of hypothermia following oxotremorine. As demonstrated in previous investigations, the C57BL/6NNia mice of all ages were considerably more sensitive to the hypothermic effects of oxotremorine than NZB/BINJ mice. The finding that mature NZB/BINJ mice show an age-related decline in colonic temperature may reflect an accelerated decline in thermoregulatory capacity. However, senescent C57BL/6NNia mice evidenced no change in oxotremorine-induced hypothermia, and the attenuated oxotremorine-hypothermia response of NZB/BINJ mice showed no relationship with age. [Supported by NIH grant AGO6182 (M.J.F.)]

158.3

EFFECT OF AGE ON BEHAVIORAL RESPONSE TO COCAINE ADMINISTRATION IN RATS.

J.I. Javaid, R.F. Schlemmer, B. Duslak* and J.M. Davis* (SPON: G.N. Pandey). Illinois State Psychiatric Institute and University of Illinois at Chicago, Il 60612.

There is evidence in the literature that the intensity of pharmacological responses to a given dose of a drug can be affected by age. For example, Flemenbaum (Neuropsychobiology, 5, 213, 1979) reported that older rats showed significantly more stereotyped behavior with d-amphetamine administration than younger animals. In the present studies we report the behavioral effects (locomotor activity and stereotypic behaviors) of cocaine in young (ave. wt. 220 grams, approximately 50 days old) and older rats (ave. wt. 377 grams, approximately 110 days old). Animals in each group were injected intrapearetoneally with various doses (0-40 mg/kg) of cocaine (N = 4-6 at each dose). Locomotor activity was monitored by placing individual animals in Digiscan Animal Activity Monitors. Individual components of stereotypy, including sniffing and repetitive movements of the heads and limbs were rated according to their duration. In both groups 20 and 40 mg/kg cocaine injection produced behavioral effects significantly different than saline ($p < 0.05$). Older rats, however showed significantly decreased locomotor activity and stereotypy behaviors at 20 mg/kg of cocaine ($p < 0.01$). Contrary to our expectation, these results suggest a decrease responsivity of dopaminergic receptors with age as measured by stereotypy in rats.

158.4

PROGRESSIVE CHANGES PRODUCED BY RADIATION-INDUCED HIPPOCAMPAL DAMAGE

G.A. Mickley, J.L. Ferguson, M.A. Mulvihill*and T.J. Nemeth*. Behavioral Sciences Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145

Neonatal rat cerebral hemispheres were partially exposed to a fractionated dose of x-rays. This procedure produced hypoplasia specific to the granule cell layer of the hippocampal dentate gyrus while sparing other brain neurons. Control animals were sham irradiated. Between 71-462 days of age, we conducted 3 replications of the following behavioral tests: (1) spontaneous locomotion, (2) passive avoidance acquisition, and (3) spontaneous turning in a large bowl.

Rats with radiation-induced hippocampal damage exhibited longer bouts of slow turns (without reversals) than did control subjects. This radiation effect was statistically significant at all ages tested. However, over time, spontaneous perseverative turning was significantly potentiated in rats with hippocampal damage but increased only slightly in controls. Measures of spontaneous locomotor hyperactivity decreased in irradiated animals throughout the course of the experiment while remaining stable in controls. Passive avoidance learning did not change significantly over time.

These data suggest that radiation-induced damage to the hippocampus produces behavioral deficits that change over time as a function of subject age and test replications.

158.5

NOREPINEPHRINE NEURON GRAFTS IMPROVE SPATIAL MEMORY PERFORMANCE OF AGED RATS AND FORESTALL DEVELOPMENT OF AGE-RELATED MEMORY DEFICITS IN RATS GRAFTED AS YOUNG ADULTS. H. Barold*, J.R. Sladek Jr. and T.J. Collier. Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, N.Y. 14642.

Previous work in our laboratory indicates that 40% of 22-24 m.o. male F344 rats exhibit significant impairment of inhibitory avoidance memory performance. This deficit is accompanied by decreased norepinephrine (NE) histofluorescence in the locus coeruleus (LC), and can be ameliorated by third ventricular implants of fetal LC neurons. We report here an expansion of these studies to examine spatial memory performance in a swim maze. Six 24 m.o. F344 rats were screened for deficient performance of a spatial reference memory task, transplanted with 13-15d. fetal LC neurons, and retrained and tested to a different location in the swim maze eight weeks post-surgery. All 6 animals had grafts with well-developed clusters of NE neurons. Three rats with grafts restricted to the third ventricle showed no improvement in retention of the spatial task. In contrast, three rats with grafted cells in the parenchyma of the midline hypothalamus, thalamus and overlying cingulate cortex showed significant enhancement of retention performance. These initial results suggest specificity of targets of NE supplementation for particular memory tasks. An additional 6 rats received third ventricular grafts of fetal LC at 5 months of age, and were tested at 24 months of age on inhibitory avoidance and spatial reference memory tasks. All rats had large transplants containing clusters of NE neurons 19 mos. after grafting. All rats also exhibited maximal retention on the inhibitory avoidance task. Spatial memory performance is being analyzed and will be discussed. These findings support the view that NE neuron implants performed in adult rats continue to provide a behaviorally therapeutic effect in old age. ADRDA FSA 85-015 (TJC), AG 00847 (JRS).

158.6

THE RELATIONSHIP OF AGE-RELATED DEFICITS ACROSS SEVERAL BEHAVIORAL DOMAINS. R. Burwell*, and M. Gallagher (SPON: J. Wilson) Department of Psychology, Univ. of North Carolina, Chapel Hill, NC 27599

Young (6-8 mth) and aged (25-27 mth) male Long-Evans rats were tested on several behavioral tasks to assess the relationship among a set of measures that are sensitive to aging. These measures included spatial learning in a water maze, neophobia/recovery from neophobia for a novel taste, and the diurnal pattern of drinking. An age-related decline in spatial learning has been previously well characterized. A subpopulation of the aged rats (N=13) learned the water maze task as readily as young animals (N=29), whereas the remainder of the aged subjects (N=19) were impaired in this task. Our results indicated that young and aged rats exhibit a comparable neophobia when initially exposed to a novel taste. Aged rats were, however, significantly slower to recover from neophobia when the same taste was presented at 3 day intervals. As reflected in the diurnal pattern of drinking, there was also an age-related change in circadian rhythmicity: the percentage of water intake during the light phase of the light/dark cycle was significantly higher for the aged rats. The results of these experiments further indicated a consistent relationship between spatial learning ability and recovery from neophobia: within the aged cohort, impaired acquisition of spatial information was associated with a diminished recovery from taste neophobia. Spatial learning capacity did not, however, distinguish the degree to which diurnal behavior was affected among the aged subjects.

The results of these experiments reveal relatively distinct behavioral domains in that are sensitive to aging in rats. It is possible that neurobiological alterations in different systems may underlie those behavioral changes that do not intercorrelate. Experiments are underway to examine this hypothesis. Supported by NSF grant BNS-8719881, a Research Scientist Development Award (NIMH KO2 MH00406) to M.G., and a NSF Pre-Doctoral Fellowship to R.B.

158.7

ELEVATION OF DYNORPHIN-LIKE IMMUNOREACTIVITY IN THE AGED HIPPOCAMPUS AND SPATIAL LEARNING. M. Gallagher, H.-K. Jiang, and J.-S. Hong*. Dept. Psyc., Univ. N. Carolina, Chapel Hill, NC 27599 and Lab Molecular and Integrative Neuroscience, NIEHS/NIH, Research Triangle Park, NC 27709.

Within the hippocampal formation, the opioid peptide dynorphin is primarily localized to dentate granule cells and their mossy fiber projections to CA3. A relationship was found between dynorphin A(1-8)-like immunoreactivity (dynA(1-8)LI) in the hippocampal formation and the spatial learning ability of aged rats. An initial experiment comparing naive young and aged male Long Evans rats indicated that dynA(1-8)LI was significantly elevated in the aged hippocampus. A subsequent experiment examined dynA(1-8)LI in young (4-5 mth), middle-aged (14-16 mth), and aged (25-27 mth) rats that were trained in the Morris water maze prior to sacrifice. The behavioral results revealed an age difference in the acquisition of spatial learning: aged (but not middle-aged) rats had a significant deficit relative to the young group. These animals were subdivided into an impaired group (N=13) that performed outside the entire range of the young group, and groups of aged (N=11) and middle-aged animals that acquired the task as readily as the young group (N=18). Hippocampal dynA(1-8)LI for the unimpaired middle-aged and aged groups was not significantly different from the young group. In contrast, dynA(1-8)LI was significantly elevated in the impaired group (expressed as pmoles/g tissue, wet weight: young=11.14+0.53, impaired=16.85+1.13). Another notable finding was that dynorphin did not differ as a function of age in most other brain areas (hypothalamus, basal forebrain, striatum, parietal cortex). However, a significant age-related elevation in dynA(1-8)LI was also found in frontal cortex in two separate experiments. These data point to a dysregulation of dynorphin containing neurons in specific limbic/cortical circuitry during aging. Supported by NIMH MH35554 and a Research Career Development Award (NIMH KO2-MH00406) to MG.

158.8

HIPPOCAMPAL N-METHYL-D-ASPARTATE RECEPTORS ARE DECREASED IN AGED RATS THAT ARE ALSO IMPAIRED IN THE MORRIS WATER MAZE. M.A. Pelleymounter, G. Beatty and M. Gallagher. Dept. of Psych., Univ. of N. Carolina, Chapel Hill, N.C. 27599.

Hippocampal N-methyl-d-aspartate (NMDA) receptor characteristics of young and aged naive rats were examined by measuring the specific binding of 3H-3-[(+/-)-2-carboxypiperazin-4-propyl-1-phosphonic acid (CPP) (3.1-100 nM), a potent NMDA ligand, to frozen tissue. 10 uM L-glutamic acid was used to define non-specific binding. Bmax values for 3H-CPP binding were decreased in aged rats (Young=247.3fmol/mg protein; Aged=137.2fmol/mg protein)($p<.05$). Kd values were similar in both age groups (Young=39.54 nM; Aged=24.2 nM). A group of young and aged rats were then trained on the place version of the Morris water maze until they reached a criterion for spatial learning. Aged rats required more training to meet criterion than young rats ($p<.016$), although there was a subpopulation of aged rats that met criterion in the same number of trials as young animals. Rats were then allowed to remain in their home cages for 10 days, at which time they were sacrificed. Hippocampal Bmax values for aged rats were again significantly lower (133.2 fmol/mg protein) than those of young rats (226.6 fmol/mg protein)($p<.01$), although there was a subpopulation of aged rats that had Bmax values similar to young animals. Further, Bmax values for aged rats correlated significantly with trials to criterion ($r = -.58$; $p<.05$). There were no age-related differences in the Kd values for 3H-CPP binding (ave.= 42.0 nM). These data are interesting in light of evidence that aged rats also show altered acquisition and decay rates of long term potentiation (LTP). Further, there is evidence that NMDA receptors mediate the induction of LTP, and therefore, could be involved in the spatial learning deficits found in aged animals. Funded by NRSA Award AG-05407 to MAP, and an NIMH Research Scientist Development Award (KO2-MH00406) and NIMH grant MH39180 to M.G.

158.9

SUPERSENSITIVITY TO MORPHINE IN SENESCENT RATS FOLLOWING CHRONIC NALTREXONE. J. L. Neisewander, A. J. Nonneman, and M. T. Bardo. Dept. of Psychology, University of Kentucky, Lexington, KY 40506.

The present experiment examined whether there are age-dependent differences in sensitivity to morphine following chronic naltrexone treatment. Fisher 344 rats (3, 10, or 24 months) were implanted with a slow-release naltrexone pellet or were given sham surgery. Ten days later the pellets were removed or another sham surgery was given. Twenty-four hours after pellet removal, half the rats in each group were injected subcutaneously with either 5 mg/kg morphine sulfate or saline. Thirty min post-injection the rats were given a test for analgesia in which they were placed on a 53°C hot-plate, and latency to perform a paw lick response was recorded. Locomotor activity was then assessed in a wooden chamber equipped with photocells. The results indicated that at all ages, animals pretreated with chronic naltrexone showed enhanced sensitivity to the analgesic and locomotor depressant effects of morphine. These results suggest that middle-aged and senescent rats are capable of responding to chronic opiate antagonist treatment in a manner similar to young adult rats.

(We gratefully acknowledge Dr. Charles Mactutus for supplying the aged animals. Also supported in part by USPHS grant DA03460 and a Sigma Xi Grant-in-Aid of Research.)

158.10

BEHAVIOURAL RESPONSES TO DOPAMINERGIC STIMULATION IN AGED RATS: DECREASED YAWNING AND LOCOMOTION, BUT INCREASED STEREOTYPY A.J. Stoessl*, M.T. Martin-Iverson, T. Barth, C.T. Dourish, S.D. Iversen* (SPON: E.N.A.), Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, U.K.

We have studied the behavioural responses to apomorphine and the selective D2 agonist (+)-4-propyl-9-hydroxynaphthoxazine(PHNO) in aged (23-26mos) and mature control (6-12mos) Sprague-Dawley rats. Low doses (10-50µg/kg sc) of apomorphine, which preferentially activate dopamine autoreceptors, elicited yawning, mouth movements and penile grooming (PG). Apomorphine-induced yawning and PG were significantly decreased in the aged rats ($p = 0.01$, $p = 0.003$, respectively, 2-way ANOVA). This was associated with a 25% decrease in striatal dopamine levels, and is compatible with previous evidence for blockade of apomorphine-induced yawning in animals with 6-hydroxydopamine-induced destruction of the nigrostriatal projection. In contrast, 200 µg/kg apomorphine induced stereotyped sniffing and licking, and these behaviours were increased in the aged animals ($p \leq 0.0001$). In separate experiments, the locomotor response to infusion (14 days by osmotic minipump) of PHNO (10µg/h) was examined. PHNO increased the amplitude of circadian rhythms in locomotor activity, and daytime tolerance to this effect was reversed by stress. Both effects were significantly attenuated in aged rats ($p < 0.05$, age x drug or age x drug x stress interaction, respectively). These findings suggest that the behavioural responses to stimulation of dopamine autoreceptors and postsynaptic D2 receptors decrease with age, while the response to postsynaptic stimulation with a mixed agonist increases.

158.11

LACK OF AGING EFFECTS ON HUMAN MOVEMENT RELATED CORTICAL POTENTIALS Jaswinder Singh*, R. T. Knight, David L. Woods, D. J. Beckley* and Clay Clayworth*; (SPON: T. NAKADA). Clinical Neurophysiology Lab, Department of Neurology, VAMC; Martinez, CA 94553

We recorded movement related cortical potentials (MRCPs) in 13 young (age range =23-40, mean age=29.6) and 13 old (age range=45-78, mean age=65.8 years) normal adults in right, left (RHP and LHP) and bimanual (BMP) self-paced button press conditions. Data epochs beginning 1200 msec prior to and 800 msec after each button press were extracted from scalp sites over precentral, central and parietal regions. The initial MRCP consisted of a Readiness Potential (RP) onsetting at 950 to 1050 msecs prior to button press. RP was bilaterally distributed and maximal at the vertex in all conditions with comparable amplitudes for unimanual and bimanual conditions. A negative Motor Potential (MP) onset at 200 msec prior to movement and had a peak amplitude over scalp sites contralateral to unilateral movement. Scalp distributions were compatible with an RP source in premotor or supplementary motor cortices and an MP source in motor cortex. In contrast to prior reports, topographical distribution, onset latency and peak and mean amplitude were comparable between young and old subjects for the RP and MP components of the MRCP. The results indicate that motor programming as indexed by RP and motor cortex activity (MP) is unaffected by normal aging.

158.12

NIMODIPINE FACILITATES TRACE CONDITIONING OF THE EYE-BLINK RESPONSE IN AGING RABBITS. R.A. Deyo*, K.T. Straube* and J.F. Disterhoft. Dept. of Cell Biology and Anatomy, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Nimodipine (NIM), a Ca^{++} channel blocker, may enhance learning in aging humans but neither effect nor mechanism has been well studied. In the present experiment, we evaluated the effects of NIM on a task in which acquisition is known to be associated with reductions in a Ca^{++} mediated potassium current (Disterhoft, et al., PNAS, 83:2733-2737, 1986).

Nine young (3 month) and 11 aging (30-49 month) rabbits with intrajugular catheters were assigned to 1 of 4 groups based on age and drug received. These groups were young-vehicle (n=5), young-NIM (n=4), aging-vehicle (n=5) and aging-NIM (n=6). Beginning 10 min prior to training and continuing through each session animals received intrajugular infusion of either NIM (1.0 µg/kg/min) or vehicle. Animals were habituated and then trained in a trace conditioning paradigm in which the CS was a 100 ms 85 db, 6 KHz tone followed by a 500 ms trace interval (neither the CS or UCS present) followed by a 150 ms 2.5 psi corneal airpuff (UCS). An unconditioned response (UCR) was the eye-blink occurring in response to the UCS. A conditioned response (CR) was any response occurring after the CS onset but prior to the UCS onset. Animals were trained until performance reached a criterion of 8 CRS in any block of 10 trials.

NIM accelerated acquisition in both young and aging rabbits relative to vehicle controls ($F(1,16)= 11.69$, $p < .005$) but did not alter the amplitude of responses to the CS ($F(1,16)= 3.24$, n.s.) or UCS ($F(1,16)=3.30$, n.s.). Aging rabbits also showed significant learning impairments when compared to young animals ($F(1,16)= 7.77$, $p < .05$).

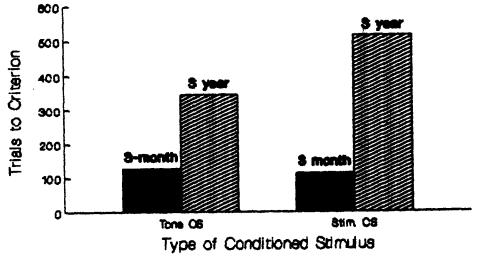
The present data do not directly test the the mechanism by which NIM facilitates associative learning. They do show that NIM enhances speed of CR acquisition directly without altering CR or UCR size, arguing against nonspecific arousal. These results are consistent with earlier reports of associative learning deficits in aging rabbits. Finally, they indicate that rabbit eye-blink conditioning is a useful model with which to study the neurobiological mechanisms underlying NIM's enhancement of learning.

Supported by the Miles Institute and The American Federation for Aging Research.

158.13

AGE DIFFERENCES IN CLASSICAL CONDITIONING OF THE NM/EYEBLINK RESPONSE IN RABBITS IN THE DELAY PARADIGM. D. S. Woodruff-Pak, Dept. of Psych., Temple Univ., Philadelphia, PA 19122

While age differences in the NM response in rabbits in the trace paradigm are clearly documented, the existence of age differences in the delay paradigm is debated. We present here data from two studies using two types of CS in young and older rabbits. Both types, (85 dB, 1 KHz tone; 200 Hz train of 0.1 msec constant current to pontine nucleus) were paired with a 3 psi airpuff US and resulted in significant age differences. [Supported by 1 F33 AG05312].



158.14

VISUAL ACUITY LOSSES IN AGED PIGEONS. W. Hodos, R.W. Miller* and K. V. Fite. Dept. of Psychology, Univ. of Maryland, College Park, MD 20742 and Dept. of Psychology, Univ. of Mass., Amherst, MA 01003.

The visual acuities of 15 pigeons ranging in age from 2-16 years were tested in an operant-conditioning chamber. The stimuli were high-contrast, square-wave gratings. A systematic decline in acuity with age was observed that was well described by a logarithmic function. Pupil diameter also decreased with age, which resulted in a decrease in retinal illumination and an increase in depth of focus. The decrease in retinal illumination and presbyopia were able to account only for about 40% of the acuity loss, but this was in part compensated for by the increased depth of focus. No relationship between corneal or lenticular density and age was observed. Ophthalmoscopic examination of the optic media revealed no abnormalities that were correlated with age. Microscopic examination of epon-embedded, thin sections of both the fovea and area dorsalis of the retina revealed no age-correlated differences in the thickness of the retinal layers nor in the densities of photoreceptors and ganglion cells. The authors speculate that losses of central neurons may in part account for the decline in acuity with age.

158.15

AGE-RELATED DIFFERENCES ON A CONTINUOUS PERFORMANCE TASK IN RHESUS MONKEYS. M.J. Callahan and R.E. Davis. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Performance of aged (23+ yrs old) and young adult (5-16 yrs old) rhesus monkeys was measured on a task which depends heavily on the sustained and efficient selection and processing of information over relatively long time epochs. Using this continuous performance task, differences in performance of young adult and aged rhesus monkeys were observed under scopolamine treatment and nondrug conditions within a variety of paradigms.

This task required a monkey to respond quickly and accurately to a colored square randomly selected and positioned on a color television monitor. Initial studies using a nondiscriminative choice-reaction test showed no difference in the ability of young adult and aged monkeys to respond to a stimulus for a food reward. With the addition of a discriminative component, aged but not young adult monkeys exhibited a decrease in performance attributable to a waning of attention as the test progressed. Parametric studies in which stimulus duration (1, 3 or 6 sec) or intertrial interval (2 or 4 sec) was varied systematically, maximized the differences in performance of young adults and aged monkeys with aged monkeys performing poorly at short stimulus durations when attentional demands are high.

Scopolamine dose dependently decreased the performance of both young and aged monkeys with young monkeys being more sensitive to the disruptive effects of this agent than aged monkeys. Results of other parametric manipulations involving increases in the numbers and colors of different stimuli and altering the duration of stimulus presentation within a test session also revealed age-related differences in rhesus monkey performance. It appears that aged-rhesus monkeys are less attentive or vigilant than young adult monkeys. These differences are maximal when a new paradigm is first introduced suggesting that aged monkeys exhibit less behavioral plasticity than young monkeys.

158.16

COGNITIVE IMPAIRMENT IN THE AGED RHESUS MONKEY. P.R. Rapp and D.G. Amaral. The Salk Institute for Biological Studies, La Jolla, CA 92037.

In the initial phase of a research program designed to provide an animal model of normal human aging, 5 aged (approximately 23-27 years old) and 4 young adult (9 years old) female rhesus monkeys were trained in a variety of behavioral testing procedures. All animals were experimentally naive prior to this research. Behavioral testing was conducted in a Wisconsin General Test Apparatus. Monkeys were initially trained in a delayed response task (DR) which required subjects to remember the spatial location of a reward presented prior to a delay interval. Although both age groups reached a learning criterion (90% correct) in an equivalent number of trials with a zero second delay, the performance of aged subjects was disproportionately impaired compared to young animals as retention intervals were increased from 0 to 30 seconds. In contrast to their performance on DR, aged subjects appeared to be impaired relative to young subjects in achieving a 90% learning criterion on a recognition memory task (i.e., delayed nonmatching to sample). When retention was tested across delays as long as 10 min., however, only relatively mild age-related deficits were apparent. These results suggest that specific aspects of memory and other cognitive functions may be differentially compromised during aging in the nonhuman primate. These findings therefore provide a framework for identifying selective neural alterations which underlie age-dependent cognitive deficits.

159.1

DIAMETERS AND PERCENTAGES OF DORSAL ROOT AXONS IMMUNOREACTIVE FOR CALCITONIN GENE-RELATED PEPTIDE (CGRP), SOMATOSTATIN (SOM), BOMBESIN (BOM) AND SUBSTANCE P (SP). R.E. Coggeshall, D.L. McNeill* and K.N. Westlund (SPON: S. Melville). Marine Biomedical Institute and the Department of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, TX 77550.

CGRP, SOM, BOM and SP immunoreactivity have been observed in small diameter primary afferent perikarya and in the superficial lamina of the dorsal horn. In the present study the diameters and percentages of axons in the L2, L4 and L6 dorsal roots immunoreactive for these peptides were obtained from normal rats. The animals were perfused with a solution of 3% glutaraldehyde, 3% paraformaldehyde and 0.1% picric acid. The roots were then removed, treated with sodium borohydride, immunostained and embedded. Thin sections were examined with an electron microscope. Preliminary data indicate that approximately 14.1% of the unmyelinated fibers are immunoreactive for CGRP, 8.6% for SP, 6.7% for SOM and 3.1% for BOM. CGRP immunoreactivity was also observed in myelinated fibers (2.3%). Immunostained unmyelinated axon diameters were approximately 0.16 $\pm$ 0.13 μm while CGRP myelinated fibers were approximately 0.60 $\pm$ 0.34 μm. These data indicate that immunocytochemical labeling of dorsal root axons can be used as an assay for experimental procedures. (Supported by grants from NIH NS11255, NS21996, Kent Waldrep National Paralysis Foundation, Marie Hall Foundation and Bristol-Meyers.)

159.2

SOMATIC SENSORY NEURONS OF THE RAT SCIATIC NERVE J.E. Swett, Y. Torigoe, V.R. Elie* and C.M. Bourassa*. Dept. of Anatomy & Neurobiol., College of Medicine, U.C.I., Irvine, CA 92717.

This laboratory reported on the locations and numbers of motoneurons (about 2000) in the rat's sciatic nerve as revealed by retrograde labeling of its branches with HRP or WGA-HRP (Swett et al., Exp. Neurol. 93:227, '86). Using identical procedures, neurons in dorsal root ganglia (DRGs) L3 to L6 were reconstructed using a mathematical factor to correct for split cell error (Hendry, J. Neurocytol., 5:337, '76; Rose & Rohrlich, J.C.N. 263:365, '87). The sciatic nerve contains 8210 ($\pm\approx$25%) DRG cells, more than 4 times the number of motoneurons. The subdivisions of the sciatic nerve contain numbers of cells as follows (mean $\pm$S.D.): sural, 1416 $\pm$397; peroneal, 2639 $\pm$576; tibial, 3804 $\pm$535. The correction factor assumes that cell shape is spherical; however, as cell shape is non-spherical, cell numbers are probably underestimated by nearly 5%. The pure muscle nerve branches supplying the triceps surae group contain 341 $\pm$30 motoneurons and 298 $\pm$25 DRG neurons; motor/sensory innervation ratios of muscle are about 1:1. Assuming that all muscles innervated by the other branches of the sciatic nerve have similar ratios, one quarter of all afferent fibers must be innervating deep tissues while the remaining 6200+ innervate skin. For each afferent supplying muscle there are 3 supplying skin. Based on perimeter measurements, mean diameter of muscle DRG neurons (36.5 $\pm$4 μm) is greater than those supplying skin (25.7 $\pm$4 μm) due to the large numbers of small DRG neurons in the latter. Thus, the population profile of skin DRG neurons is heavily skewed toward small diameters. This difference probably represents DRG neurons for C-fiber afferents, of which there are very few in muscle nerves. (Supported by NIH grants NS-17630, NS-23707)

159.3

LEVELS OF mRNA CODING FOR SUBSTANCE P, SUBSTANCE K AND SOMATOSTATIN IN SENSORY NEURONS ARE AMONG THE HIGHEST OF ANY REGION OF THE RAT OR RABBIT BRAIN. C.G. Boehmer*, R.P. Zimmerman, T.S. Gates*, and P.W. Mantyh. C.U.R.E.; Brain Research Institute; UCLA School of Medicine, LA, CA 90024

Past studies which have demonstrated the presence of certain neurotransmitters in sensory ganglia required pretreatment with colchicine to raise the neuropeptides to detectable levels. This raises the question whether sensory neurons normally synthesize these neuropeptides in quantities that are functionally significant.

We performed in-situ hybridization using ^{35}S-labeled DNA probes (30 mers) for somatostatin, preprotachykinin (substance P and substance K), vasoactive intestinal peptide and vasopressin in order to compare levels of mRNA found in individual sensory neurons with those found in neurons in other brain areas. Of the probes tested only somatostatin and preprotachykinin were detectable and their mRNA levels were among the highest of any region of the rat or rabbit brain examined. These results suggest that selected sensory neuropeptides are normally synthesized in very high levels in sensory neurons.

159.4

LECTIN BINDING TO GLYCOCONJUGATES IN CHICK SPINAL CORD AND DORSAL ROOT GANGLIA. N. Patel* and S. A. Scott. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Dorsal root ganglia (DRG) contain a diversity of sensory neurons. We are interested in identifying markers for functional subsets of DRG neurons in the chick. To this end we have screened a variety of lectins with different sugar specificities to determine whether any label distinct subpopulations of DRG neurons. Of the lectins examined only those specific for N-acetyl-galactosamine (galNAc) labeled subsets of sensory neurons in hatchling chicks. For example, Dolichos biflorus (DB) intensely labeled small diameter neurons in the dorsomedial DRG, as well as their projections in dorsal horn laminae 1 and 2 and Lissauer's tract. Specific DB staining first appeared in DRG neurons at about St.38 (d12), and in the dorsal horn at about St.42 (d16). At all stages DB staining was blocked with excess galNAc and eliminated by chloroform:methanol extraction, suggesting that in the chick a subset of DRG neurons can be distinguished by the presence of glycolipids containing galNAc residues. (Supported by NS16067 to SAS).

159.5

DIAMETER AND PERCENTAGE OF DORSAL ROOT AXONS IMMUNOREACTIVE FOR GLUTAMATE. K.N. Westlund, D.L. McNeill* and R.E. Coggeshall. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

Glutamate is a candidate excitatory amino acid neurotransmitter for spinal cord sensory processing. The diameters and percentages of dorsal root axons immunoreactive for glutamate were obtained from the L2, L4 and L6 dorsal roots from normal rats. The animals were perfused with aldehydes (3% glutaraldehyde, 3% paraformaldehyde), the roots removed and cut into 3mm lengths, and treated with sodium borohyride. The roots were then immunostained using mouse monoclonal antibody specific for fixative-modified glutamate (generously provided by A.J. Beitz) at a dilution of 1:10,000. The root segments were dehydrated, embedded, and thin sectioned. Preliminary data indicate that approximately 10% of the unmyelinated axons of the root are immunostained. The percentages of labeled axons do not differ in the sampled levels. Myelinated axons are also stained, but are fewer than the unmyelinated axons. Thus axonal labeling is predominantly in fine fibers. Axonal diameters are being determined. These data, when complete, can serve as a baseline for experimental studies. Supported by National Institutes of Health grants NS11255 and NS21996, the Kent Waldrep National Paralysis Foundation, the Marie Hall Foundation and Bristol-Meyers.

159.6

MAGNETIC SENSITIVITY OF THE AVIAN OPHTHALMIC NERVE. R.C. Beason, P. Semm* and A.J. Mackie*. Biology Dept., State Univ. of New York, Geneseo, NY 14454.

The bobolink (Dolichonyx oryzivorus), a transequatorial migratory bird, has demonstrated behavioral responses to earth-strength magnetic field (MF) stimuli (Beason, R.C. J. Ornithol., 128:317, 1987), which may involve a magnetite-based MF transducer (Beason, R.C. & Brennan, W.J. J. Exp. Biol., 125:49, 1986). Deposits of a magnetic material lie in the ethmoid region and appear to be innervated by branches of the trigeminal nerve, especially the ophthalmic branch. Extracellular recordings, made with glass microelectrodes filled with 4 M NaCl, indicate a sensitivity to changing orientations of earth-strength MF stimulation. The rate of spontaneous activity was modified in 15% of the 195 units recorded from 18 birds. The most common response was a 20% to 100% increase in firing rate, which sometimes outlasted the 4-6 s stimulus by 1-2 min. Additional fiber responses included inhibition, ON-OFF, and OFF responses.

160.1

NEOSTRIATAL UNIT ACTIVITY DURING LEARNING ENHANCED IN RABBITS WITH FIBER SPARING LESIONS IN THE ANTERIOR CINGULATE CORTEX. M. Gabriel, Y. Kubota, A. Cox*, and C. Cuppernell*. Dept. Psychol., Univ. Illinois, Champaign, IL 61820

Past studies have implicated limbic cortical and thalamic neural circuitry in the mediation of discriminative avoidance conditioning in rabbits. A theoretical working model based on these studies generates the hypothesis that conditional stimulus (CS) driven activity of Layer V corticostriatal cells in cingulate cortex is involved in triggering the conditioned response (CR, stepping in an activity wheel). A study to test this hypothesis showed that bilateral electrolytic and aspirative lesions in the cingulate cortex (Brodmann's areas 24 and 29) blocked acquisition of the avoidance response. However, the neostriatal multi-unit discharges elicited by the CSs were significantly greater during training in rabbits with lesions than in intact controls (Lambert & Gabriel, Soc. Neurosci. Abstr. 86.19, 1982). Moreover, the discharges in the rabbits with lesions were induced by the conditioning procedure and they were associative in character (i.e., greater in response to the positive CS [CS+], the stimulus that predicted a footshock unconditioned stimulus and called for the CR, than to the negative CS [CS-]). These associative properties were absent in the intact rabbits. Here, multi-unit activity in the dorsal anterior neostriatum was recorded during conditioning in 7 rabbits given bilateral ibotenic acid (IBO) lesions in area 24 during surgery for implantation of the recording electrodes. The recordings were compared to those obtained in 7 controls. Three controls received saline instead of IBO injections and four received IBO injections that caused only minimal lesions. Neostriatal training-induced discharges were significantly greater in rabbits with lesions than in controls. In this study however, with more restricted lesions, behavioral acquisition was only mildly impaired. These results confirm the previous findings and they suggest that the lesion-induced enhancement of neostriatal discharges is not due to damage to passing fibers in cingulate cortex. (Supported by NIMH Grant MH37915 to MG).

160.2

CUE ELICITED NEURONAL DISCHARGES IN LIMBIC THALAMIC NUCLEI PEAK AT DIFFERENT TRAINING LEVELS DURING AVOIDANCE LEARNING IN RABBITS. Y. Kubota, J. Shenker, M. Mignard, D. Parish*, and M. Gabriel. Dept. Psychol., Univ. Illinois, Champaign, IL 61820

Rabbits with chronically implanted multi-unit recording electrodes in the anterodorsal (AD), anteromedial (AM), anteroventral (AV), laterodorsal (LD), and mediodorsal (MD) nuclei of the thalamus were trained to avoid a shock unconditioned stimulus (US) by stepping in an activity wheel in response to a tone (positive conditioned stimulus, CS+). They also learned to ignore a different tone (negative conditioned stimulus, CS-) that was presented equally as often as the CS+. Daily training sessions consisting of 60 trials with each CS in an irregular order were given until conditioned avoidance response (CR) performance reached a criterion, and 3 additional sessions were given after criterion. In all nuclei except the AM, the neuronal discharge in response to CS presentation increased as conditioning progressed and this increase was greater to the CS+ than to the CS-. The maximal neuronal discharge in AD, LD, and AV/MD nuclei occurred in the first training session, the session of the first significant CR performance, and the session of criterial CR performance, respectively. The discharge magnitudes then declined as training continued beyond sessions of the maximal discharge. This pattern of results indicates that the spatial distribution of CS driven training-induced thalamic excitation arriving in the limbic cortex varies with the stage of training. Such variation may provide a neural code for the age of the discriminative habit, a code that is likely to be of importance in relation to retention and recall. A hypothetical mechanism for the neural coding of habit age remarkably similar to the present data has been proposed by Deutsch (In Neurobiology of Learning and Memory, G. Lynch, J. McGaugh and N. Weiberger [Eds.], 1984, 105-110). (Supported by NIMH grant MH37915 to MG).

160.3

DOPAMINERGIC AND CHOLINERGIC RECEPTOR BLOCKADE, AVOIDANCE BEHAVIOR, AND TRAINING-INDUCED LIMBIC CORTICAL AND THALAMIC UNIT ACTIVITY IN RABBITS. S. Poonawala*, V. Henzi*, Y. Kubota, and M. Gabriel (SPON: P. Johnston). Dept. Psychol., Univ. Illinois, Champaign, IL 61820

Past studies of the neuronal correlates of training and the effects of lesions have indicated that neurons of the limbic thalamus and cingulate cortex are importantly involved in mediating discriminative avoidance behavior in rabbits (Gabriel, Kubota & Shenker, in Information Processing by the Brain, H. Markowitsch [Ed.], 1988). As a prelude to manipulating dopamine and acetylcholine in these areas, we recorded the multi-unit activity of the cingulate cortex (Brodmann's areas 29c, 29b and 24) and the anterior ventral (AV) nucleus of the thalamus during conditioning in rabbits systemically injected with scopolamine hydrobromide (SH, 1, 2, & 4 mg./kg.) and haloperidol (HA, .025, .10, & .40 mg./kg.). The rabbits learned to avoid a footshock by stepping in an activity wheel in response to a tone CS+ and they learned to ignore a tone CS- of different frequency. After training to asymptotic performance each rabbit received daily training sessions preceded by subcutaneous injection of either saline, scopolamine methylbromide (SM), or one of the drug doses. A given rabbit received either HA or SH/SM injections, but not both, in a counterbalanced order. A partial reinforcement procedure was adopted to control for the effects of shock induced arousal on the neuronal response profiles for different drug treatments. Both drugs reduced significantly the frequency of conditioned avoidance responses (CRs). This effect was monotonically related to HA dose, but all doses of SH reduced CRs equally. SM did not reduce CR frequency. As in the case of CRs, all doses of SH significantly and equally attenuated Area 29 and AV nuclear training-induced neuronal discharges. HA injections which had abolished CRs had no effect on the activity in these areas. These results suggest that the behavioral impairments produced by SH resulted from impaired neuronal processes of the cingulate cortex and anterior thalamus. However, the behavioral impairment produced by HA is more likely due to disruption of nigrostriatal and mesolimbic dopaminergic circuitry. (Supported by NIMH grant MH37915 to MG).

160.4

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF DENTATE GYRUS GRANULE CELLS FOLLOWING CLASSICAL TRACE CONDITIONING. J. Black, R.A. Deyo*, and J.F. Disterhoft. Dept. of Cell Biology and Anatomy, Northwestern University Medical School, Chicago, IL, 60611.

Our laboratory has demonstrated a reduction in the spike afterhyper-polarization (AHP) in hippocampal CA1 neurons obtained from rabbits trained in a classical eye-blink task (Disterhoft et al., 1986). CA1 and dentate gyrus neurons also exhibit conditioning-related firing changes *in vivo*, but those patterns of changes differ (Berger and Weisz, 1985). Thus, it is of interest to test dentate gyrus granule cells for a conditioning-linked AHP reduction similar to that in CA1.

26 young adult male rabbits (1-1.5 kg) were trained according to 1 of 3 procedures: 1 TRACE animals (n=6) were habituated to test apparatus and then trained using a 100 ms tone CS followed by a 300 ms trace interval (neither CS or UCS present), then a 150 ms corneal air puff (UCS). Animals received daily training to a criterion of 80% CRs. 2 PSEUDO animals (n=9) were habituated and then received an equal number of randomly-presented unpaired CS and UCS trials as a matched TRACE animal. 3 NAIVE animals (n=11) received neither habituation nor training. Following training, rabbits were sacrificed and 500µm hippocampal slices prepared. During recording, slices were maintained in ACSF at 31°C in a submerged-type recording chamber. Intracellular recordings were obtained from the granule cell layer of the dentate gyrus, using microelectrodes (filled with 3M KCl, tip impedances 80-100MΩ). Investigator performing electrophysiological tests was blind to the type of training each animal had received.

GROUP	N (cells)	MEMBRANE POTENTIAL	INPUT RESISTANCE	SPIKE HEIGHT	AHP (4-SPIKE)
NAIVE	17	-72.0 ± 2.0mV	98.2 ± 8.6MΩ	90.5 ± 4.5mV	4.7 ± 0.6mV
PSEUDO	13	-73.4 ± 2.3mV	99.2 ± 9.0MΩ	96.5 ± 3.6mV	4.3 ± 0.5mV
TRACE	7	-74.3 ± 2.3mV	98.8 ± 9.6MΩ	88.0 ± 3.0mV	4.1 ± 0.9mV

These preliminary results suggest that postsynaptic conditioning-induced AHP changes reported for CA1 neurons are not seen in granule cells. Since *in vivo* data also show that changes in CA1 firing during conditioning are qualitatively different from those in dentate, these results may indicate that different mechanisms are responsible for conditioning-induced changes in different areas of the hippocampus.

Supported by NIH NS23482 and the American Federation for Aging Research.

160.5

CHANGES IN ELECTROPHYSIOLOGICAL CHARACTERISTICS OF HIPPOCAMPAL CA1 NEURONS AFTER TRACE EYE-BLINK CONDITIONING. M.C. de Jonge, R.A. Deyo*, T.P. Bleck and J.F. Disterhoft. Dept. of Cell Biology & Anatomy, Northwestern University Medical School, Chicago, IL 60611.

It has previously been shown that the magnitude of the intracellularly evoked afterhyperpolarization (AHP) in CA1 cells is reduced after short-delay eye-blink conditioning in rabbits (Disterhoft et al., PNAS, 83:2733-2737, 1986). Trace conditioning, whereby CS and UCS do not overlap, is a more difficult associative task. In the present experiments we investigated whether different types of cellular changes would occur from those observed with the delay conditioning paradigm.

Trace conditioned rabbits (N=9) were habituated and then conditioned to a 100 ms, 85 db, 6 KHz tone (CS) followed by a 300 ms trace interval before a 150 ms, 2.5 psi corneal airpuff (UCS). Animals were trained to a criterion of 80% conditioned responses during an 80 trial training session. Pseudo conditioned animals (N=11) were habituated and then received an equal number of randomly presented unpaired CSs and UCSs for the same number of days as matched Trace animals. Naive animals (N=11) received no behavioral training. One day after rabbits reached criterion, slices were prepared from the dorsal hippocampus. Responses, recorded through KCl-filled glass microelectrodes, were digitally stored and, later, computer analyzed

An ANOVA showed a significant (F(2,46)=5.2; P<.009) reduction of AHP magnitude in trace conditioned animals vs controls. This reduction was still apparent 700 ms after the

GROUP	N (cells)	MEMBRANE POTENTIAL	INPUT RESISTANCE	SPIKE HEIGHT	AHP (4-SPIKE)
NAIVE	15	-67.1± 2.3mV	55.3± 2.6MΩ	94.1± 3.0mV	4.3± 0.5mV
PSEUDO	19	-64.8± 1.4mV	59.6± 3.5MΩ	97.8± 1.3mV	4.8± 0.4mV
TRACE	15	-70.7± 2.1mV	62.4± 4.9MΩ	96.6 ± 2.9mV	2.9 ± 0.4mV

offset of the depolarizing current. No significant effects of trace conditioning on resting membrane potential, input resistance or action potential magnitude were observed.

It is concluded that the medium and late AHPs are reduced after trace as well as delay conditoning. Trace conditioning does not appear to cause any additional ionic changes to those seen in previous studies of short-delay conditioning.

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160.6

ACTIVITY OF SINGLE HIPPOCAMPAL CA1 PYRAMIDAL NEURONS DURING TRACE EYE-BLINK CONDITIONING. E. Akase*, R.A. Deyo*, and J.F. Disterhoft (SPON:T.P. Bleck). Dept. of Cell Biology and Anatomy, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Recording from single hippocampal neurons during short-delay eye-blink conditioning has demonstrated that CA1 neurons fire in a pattern mimicking the learned response (Berger & Thompson, PNAS, 75:1572-1576, 1978). The function of this neuronal response as related to performance of the conditioned response is unkown. In the present experiment, we sought to further define the conditions of neuronal modeling of behavior by examining neurons in left and right hippocampus during trace conditioning using unilateral CS and UCS.

Subjects were 5 young adult male rabbits (1-1.5 kg) implanted bilaterally with Trent Wells recording chambers overlaying each hippocampus. Animals were habituated and then trained in a trace conditioning paradigm in which the CS was a 100 ms 85 db, 6 KHz monaural tone presented to the right ear followed by a 300 ms trace interval (neither the CS or UCS present) followed by a 150 ms 2.5 psi corneal airpuff (UCS) presented to the right eye. Animals were trained until performance reached a criterion of 80% CRs. During the next 3-4 training sessions single neuron activity was recorded by alternating from right to left hippocampus every 20-40 trials. After each recording the site was lesioned and the location of the electrode tip was verified. To date, recordings have been made from a total of 18 CA1 neurons (12 left, 6 right).

Examination of data suggests that while both left and right hippocampi exhibit modeling of the conditioned response, the magnitude of the effect in the right is much less pronounced than that observed in the left hippocampus. A second interesting finding is a marked tendency (> 50% of neurons) for inhibition of firing at CS onset followed by the typical neural modeling later in the trial as the CR was elicited. These data suggest that CA1 neurons in the hippocampus contralateral to the conditioned eye are more involved in mediating the CR than are those in the ipsilateral hippocampus. These neurons also seem to be playing an important role in timing CR onset.

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160.7

PURKINJE CELL RESPONSES IN THE CEREBELLAR VERMIS DURING PAVLOVIAN FEAR CONDITIONING IN THE RABBIT. W.F. Supple and B.S. Kapp. Dept. of Psychology, University of Vermont, Burlington, VT. 05405.

Research has demonstrated that lesions of the vermis block Pavlovian conditioned heart rate responses (HR) in rats (Supple, 1986). This study therefore examined the responses of vermal Purkinje cells during differential HR conditioning in the rabbit.

Extracellular single-unit recordings were obtained from anterior lobe Purkinje cells in response to CS+ and CS- presentations during retention testing. Many of the units demonstrated differential responding to the two stimuli. The CS+ elicited a greater increase in activity compared to the CS- in 20 neurons with spontaneous rates between 1.8 - 31.6 Hz. The responses of several neurons were significantly correlated with the magnitude of the conditioned HR response. Additional groups of neurons showed greater responses to the CS- compared to the CS+, as well as differential decreases in activity during the CS's.

These data, together with the previous lesion results, suggest that the cerebellar vermis is importantly involved in the acquisition and/or expression of conditioned HR responses.

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160.8

BILATERAL TRANSFER OF CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE RESPONSE: IMPLICATIONS FOR NEURAL SUBSTRATES. M.M. Patterson, J. C. Greiner,* A. G. Hutchins* and L. A. Wilson*. Dept. of Psychology and Coll. of Osteopathic Med., Ohio Univ., Athens, OH 45701.

In previous studies (Polenchar & Patterson, Neuro. Abst., 11:1041, 1985; Polenchar, Aluko, Donahue & Patterson, Neuro. Abst., 51.8:181, 1986), we examined neural activity during transfer of the conditioned response (CR) from trained to untrained sides during classical conditioning of the rabbit's nictitating membrane (NM). R.F. Thompson and his colleagues (e.g., McCormick & Thompson, J. Neurosci., 4:2811, 1984) have shown that the ipsilateral cerebellar dentate/interpositus nuclei appear to be the site of organization of the CR, and that their destruction causes a complete abolition of the CR following conditioning. If the unconditioned stimulus (UCS) is shifted to the opposite eye after the lesion, conditioning occurs in the newly trained eye with considerable savings. In our studies, we showed that CRs occur in both eyes during training in one eye, with the pattern of CRs being somewhat lower in the untrained eye. In addition, the pattern of unit activity which occurs in the dentate/interpositus nuclei of the untrained side is similar to that of the trained side during the initial training, when both sets of nuclei display both tone and airpuff evoked neural responses. Increases in neural activity which parallel the amplitude/time-course of the CR were also present bilaterally, but of smaller magnitude on the untrained side. When training was shifted to the untrained side, activity in the nuclei of the previously trained side increased dramatically, with a smaller increase on the now trained side. CR rates on the now trained side increased almost immediately in response to the presence of the UCS on that side. The present study determined the behavioral response in both eyes to conditioning on one side to asymptote followed by continued conditioning to the previously untrained eye. Animals were given 10 days of conditioning sessions with responses recorded from both eyes and the UCS on the left eye. The UCS was then switched to the right eye for another 10 conditioning sessions. The results indicated that there was a significant difference in conditioned responding between the two eyes during the first 10 days, with fewer responses occurring in the untrained eye. Following the UCS switch, the rate of responding in the left (previously trained) eye dropped over 2-3 days to the previous level of the right (now trained) eye, while the response rate in the right eye rose to the level formerly achieved by the left eye. These results, coupled with the results of the neural recordings, suggest a spatial effect of UCR placement operating in NM conditioning, independent of simple performance factors.

160.9

INHIBITION OF DENTATE GYRUS ACTIVITY DURING APPETITIVE CLASSICAL CONDITIONING OF THE RABBIT JAW MOVEMENT RESPONSE. R.A. Swain*, C.G. Oliver* and S.D. Berry. Department of Psychology, Miami University, Oxford, Ohio 45056.

New Zealand White rabbits were surgically implanted with 50u stainless steel multiple unit recording electrodes in the dentate gyrus under general anesthesia (Ketamine, 50mg/kg and Xylazine, 10mg/kg). Topical anesthetics and antibiotics were applied as necessary to minimize discomfort during postoperative recovery. One week later, each rabbit was adapted to restraint and conditioning apparatus, and placed on a 22 Hr water deprivation schedule. Classical conditioning was established using a 350 msec, 85 dB, 1 KHz tone as the conditioned stimulus (CS) and 1 cc, 100 msec duration, .022% saccharin-water solution as the unconditioned stimulus (UCS). Control animals received explicitly unpaired presentations of tone and saccharin. Twelve blocks of 9 training trials were given in 2 sessions.

The results showed that spontaneous dentate activity was rhythmic at approximately 8 cps. Within the first block of trials, both trained and control animals displayed inhibitory responses to CS and UCS. This inhibition remained stable in unpaired animals but showed a progressive suppression in paired animals over the course of training. These data contrast with the excitatory dentate responses reported for nictitating membrane conditioning in the rabbit (Weisz, et al., 1982) suggesting a different role for dentate neurons in jaw movement training.

160.10

HIPPOCAMPAL UNIT RESPONSES TO TRIAL SEQUENCE DURING DISCRIMINATION TRAINING IN RABBITS. C.G. Oliver*, R.A. Swain* and S.D. Berry (SPON: J. Czajа). Dept. of Psychology Miami University, Oxford, Ohio 45056.

Conditioned hippocampal and dentate responses in discrimination training may depend upon a particular sequence of prior trials (Deadwyler et al., Behav. Neural Biol., 44:201 1985; Eichenbaum et al., J. Neurosci., 7:716, 1987). After training rabbits in a nictitating membrane (NM) and jaw movement (JM) discrimination paradigm, we averaged multiple unit responses in CA1 according to whether the prior trial was the same or opposite to the current trial.

Ten New Zealand White rabbits were implanted with electrodes in the CA1 region. Surgery was performed under anesthesia (Ketamine 50 mg/kg, Xylazine 10 mg/kg) and rabbits were treated with antibiotics and local anesthetics to minimize discomfort. Each animal was given 10 days of discrimination training. JM trials consisted of a tone conditioned stimulus (CS: either 1 KHz or 8 KHz, 85 dB, 800 ms) and introral saccharin as the unconditioned stimulus (US: 0.022%, 1 cc, 100 ms). NM trials consisted of the alternative tone CS and an airpuff US (210 g/cm^2, 100 ms). Unit response histograms were generated by averaging the digitized spike trains with respect to CS and US onset.

While NM trials did not differ, JM trials showed greater rhythmicity in the autocorrelation function if preceded by another JM trial than by an NM trial. While this demonstrates an effect of trial sequence, it is the opposite of the alternation enhancement found in rats.

160.11

SINGLE UNIT ACTIVITY IN PYRIFORM CORTEX OF RATS DURING OLFACTORY DISCRIMINATION LEARNING. J. McCollum, J. Larson*, T. Otto, and G. Lynch. Center for the Neurobiol. of Learning & Memory, Univ. of Calif., Irvine, CA 92717.

The olfactory system has a number of advantages for studies of cortical memory operations in rodents. Rats learn olfactory discriminations very rapidly, and relatively early processing of olfactory cues take place in primary olfactory cortex. However, there is very little information available regarding single unit activity in olfactory cortex in freely-moving animals. The present study was undertaken to characterize unit activity in pyriform cortex of rats performing olfactory discriminations.

Animals were prepared with a chronic microdrive system for lowering recording electrodes into layer II of the pyriform cortex as identified by responses to lateral olfactory tract stimulation. After recovery from surgery the animals were trained on a series of two odor successive-cue discrimination 'go-no go' tasks. Animals required approximately 6 sessions to reach criterion performance (90% correct in 20 consecutive trials) within 20-25 trials. After training on approximately 12 sessions with different odor pairs, the activity of isolated pyriform units were recorded using etched tungsten microelectrodes. The animals were given a series of odor discrimination sessions and the unit firing activity was observed for one sec before and after odor-cue onset for all odors presented. Histograms of the mean number of cell spikes for selected time bins within the 2-sec observation period were made for each individual odor tested while recording from a unit. Responses of 15 units to odors have been analyzed in detail; an average of 8 odors was tested for each cell.

Two categories of cells based on background firing rate were observed. Spontaneous activity of type I units (90% of the recorded cells) ranged from 0.5 to 5 Hz, whereas type II units fired much faster (>10 Hz). Most type I cells occasionally fired in a high frequency burst resembling the complex-spike discharges observed in hippocampal cells. Of the type I cells analyzed, 27% increased their firing rates for more than 90% of the odors tested on these cells. Most increases in firing rate began 200 msec after odor onset. Sixty percent increased their firing rates for less than 10% of the odors tested. Analysis of the type II cells also indicated a similar distinction. Two type I cells did not fall into either category responding to an average of 50% of the odors. These data suggest that whereas some pyriform cells respond to odors non-selectively, the majority are relatively specific.

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160.12

BEYOND THE TRISYNAPTIC LOOP: EVIDENCE FOR DISTRIBUTED SPATIAL REPRESENTATIONS IN HIPPOCAMPAL CIRCUITS. B.L. McNaughton, J. Meltzer, R.J. Sutherland, and C.A. Barnes. University of Colorado, Boulder CO, 80309, and University of Alberta, Lethbridge, Alberta, Canada T1K 3M4.

A predominant theme concerning information processing in hippocampal circuitry has been the notion of a "trisynaptic loop", consisting of afferents from the entorhinal cortex (perforant path), the granule cells of the fascia dentata, pyramidal cells of CA3 and, finally, CA1 pyramidal cells, organized as a series of transverse ("lamellar") subunits. Single units were recorded (using the "stereotrode" method) from fields CA1 and CA3 in freely behaving rats several weeks following selective neurotoxin (colchicine) lesions of the granule cells of the fascia dentata (Goldschmidt et al., 1982). Apparently normal spatially selective units were recorded in CA1 and CA3 after severe loss of dentate granule cells, amounting to at least 95% within several mm in the septal and temporal directions on each side of the recording electrode (and an overall loss of about 50%). Given the strict restriction of granule cell axons to less than about 400 μm in the transverse plane (Blackstad and Kjaerheim, 1961; Gaarskjaer, 1978; Claiborne et al.,1986), we conclude that most spatial selectivity must be conferred on CA3 pyramidal cells either through interactions via the longitudinally extensive CA3 intrinsic axonal collateral system (Ishizuka et al., 1986), or via the direct entorhinal-CA3 collaterals of the perforant pathway. CA1 pyramidal cells may also receive spatial information via the direct projection from layer III of the entorhinal cortex. In spite of the apparent integrity of at least some spatially selective firing, the animals were, nevertheless, impaired on spatial tasks including the Barnes circular platform, Morris swimming pool, and radial arm maze.

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160.13

INFLUENCE OF MOTOR SET ON PLACE-RELATED HIPPOCAMPAL COMPLEX SPIKE CELL ACTIVITY. T.C. Foster*, C.A. Castro, and B.L. McNaughton (SPON: A. S. Schwartz). Dept. of Psychology, University of Colorado, Boulder, Colorado 80309.

Neuronal activity in the hippocampus is known to be modulated by the animal's behavior. In the freely moving rat, both pyramidal (CS) cells and interneurons (theta cells) have been shown to fire in phase with the rhythmic EEG activity (theta,RSA) which accompanies translational movement, and both cell types are to some extent tuned to movement velocity. In the large majority of CS cells, a striking sensitivity to spatial location is superimposed on this movement correlated modulation. In the present study, rats were trained to tolerate immobilization, implemented by snugly wrapping the body and limbs in a towel fastened with clips. Single unit activity and associated EEG were recorded using the "stereotrode" technique. CS cells were tested for location and RSA correlated activity in both the freely moving state and under restraint, by simply manually moving the animal into and out of a previously determined "place field". As reported previously by Kubie, Fox and Muller (1984), CS cell place-specificity in the freely moving rat was greater during movement associated type I theta slow wave activity (> 7 Hz) ($p < .05$). Immobilization resulted in a nearly complete suppression of place-specificity for all CS cells ($p < .001$) and an over all decrease in the discharge rate of CS ($p < .001$) and theta cells ($p < .01$). Immobilization induced changes in unit activity were accompanied by a decrease in type I theta and increased low frequency and type II theta (< 7 Hz) activity which was eliminated by atropine (50 mg/kg). We conclude that CS place-specificity and discharge activity of theta and CS cells is dependent not on motion *per se*, but on the animal's motor set, and that type II theta slow wave activity is not sufficient to drive CS cell place-specificity. The dependence of location specific discharge on the animal's perceived ability to engage in active movement through space may partly account for the relatively small number of spatially selective neurons recorded from hippocampus in restrained primates and rabbits.

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160.14

LOCATION- AND DIRECTION-SPECIFIC DISCHARGE OF RAT HIPPOCAMPAL COMPLEX-SPIKE CELLS IN AN OPEN FIELD AND ON THE RADIAL 8-ARM MAZE. B. Jones Leonard, B. L. McNaughton, and C. A. Barnes. Dept. Psychology, Univ. Colorado, Boulder, CO 80309.

Several studies on the behavioral correlates of hippocampal complex-spike cell activity have shown that, on various elevated mazes, discharge is related not only to spatial location but also to the animal's directional orientation within the preferred location (e.g., Hill, 1979; McNaughton, et al., 1983; O'Keefe, 1976; Olton, et al., 1978). In the present study, we investigated whether such directional modulation is a general property or whether it is restricted to environments such as elevated mazes in which the rat's movement trajectories are highly constrained. Complex-spike cells were recorded from CA1 and fascia dentata in freely moving rats under two environmental conditions: an elevated square (1.2m) platform and an elevated 8-arm maze centered on the same spot in the same room. On the platform rats were trained to find food in a direct path when placed on its surface. This allowed trajectories of any direction to be assessed in relation to place cell discharge. On the 8-arm maze a forced choice procedure was used. Directional selectivity was assessed relative to the center of the previously determined maximum firing rate field. Rates were compared between the preferred direction and the nonpreferred direction by computing a standard measure consisting of the difference between the rates divided by their sum. Thus, no directional preference would result in a score of 0, whereas absolute directionality would result in a score of 1.0. The average score on the open platform was 0.71 ± 0.05 (SEM), $n = 9$, whereas the score for the same cells on the radial maze was 0.52 ± 0.10 (SEM), $n = 8$. (One cell did not have a field on the maze.) These results indicate that the directional modulation of "place cells" is not accounted for by the restricted orientational experience of animals on the 8 arm maze. It appears, therefore, that the role of these cells in spatial processing can more accurately be thought of as representation of local views of the animal's environment.

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160.15

PERSISTENT ALTERATIONS OF PERFORANT PATH EVOKED RESPONSES ASSOCIATED WITH MAZE RUNNING. J.R. Keith*, B.L. McNaughton, E.J. Green, S. Scott* and C.A. Barnes. (SPON: L. NADEL). Dept. Psychology, University of Colorado, Boulder, Colorado 80309.

Exploratory behaviors are associated both with an increase in the amplitude of the synaptic component (EPSP) of perforant path evoked field responses in the fascia dentata and a general decline in the area of the population spike (Sharp et al., 1987, Soc. Neurosci. Abstr.). It is generally accepted that the hippocampus plays a critical role in the processing of new spatial information. If translocation through an environment provides such information, then the population EPSP and spike magnitudes could be sensitive to the extent to which the task requires translocation through the environment. In the present experiment we collected evoked responses from animals which were running for a food reward on 2 arms of an elevated maze, situated in an environment rich in distal cues, or restricted to the center portion of the same maze.

EPSP amplitudes were greater following sessions during which subjects had run on the maze than when they had been restricted to the center of the maze. Additionally, running on the maze produced a greater (23%, $p < .01$) reduction in the area of the evoked population spike compared to sessions when the same subjects were restricted to the center of the maze. This attenuation of granule cell excitability persisted beyond the period during which animals were required to run on the maze, suggesting that this effect can not be attributed to movement *per se*. Taken together, these data suggest that movement through a spatially informative environment produces moderately persistent alterations in perforant path to granule cell synaptic strengths as well as a pronounced attenuation of granule cell excitability. These alterations may reflect a "gating" or neural selection process which modulates the flow-through in information in the hippocampal formation.

Supported by NS20331 to B. L. M.

160.16

INVOLVEMENT OF DOPAMINERGIC AND CHOLINERGIC SYSTEMS IN POSITIVE AND NEGATIVE CUE SIGNAL LEARNING BY RAT LATERAL HYPOTHALAMIC NEURONS. K. Nakamura*, T. Ono, H. Nishijo* and T.L. Zhou* (SPON: A. Sato). Dept. Physiol., Fac. Med., Toyama Med. & Pharmaceu. Univ. Sugitani, Toyama 930-01, JAPAN

Unit activity in rat lateral hypothalamus (LHA) was recorded during discrimination learning of cue tone (CTS) stimuli predicting reward (glucose or ICSS) or aversion (weak electric shock or tail pinch). Activity of 264 neurons in the LHA were investigated by electrophoretic application of dopamine (DA), ACh and their antagonists. Of these, 234 responded during CTS learning in one or more phases. Of 121 neurons tested for both reward and aversion, 86 discriminated them and their respective CTSs. DA sensitive neurons (mostly inhibition) responded to ACh (mostly excitation) more often than DA insensitive neurons. DA sensitive neurons responded to reward and aversion and their respective CTSs more than DA insensitive neurons. DA effects were similar to those of rewarding stimuli and CTS^+. ACh sensitive neurons responded to aversion and CTS^- more than ACh insensitive neuron. ACh effect were similar to those of aversion and CTS^-. Responses to CTS^+ were blocked by spiperone, while those to CTS^- were blocked by atropine. Data suggest that inputs from dopaminergic and cholinergic systems involve the same LHA neuron in CTS learning of positive and negative reinforcement, respectively.

160.17

EFFECTS OF KYNURENIC ACID INJECTIONS INTO THE NUCLEUS ACCUMBENS ON RADIAL MAZE PERFORMANCE. G.B. Schacter*, C.R. Yang*, N.K. Innis* & G.J. Mogenson (SPON: K.L. Harburn) Depts. of Physiology & Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

The nucleus accumbens (NAC), which receives glutamatergic afferents from the hippocampus (HIPP), has been implicated in integrating limbic signals to motor movements (Mogenson, 1984). The present study investigated the role of the HIPP-NAC pathway in radial maze performance.

Rats (n=6) were food deprived and trained in an 8-arm radial maze with 8 arms baited. Bilateral injections of the glutamate antagonist, kynurenic acid (5ug/0.2ul), into the NAC had no effect on performance accuracy (no. of correct responses in the first 8 choices and returning to previously visited arms, i.e. working memory). However, the rats spent an increased amount of time on the central platform ($t=4.99$, $p<0.005$) and on the arms of the maze ($t=2.75$, $p<0.05$) compared to control tests after saline injections. This effect was due to an increased latency to initiate movement and not due to a reduction in the speed of movement.

Bilateral injections of kynurenic acid into the NAC of rats trained in an 8-arm radial maze with 4 arms baited, resulted in a deficit in performance accuracy (no. of correct responses in the first 4 choices and visits to arms not baited, i.e. reference memory) as well as an increased latency to initiate movement.

(Supported by NSERC of Canada).

160.18

DIFFERENTIAL HIPPOCAMPAL AND CORTICAL CHOLINERGIC ACTIVATION AS A FUNCTION OF BOTH TYPE AND DURATION OF MEMORY TESTING OF MICE IN A RADIAL MAZE. T.Durkin, A. Toumane and R. Jaffard, Lab. Psychophysiologie, UA CNRS n° 339, Univ. Bordeaux I, Avenue des Facultés, 33405 TALENCE FRANCE

We showed previously that spatial discrimination learning by mice in an 8-arm radial maze induces differential activation of both the cholinergic septo-hippocampal and nBM-cortical pathways. Whereas the amplitudes of activation did not vary significantly in either hippocampus or cortex 30 sec post-session over the 9 days of acquisition evidence was obtained that the duration of activation in each structure might vary differentially as a function of acquisition. The hypothesis that the duration of cholinergic activation varies in each structure as a function both of the type of memory (reference or working) tested and of the duration of testing (days) was investigated by measuring sodium-dependent high affinity choline uptake in both hippocampus and cortex at different times after specific reference or working memory tests. Tests (simple two-choice procedures) were administered over 15 days. Both types of memory testing induced similar and significant cholinergic activation in each brain region 30 sec post-test each day. However cholinergic activity remained elevated particularly in frontal cortex, 15 min following reference tests over the 15 days whereas progressive attenuation of the duration of cholinergic activation was observed in both brain regions following repetitive testing for working memory.

161.1

MEASUREMENT OF MET-ENKEPHALIN AND ENCRYPTED MET-ENKEPHALIN IN HUMAN CEREBROSPINAL FLUID. D.L. Lucas[1]*, D.E. Byer[2]*, V.L.W. Go[1]* and T.L. Yaksh[3] (SPON: B.F. Westmoreland). Departments of [1]Gastroenterology, [2]Anesthesiology, and [3]Neurosurgery, Mayo Clinic, Rochester, MN 55905.

Met-enkephalin like immunoreactivity (MEI) has been observed in human CSF. To further characterize this activity and that of extended enkephalin encrypting forms, CSF samples from individuals undergoing largely elective surgery (34 males, ages 25 to 82; 17 females, ages 28 to 85) were collected on ice and subjected to radioimmunoassay for MEI and for total encrypted MEI (TMEI) after trypsin/carboxy peptidase B treatment. Mean MEI values were 232 pg/ml (±105) and the mean ratio of TMEI/MEI was 6.5 (±2.5). Several observations were made: 1) MEI values increased with age ($R = 0.54$, $p < 0.001$); 2) the ratio of TMEI/MEI varied inversely with MEI levels ($R = -0.64$, $p < 0.001$); 3) the TMEI/MEI ratio did not change significantly with age ($R = -0.11$); 4) no detectable differences between males and females were observed for MEI or TMEI/MEI ratios. Pools of several samples were concentrated with a Sep-Pak C-18 column and assayed for MEI and TMEI before and after passage through a YM-2 filter. The ratio of TMEI/MEI after filtration fell to near 1.0, suggesting that the principal fragments encrypting Met[5]-Enkephalin are larger than 2000 daltons. (Supported by grant NS-16541 (TLY).)

161.2

GALANIN-LIKE IMMUNOREACTIVITY (GLI) IN NORMAL HUMAN SPINAL CORD. S.R. Michener*, T.L. Yaksh, L.D. Aimone and V.L.W. Go* (SPON: D.W. Klass). Mayo Clinic, Rochester, MN 55905.

A radioimmunoassay (RIA) for galanin, a 29 amino acid peptide was developed using rabbit galanin antibody #31 (1:7000) and I^{125}-galanin (coupled by chloramine-T). Human spinal cord and ganglia were removed within 6 h of death from four neurologically normal subjects (ages 17-55). Rat studies showed no loss of GLI in this interval. Tissues were dissected into ventral (VH) and dorsal (DH) horns at each segment of the cervical, thoracic, lumbar and sacral regions of the cord and extracted by boiling 0.1 N HCl for galanin RIA. GLI in representative segments are reported (μ:ng/g wet weight; SEM<10% of μ).

	Cervical			Thoracic			Lumbar			Sacral		
	C1	C4	C7	T3	T6	T9	L1	L3	L5	S2	S4	S5
DH	*	*	3.4	2.4	*	*	4.1	4.2	5.4	8.8	11.7	12.8
VH	*	*	*	*	*	*	2.7	3.3	3.3	4.5	7.2	8.0

* Below assay sensitivity in two or more samples.

GLI in dorsal root ganglia ranged from 2-4 ng/g. GLI was characterized using reverse phase HPLC with detection by RIA. The principal peak of immunoreactivity in human cord coeluted with galanin standard and a smaller second peak eluted 7 minutes earlier. Antibody #31 does not crossreact with preprogalanin 65-105 and 108-123. These results suggest the presence of GLI in human spinal cord with highest levels in dorsal sacral cord. (Supported by NIDDK Core Center Grant #DK34988-D).

161.3

COMPARISON OF CHOLECYSTOKININ (CCK) CONCENTRATIONS IN RAT AND HUMAN SPINAL CORDS, USING TWO CCK ANTIBODIES. D.R. Roddy*, J.E. Bailey*, V.L.W. Go* and T.L. Yaksh (SPON: J.A. Gilbert). Mayo Clinic, Rochester, MN 55905.

We determined concentrations of CCK-8 (characterized by HPLC reverse phase chromatography) in dorsal horn (DH) and ventral horn (VH) of the spinal cords in Sprague-Dawley rats (n=6) and humans (n=4), aged 17-55, utilizing two specific antibodies: Ab216 is specific for the N-terminal of sulfated CCK-8; Ab3438 recognizes the amidated C-terminal, common to the CCK and gastrin molecules.

Spinal Cord Region			Cervical		Thoracic		Lumbar		Sacral	
			Con[1]	Ratio[2]	Con	Ratio	Con	Ratio	Con	Ratio
Ab216	Rat	DH	5.8	1.5	6.2	1.6	7.8	1.6	15.8	1.5
		VH	1.8	0.9	2.0	0.9	3.1	0.8	6.1	1.0
	Human	DH	1.6	1.4	1.5	1.2	3.7	1.6	9.1	1.9
		VH	1.5	1.5	1.5	1.4	2.9	1.7	7.6	1.9
Ab3438	Rat	DH	8.8		9.7		12.3		24.0	
		VH	1.7		1.8		2.6		6.3	
	Human	DH	2.2		1.8		6.1		17.1	
		VH	2.3		2.1		5.0		14.7	

[1]Con=ng/gm CCK; μ (SE<10% of μ). [2]CCK-LI: Ab3438 ÷ Ab216.

The Ab3438/Ab216 ratio was constant along the rostrocaudal axis, in rat and man. As expected, the ratio was greater than 1 in man (DH, VH) and in rat (DH). However, in rat, the DH ratio was greater than in VH. In the absence of evidence for spinal gastrin, this emphasizes the possibility of differential processing of the CCK family of peptides in DH and VH of the spinal cord. (Grant DK-34988-D.)

161.4

PLASMA UPTAKE AND METABOLISM OF [LEU]ENKEPHALIN FOLLOWING INTRAPERITONEAL ADMINISTRATION IN RATS. J.L. Martinez, Jr., G. Schulteis, L. Chisholm* & S.B. Weinberger. Dept. of Psych., Univ. Calif., Berkeley, CA 94720.

Male Sprague-Dawley rats received ip injections of ^{3}H-[Leu]enkephalin (LE) (3 μg/kg). Blood samples (0.4 ml) were collected through indwelling femoral artery cannulae at 1, 2, 3, 4, 5, 7.5, and 15 minutes following injection; blood volume was immediately replaced with saline. Plasma was immediately separated by microcentrifugation and then proteins were denatured with 0.1% TFA in MeOH. Degradation during the time between collection and denaturation was minimized by the presence of 500 μM bestatin. Total plasma ^{3}H was counted in a portion of each sample. The remainder of each sample was subjected to thin-layer chromatography (TLC) to separate LE from its combined metabolites.

The steepest rise in plasma levels of total ^{3}H was in the first minute following injection; peak levels were typically reached within 3-5 min. Substantial ^{3}H was still detectable in plasma at 15 min. TLC separation indicated that greater than 95% of plasma ^{3}H represented metabolic fragments of LE at all sampling time points. Since LE has an *in vitro* 1/2-life of about 2.5 min in rat plasma (*c.f.* Behav Neural Biol, 49:192-221, 1988), plasma degradation alone cannot account for the present data; rather, the data suggest that LE undergoes substantial metabolism before or during its uptake into plasma. These results have important implications for the design and interpretation of studies on the behavioral and physiological effects of LE following ip administration. (Supported by PHS grants DA #04195 [JLM], NRSA #1 F31 DA05334-01 [GS], & NRSA #1 F32 DA05313-01 [SBW])

161.5

EVIDENCE FOR DISSOCIATION OF POMC BIOSYNTHESIS AND RELEASE IN THE RAT ANTERIOR PITUITARY FOLLOWING DOPAMINERGIC BLOCKADE. J.H. Meador-Woodruff, B. Pellerito* and H. Akil. Mental Health Research Institute and Department of Psychiatry, University of Michigan Medical School, Ann Arbor, MI 48109.

Proopiomelanocortin (POMC) biosynthesis and processing has been previously demonstrated to be in part regulated by dopaminergic systems in both the anterior (AL) and intermediate (IL) lobes of the rat pituitary. We have previously reported on the effects of chronic (14 day) haloperidol administration on mRNA and POMC peptide levels as well as post-translational modifications in both IL and AL; we now extend these findings to include secretion of POMC products from IL and AL following chronic haloperidol treatment, as reflected by both circulating plasma levels and *in vitro* releasability studies. In IL, acute haloperidol treatment promotes rapid release of peptide products. Chronic treatment results in compensatory elevations of levels of both POMC mRNA and processed peptides, and the IL is able to continue to release peptides at pretreatment levels. In AL, however, a radically different and paradoxical situation develops. Acute haloperidol administration also results in rapid release of product; chronic treatment, however, results in decreased POMC mRNA levels, diminished peptide stores, and an inhibition of the production of processed forms of POMC. Both basal and stimulated release from the AL are not affected. These results suggest that biosynthesis may be dissociated from release in the AL; despite reduced levels of processed peptides, the AL is able to continue to maintain pretreatment levels of release. This may reflect the existence of different pools of POMC storage in AL: the reduction of total peptide content following haloperidol treatment may occur in a nonreleasable pool, whereas the releasable fraction is maintained at a constant level.

161.6

MORPHINE MODULATING PEPTIDES: DISTRIBUTION IN RAT CNS AND PITUITARY AND INTERSPECIES HETEROGENEITY. E.A. Majane and H.-Y.T Yang, NIMH, St. Elizabeths Hosp., Wash., D.C. 20032.

FLFQPQRF-NH_2 (F-8-F-NH_2) and AGEGLSSPFWSLAAPQRF-NH_2 (A-18-F-NH_2) are two FMRF-NH_2 like peptides we have previously, isolated from bovine brain. Using antisera developed against these peptides, the distribution of F-8-F-NH_2 in rat CNS was studied and FMRF-NH_2 like immunoreactivity (IR) in spinal cords of various species was characterized by HPLC coupled with RIA. F-8-F-NH_2 was found to be unevenly distributed with the highest concentration found in posterior pituitary and dorsal spinal cord (1008 and 502 fmol per mg prot. respectively). Hypothalamus and pons medulla contain intermediate concentrations of F-8-F-NH_2-IR (202 and 136 fmol per mg prot. respectively); lowest values are in cortex and anterior pituitary. In spinal cord of rat, mouse, bovine, guinea pig and human, at least one major F-8-F-NH_2 IR was detected, however, the retention times (RT) of the material varied from species to species. Though there is an interspecies molecular heterogeneity, F-8-F-NH_2-IR is found in high concentration in spinal cord of every species. This result and the opiate modulating activity of F-8-F-NH_2 taken together suggest that F-8-F-NH_2 may have a role in antinociception.

161.7

MODULATION OF STRIATAL ENKEPHALIN AND TACHYKININ TURNOVER BY THE D1 DOPAMINE RECEPTOR. A.E. Pollack, C.J. Cruz, R.M. Beckstead, and G.F. Wooten. Dept. of Neurosci, Univ. of Va. Sch. of Med., Charlottesville, VA 22908.

Chronic treatment of rats with the dopamine D2 antagonist haloperidol (HAL) increases striatal met-enkephalin (ME) and preproenkephalin (PPE) mRNA, but decreases striatal substance P (SP) and preprotachykinin (PPT) mRNA. Chronic treatment with the D1 antagonist, SCH23390, leads to the same change in striatal ME and PPE mRNA as does HAL. In this study, we examined the time course of SCH23390 induced changes in striatal ME, SP and their precursor mRNAs. Adult rats were treated with SCH23390 (1 mg/kg/d, s.c.) or vehicle for 1, 3, 7, or 21 d, and their striata and substantia nigrae processed for radioimmunoassay or RNA extraction. Striatal PPE and PPT mRNA of drug and vehicle treated rats were compared by dot blot hybridization using 32-P labeled oligonucleotide probes. After SCH23390 treatment for 3 or 7 d, striatal ME doubled, but fell to control levels after 21 d of treatment. Although striatal SP did not change at any time, nigral SP increased at 7 and 21 d. Striatal PPE and PPT mRNA increased after 21 d of treatment. These results suggest that the D1 receptor modulates the turnover of both striatal enkephalins and SP in the striatonigral projection. Moreover, this evidence implies that D1 and D2 receptors mediate similar effects on striatal PPE gene transcription, but opposing effects on PPT transcription.

161.8

PURIFICATION AND CHARACTERIZATION OF γ-ENDORPHIN GENERATING ENZYME ACTIVITY IN THE BRAIN. N.M. Soeter* and J.P.H. Burbach* (SPON: Tj.B. van Wimersma Greidanus). Rudolf Magnus Institute of Pharmacology, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands.

α- and γ-Endorphin are endogenous neuropeptides with opposite biological activity and it is suggested that the balance between these peptides is essential for adequate brain functioning. γ-Endorphin is generated from β-endorphin by a single cleavage of the Leu 17 - Phe 18 bond. The enzyme activity catalyzing this reaction has been called γ-endorphin generating endopeptidase (γ-EGE).

γ-EGE is a thiol-peptidase with a pH optimum between 8.5 and 9 and it is found in many brain areas. In order to obtain specific antibodies and inhibitors for biological investigations we are purifying the enzyme activity. Rat brain cytosol was applied to a Q-Sepharose Fast Flow column and a concentrated pool of this column was subsequently chromatographed on a Sephadex G-200 column. Fractions were further purified by ion-exchange (Mono Q) and size-exclusion (Zorbax GF-450) HPLC. On a gelfiltration column (Sephadex G-200, TSK G-3000, Zorbax GF-450) the enzyme behaved as a protein with a molecular weight of 200kDa. So far the enzyme seems different from known peptidases including the enkephalin-generating enzymes. Endo-oligopeptidase A (a gift from Dr. A. Camargo) did not cleave β-endorphin. The EC 3.4.24.15 inhibitor cF-A-A-F-pAB (a gift from Dr. M. Orlowski) inhibited our enzyme but the general inhibition profile of the two enzymes is different.

161.9

SUBCELLULAR FEATURES OF LHRH NEURONS IN CYCLING FEMALE RATS. G.R. Seiler*, P.G. Brunetta*, and J.C. King. Dept. of Anat. & Cell Bio., Tufts University Health Sciences Campus, Boston, MA 02111.

Release of LHRH from hypothalamic neurovascular terminals is essential to induce the preovulatory LH surge. Little is known about intracellular synthesis and processing of LHRH precursor to generate the decapeptide during the estrous cycle. We compared the ultrastructure of LHRH immunopositive cells of intact females sacrificed in the morning of each day. The most dramatic changes within LHRH neurons from the rostral preoptic area, surrounding the OVLT, occurred in diestrous and proestrous females. Protein-synthesizing organelles increased from diestrus to proestrus with polysomes predominant in the cytoplasm of LHRH neurons from diestrous females. Rough endoplasmic reticulum [RER] and Golgi complexes, with associated dense-cored and small, clear transport vesicles, were pronounced in LHRH neurons from proestrous females. In estrous females, the Golgi complex was less immunoreactive, so that only the dilated regions were immunopositive; few RER cisternae were present. Many dense bodies with crystalline inclusions were also present. Finally, nematosomes differed in texture with a greater filamentous character displayed during estrus. These ultrastructural features are consistent with the hypothesis that LHRH synthesis is increasingly augmented from diestrus to proestrus and that degradation is the primary event occurring in estrus. NSF DCB 8702388.

161.10

CYCLIC CHANGES IN NUMBERS OF LHRH NEURONS IN FEMALE RATS. E.S. Hiatt*, G.R. Seiler*, P.G. Brunetta*, and J.C. King. (SPON: D.A. Damassa), Dept. of Anat. & Cell. Bio., Tufts University Health Sciences Campus, Boston, MA 02111.

We have shown that augmented release of LHRH following gonadectomy is correlated with enhanced synthesis and/or processing of LHRH precursor [King and Seiler, Brain Res., in press]. In this immunocytochemical study we examined changes in the numbers of LHRH neurons in cycling females using LHRH antisera directed to either amino acid sequences 4-8 [Millar 1076] or 3-9 [Arimura 419]. Rats [16] were sacrificed on the morning of each day [n=4] of the estrous cycle. Between- [cycle day] and within-[antiserum and forebrain region] subject effects were analyzed in a repeated measures ANOVA. The analyses confirmed the concentration of LHRH neurons in the OVLT/POA forebrain region. The influence of cycle day on the number of LHRH neurons detected with 419 was significant, with the greatest number observed on proestrus. In contrast, AS 1076 detected a significantly greater number of LHRH neurons than did 419 on all days of the cycle. These data may reflect differences in both antiserum sensitivity and ability to detect multiple molecular forms of LHRH. Variations in abundance and subcellular distribution with cycle stage are suggested by ultrastructural studies of these neurons [Seiler et al. 1988]. The increase in LHRH neurons detected on proestrus may represent coordinated activation of a larger segment of the population. NSF DCB 8702388; NIH 5 R01 HD19803.

161.11

REGULATION OF THYROTROPIN RELEASING HORMONE DEGRADING ENZYMES BY THYROID HORMONE IN VIVO. C.S. Suen* and S. Wilk. Dept. of Pharmacology, Mount Sinai School of Medicine, New York, NY 10029.

Thyrotropin releasing hormone (TRH, pGlu-His-Pro-NH_2) is cleaved at the pGlu-His bond by two distinct pyroglutamyl peptidases. Pyroglutamyl peptidase I (EC 3.4.19.3), a cytosolic cysteine protease, has a broad substrate specificity cleaving all pGlu-amino acid bonds except pGlu-Pro. Pyroglutamyl peptidase II (EC 3.4.19.-.), a synaptosomal membrane-bound metalloprotease appears to be specific for TRH. We have previously reported on the regulation of pyroglutamyl peptidase I by L-3,5,3'-triiodothyronine (T_3) in GH_3 cells (Suen and Wilk, Endocrinology 121, 770 (1987)). We now report on the effect of T_3 on the activities of both pyroglutamyl peptidases in rat brain regions, pituitary and serum. In acute studies, rats received an sc injection of 100 μg T_3 and were killed after 24h. The activity of pyroglutamyl peptidase I was unaltered whereas pyroglutamyl peptidase II was increased 3 fold in pituitary, and 70% in frontal cortex. In chronic studies, animals received daily sc injections of 100 μg T_3 for 10 and 14 days. Pyroglutamyl peptidase I was significantly elevated in pituitary, hypothalamus, thalamus, hippocampus and olfactory bulb. Pyroglutamyl peptidase II was significantly elevated in frontal cortex and serum. This study demonstrates regulation of these enzymes by T_3 in vivo. These enzymes may play a role in the control of the biological activity of TRH and may mediate in part the negative feedback regulation of thyroid status by T_3. (Supported by an NIH grant NS-17392 and a Research Scientist Award MH 00350 to S.W.).

161.12

PYROGLUTAMATE AMINO PEPTIDASE (PGAII) IS LOCALIZED IN NEURONS. C.Cruz*, J.L.Charli*, M.A.Vargas* and P. Joseph-Bravo* (SPON: J. Alanis). CEINGEBI, Universidad Nacional Autónoma de México, A.P. 510-3, Cuernavaca, Mor. 62270, México.

PGAII is a membrane-bound enzyme mainly localized at the nerve terminal (Garat et al, Neuropeptides 6, 27, 1985 ; Torres et al, Neurochem. Int. 9, 103, 1986). It has high specificity for TRH and evidence supports PGAII as an ecto-enzyme (Charli et al, Neurochem. Int., in press). In an attempt to define its cellular localization, we have measured PGAII in primary cell cultures of fetal brain. Using 3H-TRH as substrate, PGAII activity was detected in intact cells or membrane fractions from 4 to 12 days cultures. When non-neuronal cell proliferation was abolished by cytosine arabinoside treatment PGAII/DNA values were increased (2.2± 0.3 vs. 0.9± 0.2, n=8, p 0.005). Treatment of cortical cells with the neurotoxic agent glutamate reduced PGAII activity by 80%, 48 h after treatment. Glial cell cultures expressed PGAI activity or lactate deshydrogenase but not PGAII. The localization of PGAII in neuronal cells strengthens the hypothesis that it is a neuropeptidase degrading released TRH. This is further supported by preliminary experiments where inhibition of PGAII increased TRH recovery in medium from hypothalamic slices incubated in vitro. (Supported in part by grants from CONACyT)

161.13

DIAZEPAM PRETREATMENT PREVENTS KAINIC ACID-INDUCED INCREASES IN TRH CONTENT OF RAT BRAIN. B.L. Wolfinger*, A. Winokur and M.S. Kreider. Dept of Psychiatry, Univ. of Penna., Phila., Pa. 19104

Previous studies in our laboratory have shown that induction of limbic seizures by systemic administration of kainic acid (KA) produced large increases in the concentration of TRH in limbic regions of the rat central nervous system (Kreider et al, Neurosci. Abs. 13:1656, 1986). We investigated whether pretreatment with the anticonvulsant diazepam would prevent KA-induced elevations in TRH content.

Male Sprague-Dawley rats (180-200 g) received i.p. injections of diazepam (5 mg/kg) or 0.9% NaCl/ethanol (1:1) vehicle. Thirty minutes later, rats received kainic acid (12 mg/kg) s.c. or saline. All rats were sacrificed 3 days later. Brain regions were homogenized, extracted with methanol and assayed for TRH content by radioimmunoassay.

In agreement with our previous study, significant elevations in the concentration of TRH in the corpus striatum (2 fold), anterior cortex (2.5 fold), amygdala/ piriform cortex (4 fold), posterior cortex (16 fold), and hippocampus (18 fold) were observed 3 days following KA administration. Pretreatment with diazepam significantly attenuated the increase observed in corpus striatum and posterior cortex without affecting the response in the other brain regions. These results indicate that there is a differential effect of diazepam on the responsiveness of TRH systems to limbic seizure activity.

161.14

THYROTROPIN-RELEASING HORMONE (TRH) mRNA IS PRESENT IN THE RAT HIPPOCAMPUS. M.J. Kubek, S.H. Larsen* and A. Melendez* Depts. of Anatomy, Psychiatry, Microbiology and Immunology and Program in Medical Neurobiology, Indiana University School of Medicine, Indianapolis, IN 46223.

Previous studies from our laboratory have shown that: 1) the hippocampus (HC) contains relatively low levels of TRH; 2) following generalized seizures (48 hrs), these levels increase at least 5-fold; 3) lesion studies revealed both extrinsic (60%) and intrinsic (40%) sources of HC TRH; and 4) immunocytochemical staining revealed the presence of TRH in pyramidal and granule cells of the HC (Neurosci. Abs. 13:995). If TRH is synthesized de novo in such cells, the corresponding TRH mRNA should be present in the HC. Total RNA was extracted from rat HCs. In order to increase sensitivity, we modified the polymerase chain reaction technique to amplify a 300 bp region of the mRNA sequence. A 300 bp amplified DNA fragment was obtained and shown to be similar to authentic TRH mRNA by: 1) containing three known restriction enzyme cleavage sites at the proper locations and 2) to hybridize predictably to a cloned cDNA fragment. DNase treatment of the RNA sample prior to amplification did not eliminate the 300 bp fragment, indicating that the template used for amplification was not DNA.

These results indicate that the HC contains TRH mRNA and is capable of de novo TRH synthesis.

Supported by grants from MDA, VA and NS 25661 to M.J.K.

161.15

4-NITROIMIDAZOLE-TRH: BINDING TO TRH RECEPTORS IN THE RAT BRAIN. **S. Vonhof*, V. M. Labroo*, L. A. Cohen*, I. Paakkari*, and G. Feuerstein. (SPON: H. Bryant) Dept. Neurology , USUHS, Bethesda, MD, 20814 and Laboratory of Chemistry, NIDDK, NIH, Bethesda, MD, 20892.**

In addition to its TSH- and prolactin-releasing properties, thyrotropin-releasing hormone (TRH) elicits analeptic responses affecting cardiovascular and ventilatory functions. We have previously shown that 4-NO_2-Im-TRH exerts cardiovascular responses similar to those of TRH, while its prolactin-releasing potency is substantially reduced. In order to determine whether the cardiotonic effects of this analog are mediated through TRH receptors, binding properties of 4-NO_2-Im-TRH were evaluated in different regions of the rat brain. Fresh tissue samples were incubated with [^{3}H]-3-Me-(His^2)-TRH and increasing analog concentrations. The analog displaced the radioligand only at concentrations where binding may be considered nonspecific (IC_{50} = 0.1 to 7.1 mM). The data show that the loss of prolactin-releasing activity of 4-NO_2-TRH is due to its lack of binding to pituitary TRH receptors. Its cardiotonic activity, however, seems to be mediated through mechanisms other than those involving the high-affinity TRH receptors recognized by [^{3}H]-3-Me-(His^2)-TRH.

161.16

ALTERED CATABOLISM OF THYROTROPIN RELEASING HORMONE [TRH] IN TRH-TREATED AMYOTROPHIC LATERAL SCLEROSIS [ALS] PATIENTS: INCREASED SPINAL CORD TRH-OH FORMATION. B.R. Brooks, J. Turner*, T. Schwartz*, W.W. Tourtellotte. Wm. S. Middleton Mem. VA Med. Ctr., Madison, WI 53705 and Wadsworth VA Med. Ctr., Los Angeles, CA 90073

We have studied the catabolism of [^{3}H-pro] TRH in cerebral ctx, cerebellar ctx, and spinal cord obtained from male controls [7] and ALS patients before [9], during [4], and after [2] alternate day subcutaneous TRH [2-4 mg/kg] administration. Homogenates were prepared in 100 mM KPO_4, pH 7.5; or 2 mM DTT, 100 mM KPO_4, pH 7.5, incubated with 9400 fM [1 uCi] TRH for 0, 10, 20, 30 and 60 minutes at 37°C and analysed by HPLC [Neurosci Lett 71: 209, 1986]. Cerebral ctx TRH degradation was increased [$p < 0.02$] in TRH-treated ALS patients [115 ± 14(SD) fM/min/mg protein] compared with controls [76 ± 15] and untreated ALS patients [85 ± 18] as was cerebellar ctx TRH degradation. Spinal cord TRH degradation was increased [$p < 0.03$] in TRH-treated ALS patients [65 ± 10] compared with controls [45 ± 9] but not untreated ALS patients [57 ± 21]. TRHOH formation was increased [$p < 0.02$] in TRH-treated ALS patients [464 ± 137 fM/mg protein] compared with controls [223 ± 193] or untreated ALS patients [169 ± 107]. Increased TRH degradation occurs at many CNS sites during TRH treatment but TRHOH formation is increased only in the spinal cord suggesting specific activation of the deamidation pathway at this site. [supported by MDA Midwest ALS Center grant]

161.17

COMBINED IMMUNOHISTOCHEMICAL AND *IN SITU* HYBRIDIZATION ANALYSIS OF NEUROTENSIN-PRODUCING NEURONS IN THE RAT MIDBRAIN. F. G. Williams and A. J. Beitz. (Spon: Jane Clements). Department of Veterinary Biology, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108.

A novel anti-neurotensin monoclonal antibody (NT8) was used to examine rat midbrain neurons that produce neurotensin (NT). The NT8 paratope requires that NT residues Pro_7-Arg_8-Arg_9 be intact. EM immunohistochemistry using either peroxidase-DAB staining before Epon embedding or colloidal gold staining after LR Gold embedding resulted in immuno-stained axon terminals, proximal dendrites, and rough ER within neuron perikarya. Although the distribution of stained neurons was enhanced by intra-ventricular colchicine injection, immunoreactive perikarya were also visible without colchicine. The apparent staining of protein-synthetic organelles suggests that NT8 can recognize putative NT synthetic precursors. Thus, NT8 may be useful for identifying and quantitating neurons that are active in NT biosynthesis.

To further establish that the immunostained neurons can produce NT, we performed *in situ* hybridizations on 10µ rat midbrain cryo-sections that had been incubated with NT8 and stained with peroxidase-DAB. Thirty-six base cDNAs to the peptide-coding region of canine enteric pro-NT were synthesized and 3' end-labeled with ^{35}S-dATP using terminal transferase. In 4-6 hour hybridizations followed by overnight washing at 37 °C and autoradiography, the antisense strand hybridized to neurons containing NT8 reaction product while the sense strand did not hybridize. Properties of the neuronal mRNA are currently being investigated. The ability to simultaneously visualize the products of NT transcriptional and translational biosynthetic activity is expected to permit further insights into the mechanisms that control NT biosynthesis. Supported by DE06682, NS19208 and BNS8607520.

161.18

APPEARANCE OF VASOACTIVE INTESTINAL POLYPEPTIDE-LIKE IMMUNOREACTIVITY IN THE PERFUSATE OF THE ISOLATED RAT ADRENAL MEDULLA AFTER FIELD STIMULATION. R.K. Malhotra,* M. Blank,* T.D. Wakade* and A.R. Wakade (Spon: H.L. Cohen) SUNY Health Science Center, Brooklyn, NY 11203.

Our previous studies have shown that in addition to acetylcholine (ACh), a noncholinergic substance(s) released from presynaptic neurons contributes to the secretion of catecholamine (CA) from the rat adrenal medulla (J. Physiol, 383:639, 1987). Among various peptides present in splanchnic neurons, vasoactive intestinal polyteptide (VIP) proved to be the most probable candidate (J. Physiol, 383:285, 1987). In the present study we have collected further evidence to support this idea. VIP content was estimated in perfusate of isolated adrenal gland before and after nerve stimulation. The respective values were 25.1±4.8 fg and 73.2±8.6 fg. VIP content in perfusate of denervated adrenal glands did not increase after nerve stimulation. Infusion of Ach in normal and denervated adrenal glands did not release VIP. CA secretion evoked by field stimulation was partially blocked (65%) by cholinergic antagonists, and inclusion of VIP antagonist (Ac-Tyr^{1}h-GRF, 10 µM) further inhibited (90%) the secretion. These findings provide direct evidence that VIP is released during stimulation of splanchnic neurons and may act as a neurotransmitter in evoking CA secretion from adrenal medullary cells.

162.1

REGULATION OF INSULIN AND GLUCOSE PLASMA LEVELS BY CNS BETA-ENDORPHIN IN PREWEANLING RATS. J. V. Bartolome*, M. B. Bartolome*, E. B. Harris* and S. M. Schanberg*. (SPON: J. M. Bell). Dept. Pharmacol. Duke Univ. Med. Ctr., Durham, N.C. 27710.

Insulin administration to rat pups increases ornithine decarboxylase (ODC) activity, a "critical" growth-related enzyme, throughout the body. This agrees with the well established role for insulin as a major regulator of somatic growth. Recently, we have found that central administration of beta-endorphin (BE) markedly alters tissue ODC responses to insulin and decreases metabolic activity in vital organs including liver and kidney. Since these organs are the major sites of insulin catabolism it seemed possible that plasma insulin levels could also be affected. To test this hypothesis rat pups were injected with BE i.c. followed by insulin s.c. and plasma levels of insulin were measured at different times after insulin.

Pretreatment of 6-day-old rat pups with BE i.c. markedly increased the apparent half-life of administered insulin. BE also prolonged the half-life of endogenous insulin. Hypoglycemia in rats injected with BE and insulin was more marked than in animals given insulin alone. Peripheral administration of BE had no effect. BE influences on plasma insulin/glucose levels seem to be restricted to early development as no changes were noticed in similarly treated 30-day-old rats.

In as much as insulin is necessary for normal growth during early life, these findings suggest that CNS BE by modulating insulin catabolism could have an important role in the regulation of perinatal development.

(Sp. by NIH Grants R01-NS25738 and 5R01-MH13688)

162.2

HYPOTHALAMO-PITUITARY-ADRENAL (HPA) AXIS TOLERANCE TO MU AND KAPPA AGONISTS IN NEONATAL RATS. D.M. Ignar and C.M. Kuhn. Pharmacology Dept., Duke Univ. Med. Ctr., Durham, NC 27710

We have shown that chronic treatment of adult rats with morphine (M) or U50,488 (U) results in specific mu or kappa HPA tolerance, respectively and that HPA stimulation by serotonergic and cholinergic agonists is decreased after chronic U, but not M. In the present study, we have compared mu and kappa HPA tolerance in neonatal rats to that seen in adults. Neonatal rats were injected sc twice daily from day 5 to 9 with M (5-25 mg/kg) or U (0.5-2.5 mg/kg). Thirty six hrs after the last treatment, animals were injected with M, U, quipazine (Q), or physostigmine (P), and serum CS determined 45 min later. M treatment affected only responses to M. Chronic U diminished responses to M, Q, and P, but not U. Acute CS elevation after M or U was more prolonged in pups than adults. The specific mu antagonist naloxonazine completely blocked responses to M and partially blocked responses to U. These results suggest that mu tolerance develops in neonatal rats, but that kappa tolerance is not observed despite the development of mu cross-tolerance and the decreased cholinergic and serotonergic responses seen in adults.

162.3

STRYCHNINE BLOCKS DYNORPHIN-INDUCED HINDLIMB PARALYSIS AND LOSS OF THE TAIL-FLICK REFLEX IN RATS. P. Stewart and L. Isaac, Depts. of Occup. Ther. and Medical Pharmacol., Univ. of Ill. at Chicago, Chicago, IL 60612.

Previously, we showed that repeated intrathecal (i.t.) injection of dynorphin A (1-13) (DYN) in rats results in a temporary (30 min) hindlimb paralysis (HP) after each injection with a permanent loss of the tail-flick reflex (TFR) after the 1st injection. We questioned whether an inhibitory pathway was involved in the HP. Rats (n=31) were implanted with an i.t. cannula and injected 3 days later with either I. (n=11) strychnine sulfate (10 nMoles) (STRY) and 10-20 min later saline, or II. (n=10) saline followed with DYN (25 nMoles), or III. (n=10) STRY followed with DYN. TFR and motor performance on an inclined plane were assessed over 24 hrs.

STRY-SAL injection resulted in a decreased TFR latency (5.3 to 3.6 sec, $p<0.05$) for 30 min without altering motor performance. Of the rats injected with the SAL-DYN combination, 80% lost TFR and exhibited HP. Only 40% of the rats in the STRY-DYN group lost TFR and demonstrated HP ($p<0.05$).

Because a major action of STRY is to antagonize glycine sites, we concluded that a glycinergic pathway is involved in both the HP and loss of TFR to i.t. DYN. Thus, it is conceivable that spinal cord DYN may modulate Renshaw cell activity in vivo.

162.4

DYNORPHIN A-INDUCED RAT HINDLIMB PARALYSIS IS NOT ALTERED BY THE κ OPIOID ANTAGONIST NOR-BINALTORPHIMINE (nBNI). J.B. Long, B. deCosta*[1], A. Martinez, R.E. Tidwell*, K. Dosaka*, K.C. Rice*[1] and J.W. Holaday, Dept. of Med. Neurosci., WRAIR, Wash., D.C. 20307, and [1]Sect. on Drug Design and Synth., NIDDK, Bethesda, MD 20205.

The κ opioid agonist dynorphin A (1-17) [DYN] causes hindlimb (HL) paralysis and nociceptive loss when injected into the rat lumbar subarachnoid space. Since these effects are shared by non-opioid DYN fragments, they appear not to require interaction with opioid receptors. However, the opioid antagonist naloxone has been reported to block paralytic effects of DYN, indicating possible involvement of both opioid and non-opioid actions. To distinguish a κ opioid component of DYN-induced HL paralysis, we directly compared dose-related HL paralytic effects of DYN and the non-opioid DYN fragment (2-17), and attempted to antagonize the effects of DYN with the selective κ opioid receptor antagonist nBNI. HL motor function and nociceptive responsiveness were evaluated in male SD rats following L4-L5 spinal subarachnoid injections of DYN or DYN (2-17) [1.6-25 nmols]. The potency and persistence of DYN and DYN (2-17) effects on motor and nociceptive function were indistinguishable. DYN and DYN (2-17) had paralytic ED_{50}s (95% C.I.s) of of 3.3 (1.9-5.8) and 6.0 (3.4-10.6) nmols, respectively, at 10 min postinjection. Furthermore, immediate preinjection of nBNI (20 nmol, i.t.) failed to alter the neurological disruptions produced by 5 and 20 nmols of DYN. Thus, DYN-induced HL motor and nociceptive loss appears to be totally attributable to its non-opioid action(s).

162.5

STIMULATION OF MU RECEPTORS INDUCES CHANGES IN HIPPOCAMPAL OPIOID PEPTIDE METABOLISM. W. Lason*, J.N. Simpson*, J.B. Daunais, J.F. McGinty, Department of Anatomy and Cell Biology, School of Medicine, East Carolina University, Greenville, NC 27858-4354 USA (SPON: W.R. Wooles).

When injected into the ventral hippocampus, mu opioid receptor agonists induce wet dog shakes and convulsions (Lee et al., Brain Res., 441: 381-385, 1988). In this study, wet dog shakes and convulsions induced by a single intrahippocampal injection of a specific mu agonist, DAGO (10 ug/0.5 ul), are accompanied by changes in hippocampal opioid peptide metabolism which persist for several days. Both behavioral and biochemical changes were prevented by naloxone (1 mg/kg, s.c.). Immunohistochemical analysis revealed that Leu^5-enkephalin (L-ENK) and dynorphin A 1-8 (DYN) immunoreactivity (IR) in mossy fibers and perforant path fibers was decreased by 1 hr and was increased markedly at 3, 24, and 72 hr after DAGO administration. By 6 days, L-ENK and DYN IR had returned to normal. In situ hybridization, using a synthetic oligonucleotide probe (Young et al., PNAS 83: 9827, 1986) revealed that the proenkephalin A mRNA in the dentate gyrus and in entorhinal cortex was increased markedly at 1 hr, 3 hr, and 24 hr following the DAGO injection. These changes in hippocampal opioid peptide metabolism are similar to those observed after kainic acid administration (Kanamatsu et al., J. Neurosci. 6: 3094, 1986), a fact which supports a role for mu receptor stimulation in limbic seizures.

162.6

OPIOIDS DECREASE MEMBRANE CONDUCTANCE DURING EARLY AND LATE IPSPs IN HIPPOCAMPAL CA1 AND CA3 PYRAMIDAL CELLS. E.S. Swearengen, R.M. Caudle* and C. Chavkin. Dept. Pharmacol., Univ. of Washington, Seattle, WA 98195.

We measured significant decreases in CA1 and CA3 pyramidal cell membrane conductance during both early and late IPSPs following bath application of opioids to submerged rat hippocampal slices. Membrane conductance was measured before and after activation of IPSPs by the voltage deflection produced by hyperpolarizing current pulses. In CA1, 10 μM normorphine or 1 μM PL017 reduced membrane conductance during early and late IPSPs, increased EPSP width and produced a second action potential during the EPSP. Addition of the NMDA receptor antagonist, D-APV (50 μM), blocked the second action potential and further blocked the membrane conductance resulting from NMDA receptor-activated currents unmasked by normorphine. Bicuculline (3 μM) decreased early IPSP conductance (to the same extent as normorphine plus D-APV) and produced a second action potential during the EPSP; neither action of bicuculline was affected by D-APV. These findings are consistent with the lessor inhibitory effect of D-APV on bicuculline- compared to normorphine-induced afterpotentials detected in extracellular recordings. In CA3, pyramidal cells with identifiable early and late IPSPs responded to 1 μM PL017 with decreased conductance during both IPSPs. In both CA1 and CA3, the predominant effect of opioid receptor activation is presynaptic inhibition of inhibitory input. Our findings suggest that opioids, presumably by inhibiting GABA release from interneurons, reduce activation of Cl^- and K^+ conductances associated with $GABA_A$ and $GABA_B$ receptors respectively. In CA1, these opioid actions are accompanied by the opening of NMDA receptor-associated channels.

Supported by NS-23483 and GM-07270.

162.7

MORPHINE TOLERANCE DECREASED MAXIMAL EXCITATION PRODUCED BY μ AND ∂ SELECTIVE OPIOID AGONISTS IN RAT HIPPOCAMPUS. T.L.Wimpey* and C. Chavkin. Dept. of Pharmacology, University of Washington, Seattle, WA 98195

Chronic morphine treatment reduced opioid effects on hippocampal CA1 pyramidal cells. Extracellular recordings of population spikes in hippocampus were compared in slices prepared from rats either implanted 7 days with morphine pellets, sham-pelleted, or untreated animals. In control groups, both μ and ∂ selective opioid agonists decreased the S1/2 (stimulus intensity producing 1/2 maximum primary response). The μ agonist PL017 induced a greater shift in S1/2 and caused a larger secondary spike than the ∂ agonist DPLPE. Maximal doses of DPLPE and PL017 together did not produce additive effects. Following morphine treatment, the maximal effects of PL017, DPLPE, normorphine and DAGO on the S1/2 were reduced without significantly affecting agonist ED_{50}. No apparent signs of withdrawal or residual morphine were detected in tolerant slices by the addition of the antagonist naloxone. Treatment of slices from naive animals with the irreversible opioid receptor alkylating agent ß-CNA produced changes that mimicked the tolerant state. ß-CNA treatment reduced the maximal change in the S1/2 caused by PL017. ß-CNA treatment also reduced or eliminated secondary population spikes induced by PL017. The ß-CNA experiments and the reduction in maximal opioid response caused by tolerance indicate that there is not a significant number of spare receptors controlling opioid response in CA1. Our results also suggest that tolerance is caused either by a change in functional receptor number or a change in receptor coupling as was shown in guinea pig ileum and locus coeruleus. Supported by DA04123.

162.8

OPIOID CONTROL OF THE RELEASE OF CALCITONIN-GENE RELATED PEPTIDE FROM PRIMARY AFFERENT FIBERS AT THE SPINAL LEVEL. Besson, J.M., Bourgoin, S., Carayon, A., Cesselin, F., Hamon, M., Lombard, M.C. and Pohl, M. INSERM, U. 161 and EPHE, 2 rue d'Alésia, 75014 Paris and INSERM, U. 288, 91 bd de l'Hôpital 75013 Paris, France.

Calcitonin-gene related peptide (CGRP) is particularly concentrated in thin primary afferent fibers and in the superficial layers of the dorsal horn and might participate in the transmission of nociceptive messages. We have examined the fate of spinal CGRP following dorsal rhizotomy in adult rats, and the possible modulation of spinal CGRP release by opioids. Ten days after the unilateral rhizotomy (from C4 to T2), a dramatic reduction of CGRP levels was found ipsilaterally in the dorsal (-89%) but not in the ventral zone of the cervical enlargement (CE). In contrast, no alteration of CGRP levels was noted controlaterally. In vitro superfusion of CE slices allowed the measurement of the spontaneous and K^+ (30mM)-evoked release of CGRP. Dorsal rhizotomy reduced CGRP outflow to undetectable levels, indicating that the peptide originates exclusively from primary afferent fibers. A marked reduction of the K^+-evoked CGRP overflow was found in the presence of 3 μM DTLET or 10 μM DAGO, each effect being antagonized by 50 μM ICI-154129 or 3 μM naloxone respectively. These results suggest that δ and μ opioids exert a presynaptic control of CGRP release from primary afferent fibers.

162.9

OPIOID MODULATING PEPTIDE INDUCES QUASI-ABSTINENCE SYNDROME IN RAT. D.H. Malin, J.R. Lake*, P.E. Prasco*, V. Balasubramanyam*, W.H. Potter*, M.K. Wiggins*, and J.E. Leyva*. Univ. of Houston-Clear Lake, Houston, TX 77058.

Malin et al (Life Sci. 41:377, 1987) found that CSF from morphine dependent rats contained a peptide that precipitated opiate abstinence syndrome when infused icv. Yang et al (PNAS 82:7757, 1985) have isolated an endogenous peptide ("opioid modulating" or "morphine modulating" or "FMRF-like peptide") with strong anti-opiate effects. In the present study, icv infusion of this peptide into normal rats induced a strong abstinence-like syndrome.

Eighteen rats were divided into three groups: A. received opioid modulating octapeptide (2 μg/min. for 7.5 min. in saline with 0.002 ug/μl bestatin); B. received the above plus 3.5 mg/kg morphine sulfate s.c.; C. received the bestatin vehicle alone. Rats were observed for a standard checklist of opiate abstinence signs for 30 min. under "blind" conditions with the following results:

OVERALL ABSTINENCE SIGNS M ± SEM		
OCTAPEPTIDE ICV	OCTAPEPTIDE ICV + MORPHINE SC	VEHICLE ICV
67.8 ± 12.3 **	3.8 ± 1.7	16.2 ± 7.0

The first group was different, $p<.01$, from both others (Tukey's HSD). The behavioral syndrome resembled opiate abstinence in that it was readily reversed by morphine and included shakes, writhes, teeth chatter, chewing, scratching, ataxia, ptosis, diarrhea, tremor, yawning, ejaculation and jumping.

162.10

SINGLE POTASSIUM CHANNELS OPENED BY OPIOIDS IN RAT CENTRAL NEURONS. M. Miyake,* M.J. Christie, and R.A. North, Vollum Institute, Oregon Health Sciences University, Portland, OR 97201

Using the cell-attached patch clamp technique, we have studied single K channels in neurons freshly dissociated from locus coeruleus of postnatal rats. The patch electrode contained (mM) 150 K gluconate, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 3 TEA-Cl and 5 Mops-KOH and the μ-opioid agonist Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO, 0.1 - 2 μM). Inward currents flowed through channels of unit conductance about 80 pS in the potential range from -50 to -120 mV, and usually occurred in bursts. This activity was eliminated by 1 μM naloxone in the electrode, and was not observed by agonist application outside the electrode. During the bursting periods (10 - 200 s), the open times of the channel were well fitted by a single exponential distribution; the closed times were fitted by the sum of two exponential functions. The mean open time was strongly dependent on the agonist concentration (τ = 2.3 ms at 0.1 μM DAGO, and 10.5 ms at 0.3 μM DAGO). By contrast, there was no obvious correlation between the DAGO concentrations and the close times (τ = 1.2 ms and 15-40 ms). Channels having very similar properties could also be activated by α_2 receptor agonists and somatostatin. The results indicate the μ-opioid, α_2-adrenaline and somatostatin receptors couple to the same potassium channels without a diffusible cytoplasmic messenger.

162.11

OPIOID PHARMACOLOGY OF THE CAPSAICIN-EVOKED RELEASE OF SUBSTANCE P (SP) FROM THE RAT SPINAL CORD IN VIVO. L.D. Aimone and T.L. Yaksh, Dept. of Neurosurgical Research, Mayo Clinic, Rochester, MN 55905.

Capsaicin has been shown to evoke the release of SP from small primary afferents. This study examined the pharmacology of opioid receptor systems which could modulate this capsaicin-evoked release. In chloralose-urethane anesthetized rats, an intrathecal catheter (PE-10) was inserted to the level of the sacral spinal cord, while a second catheter was placed at the level of the atlanto-occipital membrane to allow the push-pull perfusion (100 μl/min) of artificial cerebrospinal fluid. The addition of capsaicin (200 μM) to the perfusate raised the SP-like immunoreactivity (SP-LI) from resting levels which were below assay sensitivity (<15 pg/ml) to 43±10 pg/ml. The identity of released SP-LI was verified using reverse phase chromatography. Opioid receptor agonists were added to the capsaicin perfusate and their ability to inhibit the capsaicin-evoked release of SP was assessed. Morphine (10-100 μM), DAGO (1-100 μM), DPLPE (10-100 μM), but not U50488H (100 μM) produced a dose-dependent reduction in the capsaicin-evoked release of SP. Pretreatment with naloxone (1 mg/kg IP) antagonized the opioid-produced inhibition of capsaicin-evoked SP release. These data indicate that the release of SP from primary afferent fibers can be modulated by μ or δ but not κ opioid receptors.
(Supported by NS-16541 (TLY) and DA-05329 (LDA).)

162.12

PERIAQUEDUCTAL LEVELS OF CHOLECYSTOKININ OCTAPEPTIDE INCREASE AFTER ACUTE BUT NOT CHRONIC MORPHINE. M. Rattray*1and J. de Belleroche*2(SPON:J.E.Johnson).1:Gene Neuroscience Unit,NIDA, Addiction Research Center, Baltimore MD21224 & 2:Dept. Biochemistry., Charing Cross & Westminster Medical School, London W6 8RF, U.K.

The periaqueductal grey (PAG), a primary locus for opiate modulation of pain thresholds contains densities of opioid receptors and moderate concentrations of cholecystokinin octapeptide (CCK). PAG pools of CCK may be involved in the modulation of pain thresholds since locally applied CCK produces antinociception and CCK has been shown to antagonise opiate-dependent antinociception. We have previously studied opiate - CCK interactions in PAG slices and shown that CCK release from PAG slices *in vitro* can be modulated by morphine: after initial inhibition of CCK release, morphine enhances K+-evoked CCK release in a time-dependent manner.

Here we report that opiates can modulate CCK levels *in vivo*. Groups of adult male CFY rats were injected with morphine (10 mg/kg, i.p.) 5 min, 30 min and 1 hour before sacrifice. PAG CCK levels as measured by RIA. increased to 182% of controls one hour after injection (control levels were 0.98 ± 0.18 ng CCK/mg protein, n = 5). Similar significant increases in PAG CCK levels occured 1 hour - 3 hours post injection in weanling female LH rats. However no such rises were observed in groups of rats treated chronically with morphine (daily injection of 10 mg/kg morphine-HCl, i.p. for 4 days).

The increases observed after acute injection agree with the findings of others in hypothalamus and spinal cord, although unlike those studies, we observed no increases in CCK levels after chronic treatment. In view of our *in vitro* data, it is likely that morphine increases PAG CCK levels by modulating CCK synthesis rather than release.

162.13

OPIOIDS CAN ENHANCE AND INHIBIT THE ELECTRICALLY EVOKED RELEASE OF METHIONINE-ENKEPHALIN FROM THE ENTERIC NERVOUS SYSTEM. H. Xu*, I. Smolens* and A.R. Gintzler. Depts. of Biochemistry, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

This laboratory has previously demonstrated that evoked release of myenteric met-enkephalin is negatively modulated by morphine (Glass, et.al., J.P.E.T. 239:742-747, 1986). Recent experiments suggest that mu, delta and kappa opiate receptors, all of which are present in the myenteric plexus can modulate the electrically evoked release (40 Hz) of met-enkephalin. The direction of this modulation is not fixed but is bimodal. Both an inhibition and an enhancement of evoked release has been observed depending upon the concentration of opioid agonist that is utilized. Low concentrations enhance release while higher concentrations substantially attenuate release. The bimodal regulation of evoked enkephalin release could suggest that an alteration in the balance of these two opposing responses resulting from long term opiate exposure could be relevant to some of the physiological sequelae of tolerance and dependence that has been observed in this preparation. Specifically, it could provide a basis for the observation that the presence of morphine is a prerequisite for the manifestation of evoked enkephalin release from 15 min withdrawn tolerant/dependent preparations (Gintzler, et al., PNAS 84:2537-2539, 1987).

162.14

INCREASED CONTENT OF IMMUNOREACTIVE LEU-ENKEPHALIN AND ALTERATION OF δ OPIOID RECEPTOR IN HEARTS OF SPONTANEOUSLY HYPERTENSIVE RATS. M. Dumont and S. Lemaire. Dept. of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5.

The levels of immunoreactive Leu-Enkephalin (ir-Leu-Enk) and binding characteristics of δ opioid receptors were investigated in hearts of adult normotensive (WKY) and spontaneously hypertensive (SHR) rats. The levels of ir-Leu-Enk in acid extracts of hearts of adult SHR rats were significantly higher (2.67 pmol/g protein) than those in hearts of WKY rats (1.5 pmol/g protein). Reverse phase high performance liquid chromatography of heart extracts indicated that the increase in ir-Leu-Enk seen in whole heart extracts of SHR rats is an authentic increase in Leu-Enk since it coelutes with synthetic Leu-Enk. Binding characteristics of the δ opioid receptors were obtained at 37°C for 30 min with [^{3}H]-[D-Pen^2,D-Pen^5]-Enk. Saturation studies indicate the presence of two distincts receptors in membrane preparations of hearts of WKY rats with K_D of 1.2 and 22.8 nM and B_{max} of 3.5 and 15 pmol/g protein, respectively. However, the binding with the heart membrane preparation of SHR rats displayed only one high affinity δ receptor with a K_D of 1.5 nM and B_{max} of 6.5 pmol/g protein. These results indicate that the hypertensive state in SHR rats is accompanied by a rise in heart content of Leu-Enk and a down regulation of the low affinity δ opioid receptor. Supported by HSFO.

162.15

OPIOID PEPTIDE REGULATION OF NEURONS IN THE BED NUCLEUS OF THE STRIA TERMINALIS. **M. Dalsass and A. Siegel. Neurology, V.A. Medical Center, E. Orange, N.J. 07019, and Dept. of Neurosciences, UMDNJ, Newark, N.J. 07103.**

Recently, we described the electrophysiological properties of neurons in the bed n. of the stria terminalis (BNST) [Brain Res., 425:346, 1987]. Since the BNST contains numerous enkephalinergic axon terminals and cell bodies, we sought to examine the effects of iontophoretic application of opioid peptides upon these neurons.

Rats were anesthetized with chloral hydrate [400mg/kg]. A multibarrel pipette, designed for single unit recording [5-20 Mohms] and drug ejection, was utilized to sample neurons from the BNST in response to single or combined delivery [2-30 nA] of naloxone hydrochloride [50 mM] and D-ALA^2-MET^5-enkephalinamide (DAME) [10 mM]. Of 29 neurons examined to date, 20 responded with increased firing rates (>40%) following the application of DAME but were largely unaffected by naloxone. However, when both DAME and naloxone were delivered concurrently, the excitatory effects produced by DAME were significantly attenuated within 30 sec. of drug administration.

These results demonstrate that BNST neurons show a predominantly excitatory response to the application of opioid peptides. The mechanisms for this phenomenon are currently under examination.

[Supported by NIH Grant NS 07941-19 and by the Research Service of the Veterans Administration].

162.16

DERMORPHIN ANALOG TYR-D-ARG^2-PHE-SAR: OPIOID ANALGESIA WITHOUT RESPIRATORY DEPRESSION. **P. Paakkari*, I. Paakkari* and G. Feuerstein. Department of Neurology, USUHS, Bethesda, MD 20814.**

Tyr-D-Arg^2-Phe-Sar^4 (TAPS), a N-terminal tetrapeptide analog of the specific μ-opioid agonist dermorphin, exceeds in potency the parent heptapeptide Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH$_2$ (De Castiglione, R. and Rossi, A.C., Peptides 6,suppl.3:117,1985). In the present work the analgesic and respiratory effects of TAPS were studied in conscious, unrestrained male Sprague-Dawley rats (300±40 g). Analgesia was evaluated by tail-flick method, and respiration was recorded by a noninvasive Oxymax 85 system (Columbus Instrument).

TAPS 0.9 and 3 μmol/kg i.v. induced analgesia which was 40 % and 100 % of the maximal possible effect (MPE) for about 60 min. The effect of i.c.v. administered TAPS was longer lasting: analgesia induced by 1 nmol/kg i.c.v. was maximal for 90 min and >80 % MPE for 180 min. The rats were also cataleptic for about 60 min. The dose of 3 μmol/kg i.v. increased ventilatory minute volume by 30 % mainly due to increase in respiration rate (RR). I.c.v. injection of 1 nmol/kg decreased tidal volume by 25 %, but since RR increased simultaneously by 30%, the net effect was a slight increase (10%) in minute volume. According to these results the opioid peptide TAPS causes no respiratory depression at doses which induce complete, long lasting analgesia and catalepsy.

162.17

EFFECTS OF EXERCISE ON PLASMA ß-ENDORPHIN-LIKE IMMUNOREACTIVITY IN MEN AND WOMEN. D. Treat* and C. Cahill. Univ. of MD Sch. of Nsg. Baltimore, MD 21218

Stressors activate the hypothalamic/pituitary/adrenal axis, resulting in the secretion of ß-Endorphin-like immunoreactivity into the plasma. The study of stress in humans has primarily been limited to those naturally occurring events presumed to be stressful. This approach limits the researchers ability to describe the entire phenomenon from beginning to end. Exercise is a physical stressor which can be quantified through control of length of exercise and maximal heart rate achieved. The purpose of this study was to determine the effect of the standardized stressor of exercise on ß-END-1-i in men and women. 40 men and women were asked to exercise on an Ergometer exercise bicycle for twenty minutes at a rate sufficient to increase their heart rate to 75% of the calculated maximal level. Blood samples were collected before exercise began and immediately after the session. An indwelling catheter was inserted into a vein thirty minutes prior to the beginning of exercise. Plasma samples will be extracted and ß-END-1-i measured by radio-immunoassay. Our previous studies suggest that ß-END-1-i increases following the physical stress of exercise.

162.18

NEONATAL PLASMA LEVELS OF ß-ENDORPHIN IN PREMATURE AND TERM INFANTS WHO DEVELOP APNEA M. P. Leuschen, L. S. Nelson*, D.L. Bolam*, L.D. Willett*, R. M. Nelson Jr.*, Division of Newborn Medicine, University of Nebraska Medical Center Omaha, NE 68105.

Plasma ß-endorphin (ß-E) levels were measured during the immediate post-natal period in 30 term and 62 premature (33-38 wk) infants. At discharge, the Medical Director of the Center for Breathing Disorders (blinded to the ß-E levels at birth, reviewed the charts of all infants and determined whether an infant had experienced a documented apneic episode (instrument documented cessation of breathing for at least 20 seconds). In term infants, neither gender nor the presence of apnea were significant variables in predicting changes in ß-E levels (P=.77). In premature infants, both gender and apnea were significant for prediciting ß-E levels (P=.03). Premature males who did not experience apnea while hospitalized had ß-Es of 102.1 pg/ml ± 65.2 SD at birth; males who later had apneic episodes had ß-E levels of 71.4 pg/ml ± 50.5 SD. Premature male infants without apnea had significantly higher ß-E levels than similar females (64.4 pg/ml ±36.7 SD, P=.01). Premature male infants who developed apnea did not have significantly different ß-E levels than either premature females with apnea or any term NICU infant group; their levels were significantly higher than levels reported for term well babies. The results support the concept that ß-E may modulate the perinatal stress response but developmental status, particularly in respiratory control, as well as gender, may affect that modulation.

162.19

PET QUANTIFICATION OF ALTERED OPIATE RECEPTOR BINDING OF C-11 DIPRENORPHINE AND C-11 CARFENTANIL IN EPILEPSY. J.J. Frost, H.S. Mayberg, B. Sadzot, R. Fisher, R.F. Dannals, A.A. Wilson, J. Lever, H.T. Ravert, H.N. Wagner, Jr. The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Increases in mu opiate receptors in temporal lobe epilepsy have been demonstrated previously using C-11 carfentanil (CFN) and PET (Frost, et al. Ann Neurol 23:231-237, 1988).

C-11 diprenorphine (DPN) labels opiate receptor subtypes in addition to mu receptors. Six patients with unilateral temporal lobe epilepsy were studied using CFN, DPN and FDG to measure cerebral glucose utilization. DPN demonstrated increases in the temporal cortex ipsilateral to the seizure focus and in a wider distribution as compared to CFN. Two patients showed ipsilateral increases in CFN and DPN binding and hypometabolism in the contralateral temporal lobe.

DPN demonstrates more widespread abnormalities in temporal lobe epilepsy probably due to elevation in kappa opiate receptors in the temporal neocortex. Quantification of opiate receptors may aid in the localization of seizure foci and supplement metabolic imaging.

162.20

NALOXONE EFFECTS ON IDENTIFIED ENKEPHALINERGIC AND NON-ENKEPHALINERGIC NEURONS OF HELIX ASPERSA. R.Gutiérrez (SPON: A.Fernández-Guardiola) Lab. Neurophysiology, Instituto Mexicano de Psiquiatría. Calzada México-Xochimilco 101 CP 14370 México, D.F.

Evidences from several methodological approaches point out that it is unlikely that naloxone at high doses exerts a pure antagonistic opiate activity. There is strong experimental work dealing with its actions on several neurotransmitter systems other than the opiate one. Moreover, actions upon the Na-K pump and calcium fluxes have been documented. In order to further analyze whether the excitatory actions of naloxone seen in epileptogenesis studies (Fernández-Guardiola et al., 1988*) are due to an opioid-mediated mechanism or a non-specific action on membrane properties, experiments were conducted on identified neurons of the snail Helix aspersa. Intracellular recordings were carried out in the presence and absence of different concentrations of naloxone hydrochloride. It was found that naloxone at low concentrations† is able to produce membrane polarization changes in both directions, being depolarization the more frequent, with a concomitant increase in R_{input} and reduction of τ. Naloxone effects were not always reversible by either opiate agonists or wash out. A possible action of naloxone upon Ca^{++} currents is stressed since the afterhyperpolarization and the pacemaker potential, primarily due to $I_{K[Ca]}$ are affected.

* Fernández-Guardiola et al., Exp. Neurol., 1988 in press)
† (<1μM)

CATECHOLAMINES II

163.1

NEW INSIGHTS INTO THE CATECHOLAMINERGIC SYSTEMS OF REPTILES. W.J.A.J.Smeets[1] and H.W.M.Steinbusch[2], Department of Anatomy[1] and Department of Pharmacology[2], Vrije Universiteit, 1007 MC Amsterdam, The Netherlands.

The catecholaminergic systems in the lizard Gekko gecko have been studied with specific and sensitive antibodies against dopamine (DA), noradrenaline (NA), TH, DBH, and PNMT. Our results demonstrate that: (1) the periventricular organ (PVO) contains both DA and NA CSF-contacting cells; (2) the cells are immunonegative for TH and DBH which indicates that they accumulate rather than produce these amines; (3) at several places, particularly in the olfactory bulb, the hypothalamus and the pretectal region, only TH immunoreactive neurons were observed suggesting that these neurons contain DOPA; (4) the lateral reticular formation at obex levels, contains adrenergic and dopaminergic cells, whereas the nucleus of the solitary tract contains DA and NA immunoreactive cells; (5) antibodies against TH demonstrate primarily DA fibers and terminals, whereas the distribution of DBH immunoreactive fibers corresponds to that shown by antibodies against NA. In conclusion: combined studies with antibodies against the various catecholamines and their synthetizing enzymes provide detailed information on the distribution of the major catecholaminergic systems. Some indication is found for a, as yet, unknown DOPA-system.

163.2

SPECIES VARIATION IN EXPRESSION OF AROMATIC L-AMINO ACID DECARBOXYLASE IN CEREBELLUM, OLFACTORY BULB AND ADRENAL MEDULLA. J. Lugo*, C. Abate, T. Joh, and H. Baker. Lab. Mol. Neurobiology, Cornell Univ. Med. Coll. White Plains, NY 10605.

The enzyme aromatic l-amino acid decarboxylase (AADC) is expressed in monoamine neurons as well as a number of other cell types in brain and periphery. Recently, species differences were found among rat, mouse and hamster in both the distribution and amount of AADC-immunostaining. The present studies confirmed and quantitated the differences in AADC expression. In hamster, but not mouse or rat, AADC was co-localized in some GABA neurons in cerebellum. Western blot analysis and AADC activity measurements confirmed that the immunogen was AADC. The AADC cells in cerebellum did not exhibit immunostaining for the other monoamine markers, tyrosine hydroxylase (TH) and serotonin. In addition, the zona fasciculata of the male hamster adrenal cortex exhibited anomalous AADC staining and activity. AADC and TH staining in adrenal medulla did not differ from that in rat or mouse. Lastly, AADC-immunostaining and activity, while robust in rat and hamster, was only weakly detected in mouse olfactory bulb. These data demonstrate species and tissue specific expression of AADC. Supported by #NS23103 & #MH42626.

163.3

DOPAMINE NEURONS IN THE MIDBRAIN PERIAQUEDUCTAL GREY. B.R. Frydel*, M.T. Shipley and M.M. Behbehani. Univ. of Cincinnati Med. School, Cincinnati, OH 45267.(SPON:S.-H. Tsai.)

One of the most well characterized subpopulations of neurons in the midbrain periaqueductal grey (PAG) is the dorsal raphe nucleus (DR). The DR contains an abundance of serotonergic (5HT) neurons that perferentially innervate the forebrain. The existence of dopamine containing neurons in the dorsal raphe has been noted, but comparatively little attention has been paid to these cells.

Using antibodies specific for TH, DBH, PNMT and 5HT, we have confirmed the probable DA identity of neurons in DR and have observed that these neurons comprise a surprisingly large proportion of the neurons in DR. Estimates of the numbers of 5HT and DA neurons and their sizes will be presented. Plots of 5HT and DA neurons stained in serial, adjacent series of sections suggest that the two populations of cells are largely separate. One subpopulation of DA neurons is localized immediately adjacent to the ventral part of the aqueduct where there are very few 5HT cells. Double label ICC studies are needed to determine if some neurons contain both 5HT and DA.

The extrinsic projections of DA neurons in DR are unknown. The dendrites and possibly some axon collaterals of DR-DA neurons arborize in the ventral part of PAG where there are neurotensingeric (NT) neurons and dense plexis of NT terminals. There is evidence that NT modulates DA release in other parts of the CNS. We have shown that NT activates neurons in PAG and causes analgesia. NT-induced central anagesia is not reversed by naloxone and thus appears to be a non-opiate analgesic mechanism. The present observations suggest the DR-DA neurons could be involved in the modulation of NT anti-nociceptive circuitry in PAG. (Supported by NIH NS 20643, 23348 and 24698.)

163.4

PROPERTIES OF AM ADRENERGIC NETWORK IN THE RAT PERIAQUEDUCTAL GRAY (PAG). S.D. Chandler*, M.M. Behbehani and M.T.Shipley (SPON T.I. Mandybur). Dept. of Physiology and Biophysics and Dept. of Anatomy and cell biology. U. Cincinnati College of Med. Cincinnati OH 45267-0576.

Using anatomical and physiological techniques we have examined the characteristics of an adrenergic network within the PAG. In the anatomical studies the location of cell bodies, fibers and terminal that were immunoreactive to specific antibody to PNMT were mapped. The results indicated that a circumscribed forms of terminals and fibers were located in the ventral and the ventrolateral parts of mid rostral PAG. There was only sparse labeling in the dorsal half of PAG. The density of fibers decreased at the rostral levels of the PAG.

Physiological experiments showed that the PAG neurons are responsive to both epinephrine (EP) and norepinephrine(NE). The response to NE was mixed; 46% (29/71) of the cells were excited, 38% (27/71) were inhibited and the remaining cells did not respond. The excitatory effect was long lasting and in many instances the baseline firing rate remained elevated for 2 to 20 minutes. The inhibitory effect had a shorter duration than the excitatory response. Both effects could be partially reversed by phentolamine and prazosin. Both types of responses were observed in all regions of the PAG. However the majority (53%) of neurons in the rostral PAG responded by excitation. It is concluded that the PAG contains an adrenergic network that is anatomically concentrated in its caudal ventral and ventrolateral regions. However, neurons in all regions of the PAG are responsive to EP and NE suggesting that an adrenergic network may be involved in the PAG local circuitry. Supported by PHS grant #NS 20643, 24698 and US armynDAMD-17-86c-6005.

163.5

CATECHOLAMINERGIC PROJECTION FROM THE MEDULLA TO THE MIDBRAIN CENTRAL GRAY MATTER. H. Herbert and C.B. Saper, Dept. of Animal Physiol., Univ. of Tübingen, 7400 Tubingen, FRG and Dept. of Pharm. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637

The periaqueductal gray matter (PAG) in the rat contains large numbers of catecholaminergic fibers. We traced the origin of this innervation using combined neuronal tracers and immunohistochemistry for tyrosine hydroxylase, dopamine β-hydroxylase and phenyletholamine N-methyltransferase.

After injections of fast blue or fluorogold into the ventrolateral PAG, we found retrogradely labeled noradrenergic neurons in the A1 and A2 groups, and adrenergic neurons in the C1-C3 groups. After injections of PHA-L into these cell groups, a double immunofluorescence method was used to identify anterogradely labeled (PHA-L immunoreactive) fibers that also stained for tyrosine hydroxylase. Individual varicose dual-labeled fibers were found throughout the PAG. These results indicate that the PAG is a major site of catecholaminergic innervation from medullary noradrenergic and adrenergic cell groups.

Supported by NINCDS 22835 and the DAAD.

163.6

ARCHITECTURE OF THE PERICOERULIAR REGION IN THE RAT. Li-bang Fu*[1], M.T. Shipley[1], G. Aston-Jones[2], C. Chiang[2] and V. Pierjbone[2] [1]Univ. of Cinti College of Med., Cinti, OH 45267,[2]New York Univ., New York, NY 10003.(SPON:G.D.Adamek)

In the rat noradrenergic neurons (NE) in LC project throughout the neuraxis and provide all NE innervation of the forebrain. Our recent studies indicate that the major afferents to LC derive from only two medullary nuclei, nucleus paragigantocellularis and the region of nucleus prepositus hypoglossie. Many previously reported afferents to LC do not impinge on this nucleus but rather in adjacent pontine regions. New physiological data indicate that some inputs adjacent to LC do not directly influence LC neurons. In contrast to the wealth of anatomical observations on LC there is surprisingly little information on the architecture of the regions immediately adjacent to this nucleus.

Immunocytochemistry, Nissl and myelin staining were used to investigate the architecture of the pericoeruleus region in the rat. There are several neuronal groups in close association with LC. Rostrally, dense Nissl stained neurons of the lateral dorsal tegmental nucleus are adjacent to LC; a second population of densely stained cells, tentatively identified as Barrington's nucleus nestle along the ventromedial border of LC. At most rostrocaudal levels there are two or three additional populations of pale, small-to-medium size neurons adjacent to LC. Dendritics of LC neurons preferentially extend medially and ventrally among these pericoerulear neurons.

Afferent terminals in the region medial to the cellular core of LC could contact LC dendrites, peri-LC neurons or both. Experiments are needed to determine if there are anatomical and functional interactions among these inputs, LC and peri-LC neurons.

(Supported by: NS20643, 24698, and US Army DAMD 17-86-C-6005.)

163.7

N-METHYLADRENALINE IN RAT RETINA, BRAIN AND SPINAL CORD. C.J. Plummer,* I.C. Kilpatrick,* M.W. Jones,* B. Goodwin* and O.T. Phillipson.* (SPON. R. Bruce Holman) Dept. of Anatomy, University of Bristol, BS8 1TD. U.K.

Phenylethanolamine N-methyltransferase (PNMT) is found in high concentratons in localised regions of both retina and brain. However, only low concentrations of adrenaline are detectable. In vitro, PNMT may further methylate adrenaline to form N-methyladrenaline (MA), (Axelrod, 1966 Pharm. Rev. 18:95). We now report the preliminary identification of MA in the central nervous system.

Brain regions and retinae were prepared from adult Wistar-derived rats and analysed by HPLC (Kilpatrick et al., 1986 J. Neurochem. 46:1865). In retina, as well as the expected catechol and indole derivatives, an additional peak was encountered. Its chromatographic behaviour was studied over a wide range of mobile phase pH and sodium-1-octanesulphonic acid concentrations. In each condition the unknown peak and MA standard coeluted precisely. Oxidative voltammetric spectra of the endogenous peak and standard MA were similar. Alumina adsorption confirmed that the unknown contained a catechol moiety since reruns of acidified alumina eluate spiked with MA again showed perfect coelution.

PNMT-positive brain areas were assayed for MA content. Thalamus, hypothalamus, pontine central grey, medulla (including C1 and C2/3) and T1/T2 spinal cord all displayed peaks which coeluted precisely with standard MA. Conversely, samples of rat adrenal gland were found to contain barely detectable quantities of MA.

Thus, MA is reported in the CNS for the first time. Its production and pharmacology are now being studied.

163.8

PHENYLETHANOLAMINE N-METHYLTRANSFERASE (PNMT)-IMMUNOREACTIVE (IR) TERMINALS SYNAPSE ON ADRENAL PREGANGLIONIC NEURONS IN THE RAT SPINAL CORD. H. Bernstein-Goral & M.C. Bohn. Dept. of Neurobiology and Behavior, State University of New York, Stony Brook, 11794 and Dept. of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, New York 14642.

Adrenergic neurons in the C1 region in the ventrolateral medulla oblongata project to the intermediolateral cell column (IML) of thoracic spinal cord. The ultrastructure of adrenergic axons and synaptic boutons in the IML of mid-thoracic spinal cord was studied using immunocytochemistry for PNMT, the epinephrine-synthesizing enzyme. PNMT-IR terminals form synaptic contacts with dendritic processes at all postnatal ages examined, including 7, 9, 24, 30, 60, and 90 days. In addition, axo-somatic synaptic contacts were observed in young rats, but rarely observed in adult rats.

To determine whether adrenergic axons synapse with preganglionic neurons which project to the adrenal medulla, adrenal preganglionic neurons (APGN) in IML were retrogradely labeled with HRP. At postnatal ages 7, 9, 24 and 30 days, PNMT-IR boutons were observed to form symmetrical axo-somatic and axo-dendritic synaptic contacts with HRP labeled APGN, as well as with other unidentified neurons in IML. This synaptic association provides an anatomical substrate for central adrenergic regulation of adrenal medullary development and function.

Supported by NIH grant NS20832, the Dysautonomia Foundation and a Research Career Development Award NS00919 to M.C.B.

163.9

TOPOGRAPHY OF RAT BRAINSTEM NEURONS IMMUNOLABELED WITH ANTISERA RAISED AGAINST PREDICTED SYNTHETIC EPITOPES OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE. T.J. Gorcs*, A. Csiffáry* and é. Mezey*,*. (SPON: A. R. Light). 1st Dept. of Anat., Semmelweis Univ.Med.School, Budapest, Hungary and *LCB-NIMH, Bethesda, MD 20892, USA.

The nucleotide and deduced amino acid sequence of phenylethanolamine N-methyltransferase (PNMT) catalyzing the synthesis of epinephrine from norepinephrine shares significant homology with the sequence of tyrosine hydroxylase (TH). Using combined antigenic site prediction analyses 4, probably continuous epitopes of PNMT were determined (PNMT-SP-1 to 4) without major sequence homology to TH. These peptides were synthesized by a solid phase method and antisera (AS) were raised in rabbits. Neurons resembling adrenaline- or PNMT-immunostained neurons were immunolabeled by AS-PNMT-SP-2 and 4 in the lower brainstem in rats without colchicine treatment. In contrast to these, AS-PNMT-SP-3 revealed only dense neuronal network but no neuronal perikarya. The specificity of AS-PNMT-SP-2 to 4 for PNMT was proved both with appropriate ELISA and immunohistochemical methods.Epitope prediction analysis combined with sequence homology search provides a useful tool to raise specific antisera to proteins of known or deduced amino acid sequences.

163.10

EVIDENCE FOR THE PRESENCE OF PNMT-CONTAINING CELL BODIES IN HYPOTHALAMUS. M.I. Masana,* I.N. Mefford* (SPON: P. Newhouse). Lab. Clin. Science, NIMH, Bethesda, MD 20892.

The presence of the epinephrine (E)-converting enzyme phenylethanolamine-N-methyltransferase (PNMT) in hypothalamic cell bodies is still a matter of controversy. In the present study, 2 µg of kainic acid (KA) (which selectively destroys local neurons) was injected into the lateral hypothalamus, where the presence of PNMT-containing neurons has been reported (Ruggiero et al., J. Comparat. Neurol. 239: 127, 1985). Male Sprague-Dawley rats (200-300 g) were killed 1, 2, 4, 8, and 12 days post-injection, the PNMT activity determined as described by Trocewicz et al. (J. Chromat. 227:407, 1982) and the catecholamines measured by HPLC with electrochemical detection. E content was reduced at 1 and 2 days post-injection (control 35.1 ± 2.5 ng/g, n = 10, KA lesioned 19.4 ± 2.0 ng/g, n = 4, contralateral control 18.2 ± 2.3 ng/g, n = 4, $p < 0.05$, 2 days) probably due to the extensive releasing effect of KA and the stress provoked by the treatment. At day 1 post-injection, there was a significant decrease in PNMT activity (KA lesioned 48.4 ± 11.9% of the contralateral, n = 4, $p < 0.05$). The decrease was maintained over the 12 days of the study (KA lesioned 81.9 ± 10.5%, n = 7, 2 days; 65.6 ± 9.8%, n = 9, 4 days; 68.8 ± 23.6%, n = 4, 8 days; 71.4 ± 15.4%, n = 4, 12 days). These results suggest that there are PNMT-containing cells in the hypothalamus, although, as the KA treatment is nonspecific, it is not possible to elucidate which type of neurons or other cells, such as astrocytes or ependymal cells, are involved.

163.11

PROJECTIONS FROM THE VENTROMEDIAL MEDULLARY RETICULAR FORMATION TO THE ROSTRAL A5 GROUP. J.F. Miller, M.R. Monsen, and H.K. Proudfit. Dept. of Pharmacology, Univ. of Illinois at Chicago, Box 6998, Chicago, IL 60680.

The afferent brainstem connections of the A5 noradrenergic cells of the rostral ventrolateral pons were investigated by retrograde tracing techniques. Adult female Sprague-Dawley rats received a unilateral iontophoretic injection of the retrograde tracer Fluoro-Gold into the ventrolateral pontine tegmentum. Survival time was 5-7 days. The brainstem was sectioned at 30-40 um and every other coronal section was thaw-mounted, cleared, and examined using trans- and epifluorescence microscopy. Alternate sections through the injection site were processed for dopamine-beta-hydroxylase (DBH) immunocytochemistry by the indirect fluorescent method of Coons. In those rats in which the tracer injection encompassed DBH-immunoreactive neurons, retrogradely-labelled neurons were observed bilaterally within the nucleus reticularis paragigantocellularis lateralis throughout most of its rostral-caudal extent. In addition, smaller numbers of retrogradely-labelled neurons were observed within the ipsilateral nucleus raphe magnus and adjacent nucleus reticularis gigantocellularis, pars alpha. Labelling in these medullary areas was minimal with tracer deposits situated lateral to the A5 group. These results suggest that the ventromedial medulla may exert an important regulatory control on the A5 cell group. (supported by USPHS Grant DA 03980)

163.12

LACK OF COORDINATE EXPRESSION OF TYROSINE HYDROXYLASE (TH) AND DOPAMINE-β-HYDROXYLASE (DBH) IMMUNOREACTIVITIES IN ADULT ENTERIC NEURONS. G. Baetge* and M.D. Gershon (SPON: H. D. Coulter). Dept. Anat. & Cell Biol., Columbia Univ., New York, NY 10032.

Transient catecholaminergic cells appear in the mesenchyme of the developing bowel; nevertheless, with few exceptions, such as the avian gizzard and the guinea pig proximal colon, intrinsic catecholamine neurons have not been reported to be present in the gut. On the other hand, reports of the immunocytochemical detection of catecholamine biosynthetic enzymes in some enteric neurons have appeared. The present study was done to determine whether TH or DBH immunoreactivities are valid markers of noradrenergic neurons in the bowel. Antibodies to TH or DBH raised in different species were used for the simultaneous visualization of each marker in tissue whole mounts or frozen sections. TH-immunoreactive (TH+) neurons were found in adult mouse, rat, guinea pig and rabbit colon as well as in the adult mouse stomach. These neurons measured 20-40 μm in diameter, occurred singly or in pairs, and were present in only a subset of myenteric ganglia. In guinea pig all TH+ neurons were also DBH+. In contrast, in the rat colon there were many more DBH+ than TH+ neurons, because many neurons were found that were DBH+, but TH-. Most of these DBH+/TH- neurons were surrounded by DBH+/TH+ sympathetic nerve fibers. The rat colon also contained a third category of TH+/DBH- cell. These observations indicate that neither TH nor DBH immunoreactivities, by themselves, are reliable markers for noradrenergic neurons in the bowel. The expression in adult enteric neurons of some, but not all, of the properties of a noradrenergic neuron may reflect the persistence of these traits in non-catecholaminergic neurons that are derived from a transiently catecholaminergic precursor. Supported by NIH grants #NS 15546 and NS 07062.

163.13

Flow Cytometric Detection of Tyrosine Hydroxylase Immunoreactivity on the Surface of Striatal Synaptosomes: Paradoxical Presence on Non-Dopaminergic Synaptosomes. Hiroyuki Hasegawa, Marina E. Wolf and Gregory Kapatos. Lab. of Neurochemistry, Center for Cell Biology, Sinai Research Inst., Detroit, MI 48235.

Complement-dependent lysis studies (Docherty et al., Brain Res., 1985) have suggested that tyrosine hydroxylase (TH) or a related molecule may be present on the surface of dopaminergic (DA) synaptosomes. To investigate this possibility, striatal synaptosomes were incubated with a polyclonal antibody to TH followed by a fluorescein-conjugated secondary antibody. Labeled synaptosomes were detected using a fluorescence-activated cell sorter (FACS). Approximately 10-20% of striatal synaptosomes expressed TH immunoreactivity on their surface. However, when labeled synaptosomes were isolated by FACS and analyzed for TH content using SDS-PAGE and Western blot techniques, they were depleted for TH relative to control synaptosomes. Labeled synaptosomes may represent non-DA terminals which were apposed to DA terminals in the striatum. During synaptosome preparation, pieces of DA terminal membrane with TH attached to their cytoplasmic side may have remained associated with the surface of apposed non-DA terminals. Thus, labeled synaptosomes may be derived from non-DA terminals which were in synaptic contact with DA terminals.

163.14

DIFFERENCES BETWEEN ALBINO AND PIGMENTED RAT STRAINS IN TYROSINE HYDROXYLASE, DIHYDROXYPHENYLALANINE DECARBOXYLASE, PHENYLETHANOLAMINE N- METHYLTRANSFERASE AND TRYPTOPHAN HYDROXYLASE ACTIVITIES OF BRAIN REGIONS AND ADRENAL GLANDS. H.S. Park*, T.H. Joh and D.H. Park (SPON: J. Carroll). Lab. Mol. Neurobiology, Cornell Univ. Med. Coll., Burke Reh. Ctr., White Plains, NY 10605.

Strain-dependent differences in the activities of catecholamine biosynthetic enzymes in tissues of rats and mice have been previously reported. However, there has been no study specifically directed at possible variance of the enzyme activities between albino and pigmented animals. In the present study, we selected two strains of rats, albino Sprague-Dawley (SD) rats and Long Evans pigmented (LE) rats and examined activities of tyrosine hydroxylase(TH), dihydroxyphenylalanine decarboxylase(DDC), phenylethanolamine N-methyltransferase (PNMT) and tryptophan hydroxylase (TPH). Brainstem and hypothalamic TH activity was higher in LE rats. Hypothalamic PNMT and brainstem DDC activity was higher in SD rats. All other enzyme levels were equivalent. Adrenal TH, DDC and PNMT activities of LE rats are lower than the respective enzyme activities of SD rats. Our results indicate that there is no obvious relationship between enzyme activities in albino and pigmented rat strains. Difference ,if any, in the enzyme activities of strains seems to depend upon both tissue and brain regions. Supported by NIH grants, MH 24285 and 1PO1MH 42626.

163.15

LOCUS COERULEUS LESIONS FACILITATE RECOVERY OF LOCOMOTOR FUNCTION AFTER SENSORIMOTOR CORTEX CONTUSION IN RAT. M.S. Weaver*, M.J. Chen, V.S. Westerberg* and D.M. Feeney (SPON: G.K. Hodge). Depts. of Psychol. and Physiol., UNM, Albuquerque, NM 87131.

Norepinephrine (NE) appears to have an important role in recovery of function after cortical injury (see CRC Critical Reviews in Neurobiol., 3(2):135-197, 1987). This study examined effects of bilateral locus coeruleus (LC) lesions on recovery of beam-walking ability 2 weeks prior to a right sensorimotor cortex contusion in rats. Bilateral injections of 6-hydroxydopamine (6-OHDA; 5 ug in 2.5 ul per side) decreased NE levels bilaterally (HPLC) in frontal and cerebellar cortex as compared to sham controls but did not affect beam-walking. Cortical contusion 2 weeks after LC lesions produced similar decreases in NE (measured 16 days post contusion). Contusion alone also bilaterally reduced NE in frontal and cerebellar cortices and these preliminary results indicate greater reduction in the right than left cerebellar cortex. One day after contusion alone or LC lesions followed by contusion, beam-walking was significantly impaired. Although recovery from the locomotor deficit was significantly faster for rats receiving LC lesions prior to contusion than for those receiving only contusion, perhaps more complete lesions will result in a different effect on recovery. Supported by U.S. Army Contract No. DAMD17-86-C-6144.

163.16

THE NEUROTOXIC EFFECTS OF DSP-4 ON NORADRENERGIC NEURONS ARE RESTRICTED TO AXON TERMINALS OF THE LOCUS COERULEUS. J.M. Fritschy and R. Grzanna. Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Systemic administration of DSP-4, a selective noradrenergic (NA) neurotoxin, produces nearly complete depletion of norepinephrine in neocortex, hippocampus, cerebellum and spinal cord, but only partial depletion in hypothalamus and brainstem. In the present study we have used immunohistochemistry (i) to assess to structural damage produced by DSP-4 on NA neurons and (ii) to determine whether a subset of NA axons is spared in regions only partially depleted of norepinephrine by DSP-4. Two weeks after treatment of rats with 50 mg/kg of DSP-4, brain and spinal cord sections were processed for dopamine-ß-hydroxylase immunohistochemistry using the avidin-biotin method.

The staining results identified terminal axons of NA neurons as the principal site of action of DSP-4; NA cell bodies and preterminal axons were not affected. DSP-4 produced an almost all or none neurotoxic effect on NA axon terminals in different brain regions. Nearly all NA axon terminals were destroyed in neocortex, hippocampus, olfactory bulb, thalamus, tectum, cerebellum, spinal cord dorsal horn. In contrast, NA axons were almost completely unaffected in the basal forebrain, hypothalamus, reticular formation, brainstem motor nuclei, and spinal cord ventral horn. These remaining NA axon terminals differed morphologically from DSP-4 sensitive axons by their thickness, size and spacing of their varicosities and their dense arborizations within terminal fields. The distribution of NA axons ablated by DSP-4 correlates very closely with the distribution of locus coeruleus axons. Conversely, most NA axon terminals unaffected by DSP-4 are likely to originate in non-locus coeruleus NA neurons (A1, A2, A5 and A7 cell groups).

This study provides the first direct evidence that DSP-4 destroys axon terminals of the locus coeruleus, but not those of non-locus coeruleus NA neurons. Based upon this profound differential sensitivity to DSP-4, NA axon terminals may be divided into two populations which differ in their cells of origin, topographic distribution, and morphology. DSP-4 thus offers a unique tool to study the functional organization of non-locus coeruleus NA projections. Support: NIH Grant MH41977.

163.17

NORADRENERGIC CELLS OF A5 AND A7 GROUPS BUT NOT THOSE OF THE LOCUS COERULEUS PROJECT TO THE VENTRAL HORN OF THE SPINAL CORD. W. E. Lyons*, J. M. Fritschy, and R. Grzanna. Johns Hopkins University School of Medicine, Baltimore, Md. 21205.

Descending noradrenergic (NA) projections to the rat spinal cord originate in cells of the locus coeruleus (LC), subcoeruleus, and A5 and A7 groups. We have previously shown that the LC distributes axons to the dorsal horn but not to the ventral horn of the spinal cord (Fritschy et al., Brain Res. 437: 176, 1987).

In this study we have taken advantage of our recent observation that systemic treatment of rats with the norepinephrine neurotoxin DSP-4 results in a complete loss of NA axon staining in the dorsal horn and the intermediate zone of the spinal cord with little effect on the staining of NA axon terminals in the ventral horn and the intermediolateral cell column. Following systemic administration of 50 mg/kg of DSP-4, retrograde transport of the fluorescent tracer True Blue (TB) was used in combination with dopamine-β-hydroxylase (DBH) immunohistochemistry to determine the location and number of NA cells that project to the ventral horn and intermediolateral cell column. Fourteen days after drug treatment, rats were injected with a suspension of TB into either thoracic or lumbar segments of the spinal cord. Identical injections were made in untreated rats. Following a two week survival period, 40 micron sections through the brainstem were processed for DBH staining. In DSP-4 treated rats, the number of retrogradely labeled neurons in the LC was reduced to 8% of that observed in control rats while the number of retrogradely labeled cells in the A5 and A7 groups decreased to 50-70% of control. The results reveal a distinct topography of the descending NA projections to the spinal cord: the coeruleo-spinal projection terminates mainly in the dorsal horn and the intermediate zone while cells of the A5 and A7 groups project to somatic motor neurons and preganglionic sympathetic neurons. (Support NIH grant MH 41977).

163.18

NEUROTOXIC EFFECTS OF DSP-4 ARE RESTRICTED TO A SUB-POPULATION OF NORADRENERGIC AXONS: STUDIES OF ACUTE AND LONG-TERM EFFECTS. U. Berger*, J.M. Fritschy and R. Grzanna. (Spon: A. Sastre). Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

The noradrenergic (NA) neurotoxin DSP-4 produces severe depletion of norepinephrine in rat cerebral cortex and cerebellum but only a moderate reduction in hypothalamus. Immunohistochemical analysis two weeks after drug treatment revealed that these regional variations are due to the existence of two populations of NA axons with differential sensitivities to DSP-4: NA axons in cortex and cerebellum are completely ablated while those in basal forebrain and hypothalamus are not affected (see Fritschy and Grzanna, this volume). In cortex, massive depletion of norepinephrine occurs within hours after drug administration. The present study was conducted to determine whether this early sign of neurotoxicity also occurs in NA axons of the basal forebrain and hypothalamus or whether this acute effect is restricted to NA axons ablated by DSP-4.

Rats (8/group) were sacrificed 6 hours, 24 hours or 2 weeks after systemic injections of 50 mg/kg DSP-4. Norepinephrine concentrations in cortex, cerebellum, basal forebrain and hypothalamus were determined by HPLC with electrochemical detection. In DSP-4 treated rats, norepinephrine levels in cortex and cerebellum were reduced to 31% and 38% of control at 6 hours, to 22% and 31% at 24 hours and to 5% and 13% at 2 weeks. In contrast, levels in basal forebrain and hypothalamus were reduced to 78% of control at 6 hours, 80% and 70% at 24 hours and 81% and 71% at 2 weeks.

Compared to cortex and cerebellum, levels of norepinephrine in basal forebrain and hypothalamus were affected only to a small degree and did not change between 6 hours and 2 weeks after treatment. We interpret this finding as evidence that DSP-4 does not produce acute depletion of norepinephrine in those NA axons found to be spared by DSP-4 in our immunohistochemical analysis. These data provide further support for the existence of two distinct populations of NA neurons that differ in the pharmacological properties of their axons. (Support NIH grant MH 41977).

163.19

GABA-ERGIC INNERVATION OF THE RAT LOCUS COERULEUS M.T. Shipley[1], V. Pieribone[2], G. Aston-Jones[2] and M. Ennis[1] 1. Univ. of Cincinnati Coll. Med., Cincinnati, OH 45267. 2. New York University, New York, NY 10003.

The nucleus locus coeruleus (LC) provides noradrengeric (NE) innervation for the entire neuraxis and is the sole source of NE inputs to the forebrain. Neurons in LC are preferentially activated by novel events and they appear to be discharge concomitant with sympathetic activation. The regulation of LC activity has been hypothesized to depend upon phasic excitatory and tonic inhibitory inputs. Recently we reported that the major afferent projections to LC arise in two medullary regions - the nucleus paragigantocellularis (PGi) in the rostro-ventro-lateral medulla and the region of the nucleus prepositus hypoglossi (PrH) in the dorsomedial medulla. Our physiological studies demonstrate that there is a potent excitatory amino acid synaptic input to LC from PGi; at this meeting we show that there is a strong inhibitory synaptic input from PrH.

The present study used GAD and GABA immunocytochemistry to demonstrate a dense GABAergic terminal-like staining at all rostrocaudal levels of LC. There are large and small punctate profiles in LC. The small puncta fill the entire central core of the nucleus; the larger puncta surround the cell dense core of the nucleus particularly along the dorsomedial border of LC. Adjacent structures in the pontine tegmentum also contain GABA-ergic terminals.

These results suggest that there is a dense GABAergic terminal plexus in LC. These terminals could provide tonic inhibitory regulation of LC neurons. Experiments to determine whether this innervation derives from PrH, activation of which potently inhibits LC neurons, are in progress. (Supported by: NS20643, 24698 and US Army DAMD-17-86C-6005.)

163.20

A GABAERGIC INPUT TO LOCUS COERULEUS FROM THE ROSTRAL DORSOMEDIAL MEDULLA. M. Ennis[1], G. Aston-Jones[2] and M.T. Shipley[1] Dept. Biol., New York Univ.[2], New York, NY 10003, Dept. Physiol., U. Cincinnati Coll. Med.[1], Cincinnati, OH 45267.

Our recent experiments identified a strong projection from nucleus prepositus hypoglossi in the rostral dorsomedial medulla to rat locus coeruleus (LC). Here, we have characterized the effects of PrH activation on LC discharge and examined the transmitter(s) in this pathway.

PrH activation inhibited 84% of LC cells (85/101) at a mean latency of 19.5 msec; 2 cells were activated, 1 cell was antidromically driven, and 13 cells were unaffected. PrH activation attenuated excitation of LC from sciatic nerve stimulation ($p < 0.02$).

PrH-evoked inhibition of 5/7 LC neurons was blocked by the $GABA_A$ antagonist picrotoxin (3-6 mg/kg, iv; $p < 0.03$). Local microinfusion (0.5-5.0 ng) or microiontophoretic application of the $GABA_A$ receptor antagonist bicuculline (BIC) blocked PrH evoked inhibition of 10/11 ($p < 0.02$) and 19/22 cells ($p < 0.01$), respectively. Iontophoretically-applied strychnine did not affect PrH-evoked inhibition of LC. In contrast, excitation of LC from sciatic nerve stimulation and postactivation inhibition following such excitation were not significantly altered by picrotoxin of BIC. In addition, PrH-evoked inhibition of LC was not affected by the alpha-2 receptor antagonist idazoxan or the opiate receptor antagonist naloxone (0.1-0.5 mg/kg and 1.0 mg/kg, iv, respectively).

These results demonstrate that PrH potently inhibits LC through a GABAergic mechanism, and are consistent with the findings of Shipley et al. (this meeting) of a rich GABAergic terminal plexus in LC. Supported by PHS Grants MH09381, NS24698, ONR N00014-86-K-0493, PO123348 and US Army DAMD 17-86-C-6005.

CATECHOLAMINES: ELECTROPHYSIOLOGY I

164.1

PREFRONTAL CORTEX (PFC) DOPAMINERGIC MODULATION OF ACETYLCHOLINE-EVOKED, AND STIMULUS-EVOKED RESPONSES TO VENTRAL PALLIDAL/NUCLEUS BASALIS (VP/NB) STIMULATION. C.R. Yang* & G.J. Mogenson Dept. of Physiology, Univ. Western Ontario, London, CANADA, N6A 5C1. (SPON: W. Blume).

Medial PFC receives converging inputs from acetylcholinergic(ACh) neurons of VP/NB and midbrain dopaminergic (DA) neurons. The electrophysiological interactions between DA and ACh in PFC was studied in urethane-anesthetized rats.

Iontophoretic application of ACh excited PFC neurons. DA (5-15nA) applied concurrently suppressed spontaneous firing (noise)relatively more than ACh-evoked excitation (signal), yielding an enhanced signal to noise ratio (S/N)(n=18). D2 receptor antagonist sulpiride(30nA) reversed this DA effect (n=7) whereas DA enhanced the S/N further after D1 receptor blockade (SCH23390,6mg/kg,n=11), suggesting D1 receptor inhibited D2 receptor functions in PFC.

Excitation elicited from VP/NB stimulation was ACh-mediated since it was atropine-reversible and ACh-sensitive. DA mainly attenuated this stimulation-evoked (S-E) response in 18 output neurons to the N.Accumbens. In DA-depleted(αmpt pretreated) rats, D2 agonist quinpirole(QNP,5-35nA)enhanced (n=22), but D1 agonist SKF38393(5-15nA) suppressed (n=10), this S-E excitation. Co-stimulation of D1 and D2 receptors resulted in opposing changes to both the ACh-evoked and S-E responses previously enhanced by QNP, or suppressed by SKF. Thus, DA modulate ACh-evoked and S-E responses in diverse ways which may be part of the cognitive-integrative processes in the PFC. (Funded by M.R.C. of Canada).

164.2

CLONIDINE REGULARIZES THE FIRING OF VENTRAL TEGMENTAL DOPAMINE NEURONS. J. Grenhoff* and T.H. Svensson. (SPON: B. Hedlund) Dept. of Pharmacology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden.

The α_2-adrenoceptor agonist clonidine is effective in the treatment of certain neuropsychiatric disorders with a presumed dopaminergic pathogenesis. We used electrophysiological methods to study effects of clonidine on midbrain dopamine (DA) neurons of the ventral tegmental area (VTA) which give rise to the mesolimbic DA system commonly implied in e.g. schizophrenia. Neuronal activity of single identified DA cells in the VTA was extracellularly recorded in the chloral hydrate anesthetized male rat before and after intravenous drug administration, with respect not only to cell firing rate, but also to burst firing and regularity of firing.

Clonidine (5-20 μg/kg) produced a marked regularization of firing without affecting the firing rate. This effect was blocked or reversed by the α_2-antagonists idazoxan and yohimbine, which by themselves caused a marked excitation of the cells with prominent burst firing. Reserpine (5 mg/kg, s.c.) 4 hrs before recording abolished the clonidine effect, indicating that the effect is mediated via monoaminergic neurons. The present results, together with our previous finding of a similar effect on nigrostriatal DA neurons (Life Sci. 42: 2003, 1988), suggest that certain therapeutic effects of clonidine could result from a neuromodulatory action on midbrain DA systems.

164.3

SELECTIVE ACTIVATION OF MESOLIMBIC DOPAMINE NEURONS BY AMPEROZIDE, A PUTATIVE ANTIPSYCHOTIC DRUG. T.H. Svensson, J. Grenhoff* and L. Ugedo*. Dept. Pharmacology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden.

The psychotropic drug amperozide, which binds selectively to 5-HT_2-receptors (Haskins et al., Brain Res Bull 19:465, 1987), is currently evaluated for antipsychotic efficacy. We used electrophysiological methods to study the acute effects of amperozide on the ascending midbrain dopamine (DA) systems commonly implied in antipsychotic drug action. Neuronal activity of single identified DA cells in the substantia nigra (SN) and ventral tegmental area (VTA) was extracellularly recorded in the chloral hydrate anesthetized male rat before and after intravenous drug administration with respect not only to firing rate, but also to firing pattern of the cells.

SN neurons (n=8) showed no significant response to amperozide (0.25-4.0 mg/kg). In contrast, VTA neurons (n=11) responded in two ways to amperozide in a similar dose range (0.5-4.0 mg/kg). Six of the neurons were markedly excited with variable effects on firing pattern, while the other five displayed a regularization of the firing pattern with no clearcut effect on firing rate. Cells of both types were inhibited by apomorphine, excluding DA autoreceptor block in the action of amperozide. These results suggest that selective activation of mesolimbic versus nigrostriatal DA function could be a basis for an antipsychotic action of amperozide.

164.4

PEDUNCULOPONTINE TEGMENTAL NUCLEUS LESIONS REDUCE THE NUMBER OF SPONTANEOUSLY ACTIVE SUBSTANTIA NIGRA PARS COMPACTA DOPAMINE CELLS. M. Beninato, H.S. Pan and J.R. Walters. NIH, Bethesda, MD 20892.

The pedunculopontine tegmental nucleus (PPN) projects to the substantia nigra (SN; Beninato and Spencer, 1987) and has an excitatory effect on this region (Scarnati et al., 1984). This study further investigated the relationship between the PPN and SN using extracellular single unit recording techniques in locally anesthetized, gallamine immoblized, artificially respired rats 7 days following 2 nmole kainic acid injections in the PPN. Lesioned animals were those in which the destruction of the PPN was >50% as determined by acetylcholinesterase histochemistry. Activity in the SN was measured in a population study using 9 electrode passes through the SN arranged in a 3x3 grid. No significant differences in firing rates between control (n=6) and lesioned (n=5) rats in either substantia nigra pars reticulata (SNr) or substantia nigra pars compacta (SNc) neurons were found. Likewise, the number of SNr cells/pass was not different between the 2 groups. However, in the SNc there was a significant 67% reduction in the number of spontaneously active cells/pass in control (1.2 $\pm$ 0.2) vs. lesioned (0.4 $\pm$ 0.05) groups. Striatal dopamine and DOPAC levels and dopamine/DOPAC ratios in lesioned (n=10) and control (n=4) rats were not significantly different. These results suggest that the reduction in the number of spontaneously active SNc cells following PPN lesions is the result of the silencing, not the death, of a large portion of this cell group. Furthermore, the PPN appears to exert a tonic influence on SNc dopamine cell activity.

164.5

ACTIVITY OF LOCUS COERULEUS NEURONS IN BEHAVING PRIMATES: RELATIONSHIP WITH VIGILANCE. G. Aston-Jones, T. Alexinsky*[1] AND S. Grant[2], Dept. Biology & Center for Neural Science, New York Univ., NY 10003; [1]Dept. Psychophysiologie, Univ. de Paris V, France; [2]Dept. Psychology, Univ. Delaware, Newark 19716.

In initial studies, recordings were obtained from locus coeruleus neurons in unanesthetized, chair-restrained monkeys during presentation of a range of sensory stimuli. Tonic impulse activity was higher during alertness or agitation than during behavioral inattentiveness and drowsiness. Low-level simple stimuli elicited minimal response in LC activity, while more intense, conspicuous stimuli evoked phasically pronounced discharge. Pronounced responses were also elicited by aversive air puffs and by naturalistic stimuli such as actions by the experimenter. While the sensory responsiveness of LC neurons in primate appeared to be more selective than that reported for rat or cat, a feature found for LC neurons in monkey as well as other species is that pronounced responses occurred to stimuli which elicited orienting behaviors. Thus, a common dimension for stimuli effective in eliciting LC responses in different species may be conspicuousness: Unexpected conspicuous stimuli which generate orientation and a change in the apparent focus of attention are most effective in eliciting responses in primate LC neurons, similar to results in other species. These findings are consistent with previous hypotheses that the LC functions in the control of vigilance and initiation of adaptive behavioral responses to unexpected imperative stimuli. Additional studies are underway to examine primate LC activity during a behavioral paradigm in which vigilance is manipulated and measured. Supported by ONR contract N00014-86-K-0493, Air Force Office Scientific Res., and Alzheimers Dis. Related Dis. Assoc.

164.6

SYSTEMIC, BUT NOT LOCAL, ADMINISTRATION OF BENZODIAZEPINES ATTENUATES EVOKED ACTIVITY OF LOCUS COERULEUS NEURONS. P. E. Simson and J. M. Weiss. Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

Recently, we provided evidence that alpha-2 receptors preferentially regulate the responsiveness of locus coeruleus (LC) neurons to excitational influences (Simson, P. E. and Weiss, J. M., J. Neurosci., 7:1732, 1987). In this study, we examined whether benzodiazepine receptors also regulate LC responsiveness.

Systemic administration of diazepam (0.5 mg/kg, i.v.), chlordiazepoxide (2 mg/kg, i.v.), and alprazolam (0.25 mg/kg, i.v.) in anesthetized rats attenuated the responsiveness of LC neurons to sensory stimulation by 55%, while decreasing spontaneous LC firing rates by 25%. Lower doses of diazepam (0.2 mg/kg, i.v.) significantly attenuated LC responsiveness while leaving spontaneous activity unchanged.

To test whether systemically administered benzodiazepines produce these effects by acting directly on receptors in the LC, benzodiazepines were infused into the LC region. Similar to systemic administration, local infusion of diazepam (50 ng) and chlordiazepoxide (100 ng) decreased spontaneous LC firing rates by 30%; however, unlike systemic administration, LC infusion produced no attenuation of LC responsiveness. These findings suggest that, when given by the normal systemic route, benzodiazepines decrease spontaneous LC firing by acting on benzodiazepine receptors in the LC, but markedly attenuate LC responsiveness by acting on receptors outside the LC to decrease excitatory input to the LC.

164.7

EFFECT OF IPSAPIRONE ON LOCUS COERULEUS (LC) ACTIVITY. J.A. Cook*, H.L. Williams*†, B.A. McMillen† and M.K. Sanghera. (SPON. H.P. Roffwarg) Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX 75235; †Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858.

The aryl-piperazine derivatives, ipsapirone (ISA) and buspirone are potent anxiolytics and exhibit high affinity for 5HT1a receptors. However, buspirone also activates impulse flow recorded from the noradrenergic LC neurons. The purpose of the present study was to determine if ISA altered LC impulse flow and brain noradrenergic metabolism (MOPEG-SO4 levels) in a manner similar to buspirone. Extracellular single cell recordings were made from electrophysiologically identified and histologically verified LC cells in chloral hydrate anesthetized male rats. Intravenous ISA produced sustained increases in LC firing rates (7-62% after 0.01 - 3.2 mg/kg) and MOPEG-SO4 levels increased 78% and 63% in cortex and brainstem after 3.0 mg/kg s.c. 90 min prior to decapitation. The effect of ISA on LC activity was completely blocked by dibozane (0.5 mg/kg; 4 of 4 cells), but was unaffected by propranolol (0.5 - 2.0 mg/kg; 7 of 7 cells). These data indicate that ISA, like buspirone, activates the LC noradrenergic system in a manner such that prior inhibition of α2-adrenoceptors, but not β-receptors, prevents the effect. (Supp. by NS-24290)

164.8

EFFECTS OF LOCAL INFUSION OF PHARMACOLOGICAL AGENTS ON TONIC ACTIVITY OF SUBSTANTIA NIGRA DOPAMINE NEURONS. J.H. Carlson and S.L. Foote. Dept. Psychiatry, Univ. Calif. San Diego, La Jolla, CA 92093.

Most electrophysiological studies of drug effects on substantia nigra (SN) dopamine (DA) neurons have used systemic or iontophoretic routes of administration; we have recorded from individual SN DA neurons while infusing small (25-150 nl) volumes of drugs at a distance of 0.3-0.4 mm. The major advantages of this method are that it permits local application without the possible artifacts of iontophoresis and affects a substantial yet selected population of neurons. The sites of action of drugs can be elucidated and the effects of pharmacologically altered DA cell activity on target areas can be analyzed. Extracellular activity of individual DA neurons was recorded with a metal microelectrode in halothane-anesthetized rats. The nonselective D-1/D-2 DA agonist apomorphine (APO) and the selective D-2 agonist LY 163502 (which was more potent) reduced and nicotine increased tonic discharge rates. Onset of these effects was 0.5-1.5 min after infusion. APO and LY 163502 could induce a complete cessation of neuronal activity, which began to recover within 1-2 min. Full recovery often required >1 hr. The duration of nicotine's effect was usually <10 min. Repeated infusions of APO or LY 163502 frequently led to desensitization to the effects of subsequent infusions or i.v. injections. These effects are compatible with previous findings using other routes of drug administration.

164.9

FAILURE OF ACUTE DIPHENYLHYDANTOIN (DPH) TO AFFECT DOPAMINE CELLS ACTIVITY. F. Marrosu*, G. Carcangiu*, M. Giagheddu* and G. Mereu. Lab. of Neurobiology, Univ. of Cagliari, Italy.

An incidence of extrapyramidal side effects (ESE) has recently been reported in epileptic patients under long-term treatment with the anticonvulsant DPH. Although the origin of these effects is unknown, their similarity to those produced by neuroleptics and the fact that chronic DPH produces in rats behavioural signs of hypersensitivity to dopamine (DA) agonists, have suggested that DPH would negatively interfere with DA receptors somehow mimicking the action of neuroleptics. To clearly evaluate this possibility DPH was tested in one of the most sensitive paradigm for DA receptors functionality within the basal ganglia, i.e. the spontaneous activity of mesencephalic DA and GABA cells. It was observed that DPH, at cumulative doses ranging from 1.0 to 40 mg/kg i.v., failed to modify the basal firing of DA cells as well as their response to DA agonists and antagonists. Therefore is unlikely that the reported ESE by DPH might be mediated by a direct inactivation of DA receptors. However the interference of chronic DPH on different neuronal systems remains to be detailed.

164.10

DOPAMINE (DA) AUTORECEPTOR (AR) INACTIVATION: COMPARISON OF IMPULSE-REGULATING AND SYNTHESIS-MODULATING ARs. M. Jeziorski, M.P. Galloway, and F.J. White, Dept. of Psychiatry, Wayne State Univ. Sch. Med., and NPRU and NPPL, Lafayette Clinic, Detroit, MI 48207.

The comparative effects of inactivation of a proportion of DA ARs by a moderate dose of the irreversible DA antagonist EEDQ (2 mg/kg) was examined for impulse-regulating somatodendritic (SD) ARs on mesolimbic (A10) DA cells and synthesis-modulating nerve terminal (NT) ARs on A10 and nigrostriatal (A9) DA cells. Extracellular single-unit recording methods in chloral hydrate-anesthetized rats revealed that neither EEDQ treatment nor repeated treatment with low (50 µg/kg) doses of the DA agonist apomorphine (APO; 2 x 7 days) altered the sensitivity of SD ARs to the inhibition caused by the D2 agonist quinpirole (QUIN) relative to control. Repeated APO combined with EEDQ did induce subsensitivity, providing evidence for the existence of spare SD ARs masking down-regulation. This was supported by the reduced sensitivity of SD ARs in APO-treated rats to the partial DA agonist (-)3-PPP. In contrast, studies of NT AR function, i.e. DOPA accumulation after treatment with the DOPA decarboxylase inhibitor NSD-1015, revealed decreased inhibition by NT ARs in EEDQ-treated rats. EEDQ also lowered the sensitivity of NT ARs to reversal by QUIN of GBL-induced DOPA elevations. These findings indicate differences between SD and NT ARs with respect to the effects of EEDQ and suggest a possible difference in the degree of receptor reserve for the two populations of ARs.

164.11

DOPAMINE NEURON ONTOGENY: ELECTROPHYSIOLOGICAL STUDIES. D.K. PITTS, A.S. FREEMAN and L.A. CHIODO. Lab. of Neurophysiology, Center for Cell Biology, Sinai Res. Inst., 6767 W. Outer Drive, Detroit, MI 48235.

The anatomy, physiology, biochemistry and pharmacology of dopamine (DA)-containing neurons have been extensively studied over the past two decades. Although the electrophysiological characteristics and neuropharmacology of single identified DA neurons have been reported by many investigators, such studies have not yet examined these neurons during postnatal development. Standard extracellular electrophysiological techniques were used to record from single antidromically-identified nigrostriatal DA neurons in rats at postnatal ages of 4 weeks (wk, n=10), 5wk (n =8) and > 7wk (n=11, adult) old. The mean baseline firing rate (spikes/sec) and conduction velocity (c.v., m/sec) for each group were as follows (baseline, c.v.): adult: 3.4 +/- 0.5, 0.54 +/- 0.03; 5wk: 3.0 +/- 0.5, 0.57 +/- .06; 4wk: 4.5 +/- 0.3, 0.58 +/- 0.04. The mean ED50 values (µg/kg, i.v.) for apomorphine cumulative dose-response (percent inhibition) curves were as follows: adult: 8.6 +/- 2.2; 5 wk: 14.0 +/- 3.6; 4 wk: *32.8 +/- 14.6 (* P<0.02, Mann-Whitney, 4 wk vs. adult). These results suggest that there is decreased somatodendritic autoreceptor function in 4wk old animals. Iontophoretic studies employing DA agonists will need to be conducted to confirm these findings. (MH41557-LAC.)

164.12

SEROTONERGIC AFFERENT REGULATION OF NIGROSTRIATAL DOPAMINE NEURONS. M.D. KELLAND, A.S. FREEMAN and L.A. CHIODO. Lab. of Neurophysiology, Center for Cell Biology, Sinai Res. Inst., Detroit, MI 48235.

We have begun to examine the influence exerted by serotonergic (5-HT) afferents from the dorsal raphe nucleus on the activity and pharmacological responsiveness of nigrostriatal dopamine (NSDA) neurons.

Lesions of 5-HT systems by 5,7-DHT or MDMA-induced reductions in 5-HT levels eliminate the rate-dependent nature of i.v. quinpirole-induced inhibition of NSDA neuronal firing, without affecting the responsiveness of these cells to quinpirole. 5,7-DHT appears to preferentially raise the quinpirole ED50 of more slowly firing NSDA neurons. In contrast, acute administration of 5-HT-1A selective compounds 8-OH-DPAT or 5-MeO-DMT preferentially excite slow NSDA cells, while failing to alter quinpirole-induced inhibition of NSDA neurons.

Sciatic nerve stimulation inhibits NSDA neurons (a response dependent upon intact 5-HT systems). We have preliminary evidence that the D1 selective agonist SKF 38393 both decreases the latency to this inhibition and increases its duration. The D1 antagonist SCH 23390, while having no effect on its own, reverses the SKF 38393-induced enhancement. Thus, a mechanism appears to exist which allows NSDA neurons not under tonic influence by sensory afferents to come under phasic control. (MH41557 [LAC], Sinai Res. Inst.).

164.13

Aging and Dopamine Electrophysiology. A.S. Freeman and L.A. Chiodo, Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235.

We have begun to examine the in vivo electrophysiology of DA neurons in rats of different age (3, 18, 24 mos). Overall A9 and A10 DA cell firing rates were examined as were the sensitivities of antidromically identified nigrostriatal DA (NSDA) cells to the inhibitory effects of i.v. apomorphine (APO) and d-amphetamine (AMP), and to iontophoretic DA. The incidence of spontaneously active DA cells was determined for the A9 and A10 regions with the cell/track sampling technique. The activities and DA agonist sensitivities of the DA neurons in the three age groups were remarkably similar. Mean A9 and A10 cell firing rates did not differ from each other and did not change with age. Dose-response profiles for APO and AMP on NSDA cells were not statistically different across ages. Similarly, the ability of iontophoretic DA to depress cell discharge was largely unchanged with age. Finally, the observed incidence of active A9 and A10 DA neurons did not change with age. Previous studies described reductions in DA receptors, levels and synthesis in rats by the age of 24 mos. Thus, apparently normal DA electrophysiological function is maintained in 24 mo. rats despite these biochemical changes. It is currently being determined whether this "compensatory maintenance" of activity erodes in still older rats or can be compromised by other drug challenges. (MH-43401-ASF.)

164.14

DISSOCIATION OF IDENTIFIED NIGROSTRIATAL DOPAMINE-CONTAINING NEURONS FROM ADULT RATS. L.A. Chiodo, Lab. Neurophysiology, Ctr Cell Biology, Sinai Res. Inst., Detroit, MI 48235.

Over the past few years, several groups have turned to a variety of in vitro preparations which contain mesencephalic dopamine (DA)-containing neurons in order to further characterize the active membrane properties of these cells. We have now established a protocol for the dissociation of identified nigrostriatal DA neurons from adult rats. In intact animals, it was observed that 24-48 hrs after the injection of rhodamine-conjugated microspheres into the head of the caudate nucleus, these beads were retrogradely transported and appeared in neurons concentrated primarily in the zona compacta subdivision of the substantia nigra. Over 95% of those neurons which were intensely labeled with microspheres were also tyrosine hydroxylase positive (as demonstrated with indirect immunocytochemical methods and employing the monoclonal antibody for this enzyme, LNC1). Using modifications of the dissociation procedure of Kay and Wong (J. Neuro. Meth. 16, 1986), we have been able to dissociate the zona compacta region of adult rats and recover individual nigrostriatal DA neurons (cells filled with microspheres). These cells remain viable and have been sampled electrophysiological using whole-cell patch recording techniques for up to 4 hrs. (Supported by MH41557)

165.1

REGIONAL EFFECTS OF PHARMACOLOGICAL REGULATION ON THE AFFINITY STATES OF THE D-1 RECEPTOR. Leslie M. Higuchi and Paul McGonigle. (SPON. S.J. Offord) Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

The affinity states of the D-1 receptor were examined by measuring inhibition of the binding of ^{3}H-SCH-23390 by dopamine. In sections of caudate putamen (CPu) mash from Sprague Dawley rats, competition curves in the absence of guanine nucleotide were markedly biphasic and the K_L to K_H ratio obtained from two-site analysis was greater than 1000. Corresponding competition curves measured in homogenates of CPu exhibited a K_L to K_H ratio of less than 100. In the presence of 300 μM GTP, dopamine competition curves were monophasic, confirming that the two components of binding resulted from agonist affinity states. Serial coronal sections containing CPu and nucleus accumbens (NAc) or substantia nigra (SN) were cut at a thickness of 20 μm. Dopamine competition curves were obtained from each region using autoradiography with care to avoid subregions that exhibit gradients of receptor density. The proportions of the affinity states were similar in all three regions, with 35% of the sites in the high affinity state and 65% in the low affinity state. The ratio of K_L to K_H was approximately 1000 in each region. To measure the effects of receptor regulation on the affinity states, animals received 12 daily injections (i.p.) of SCH-23390 (3 mg/kg) or vehicle. Chronic administration of SCH-23390 increased the density of D-1 receptors in the CPu (30%), NAc (35%) and the SN (50%). The proportion of high and low affinity states was unaffected by the increase in receptor density. In contrast, the ratio of K_L to K_H declined to 80 in the CPu and 110 in the NAc. (Supported by USPHS grant GM 34781)

165.2

DOPAMINE (DA) RECEPTOR OCCUPANCY BY DA AGONISTS AND ANTAGONISTS IN VIVO. C.F. Saller, L.D. Kreamer*, M.J. Czupryna* and A.I. Salama ICI Pharmaceuticals Group, ICI Americas Inc. Wilmington, DE 19897.

The occupation of D-1 and D-2 DA receptors by drugs was assessed by measuring their abilities to protect against DA receptor inactivation by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an irreversible receptor modifying agent. Rats were pretreated with drugs and given EEDQ (10mg/kg, i.p.). Generally, drugs were given 30 min. before EEDQ. Twenty-four hr after EEDQ, the rats were decapitated and the numbers of D-1 and D-2 receptors in striatal membrane preparations were estimated using saturating concentrations of[^{3}H] SCH 23390 and [^{3}H] spiperone, respectively.

Both DA agonists and antagonists protected DA receptors from inactivation by EEDQ in a dose-dependent manner. SKF 38393, a selective D-1 agonist, and SCH 23390, a selective D-1 antagonist, protected D-1, but not D-2, DA receptors from inactivation. Conversely, quinpirole, a selective D-2 agonist and haloperidol, a D-2 antagonist, protected D-2 receptors. Likewise, chlorpromazine and several other neuroleptics protected only D-2 sites. Apomorphine, a mixed D-1/D-2 agonist, clozapine, an atypical antipsychotic, and D-butaclamol, a typical neuroleptic, were also active at both sites, but D-butaclamol was far more tightly bound to D-2 receptors than to D-1 receptors. Thus, EEDQ protection experiments provide a simple and reliable means of assessing DA receptor occupancy in vivo.

165.3

SPECIFIC [^{3}H]SCH23390 BINDING TO DOPAMINE D_1 RECEPTORS IN CEREBRAL CORTEX AND NEOSTRIATUM: ROLE OF DISULFIDE AND SULFHYDRYL BONDS. **K.M. Dewar, L. Grondin* and T.A. Reader.** Département de physiologie, Université de Montréal, Montréal, Québec, H3C 3J7, Canada.

Receptor binding studies were performed with membrane preparations from rat cerebral cortex (CTX) and neostriatum (CPU; caudate-putamen) using the dopamine D_1 antagonist [^{3}H]SCH23390. Pretreatment of the membranes with the thiol reactives DTE, L-DTT and DTNB as well as with the alkylating agent NEM produced dose-dependent decreases of specific [^{3}H]SCH23390 binding in CTX and CPU membrane preparations. These changes were not reversible after up to two washes, so that saturation curves could be performed with pretreated membranes in the absence of reactives. The alkylation of sulfhydryl (-SH) groups by NEM reduced the binding mainly through an affinity change while L-DTT and DTNB decreased the number of sites. In both regions the dopamine D_1 receptor requires the integrity of -SH bonds to maintain a high affinity of the receptor protein while disulfides (-SS-) are essential to keep the maximum binding capacity. Based on the stereospecificity of both enantiomers of the dopamine D_1 agonist SKF38393 and the sensitivity towards -SS- and -SH reactives, it can be concluded that [^{3}H]SCH23390 binds to receptor sites in the cerebral cortex and in the neostriatum that not only share pharmacological properties but also exhibit a similar molecular configuration. [Supported by the MRC and FRSQ].

165.4

BINDING OF PERMANENTLY CHARGED CHLORPROMAZINE ANALOGS TO THE STRIATAL D-1 DOPAMINE RECEPTOR. R.A. Wallace, L.J. Wallace*, M. Harrold*, D.D. Miller*, N.J. Uretsky. College of Pharmacy, The Ohio State University, Columbus, OH 43210.

The objective of these studies was to determine the role of the side chain amine group of chlorpromazine (CPZ) in binding to the D-1 dopamine (DA) receptor. Consequently, we synthesized and studied the abilities of structural analogs of CPZ to inhibit the binding of ^{3}H-SCH 23390, a D-1 selective receptor antagonist, to rat striatal membranes. CPZ, a trimethylammonium analog (CPZ-N^+) and a dimethylsulfonium analog (CPZ-S^+) of CPZ were all found to inhibit ^{3}H-SCH 23390 binding with the maximal inhibition being the same for the 3 compounds. The steep slopes of the inhibition curves suggested that all 3 compounds were binding to a single site. However, the potencies of the analogs were much less than that of CPZ. Thus, the apparent equilibrium binding dissociation constants (K_i's) were 6.2 ± 2.0 nM for CPZ, 2.75 ± 0.37 uM for CPZ-S^+ and 12.3 ± 1.6 uM for CPZ-N^+. These studies show that the nitrogen atom on the side chain of CPZ is not required for binding to the D-1 DA receptor and, in addition, that compounds containing a permanent positive charge can bind to this receptor. This latter observation is consistent with the presence of an anionic site on the striatal D-1 DA receptor and is similar to our previous findings on the binding of CPZ and its analogs to the striatal D-2 DA receptor.

165.5

PHOTOAFFINITY LABELLING OF AN APOMORPHINE BINDING PROTEIN FROM BOVINE STRIATUM. G.M. Ross, B.E. McCarry*, K. Gatermann* and R.K. Mishra. Depts. of Psychiatry and Neurosciences, and Dept. of Chemistry, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Apomorphine has several effects on the central nervous system thought to be mediated primarily through dopamine receptors. We report here the photoaffinity crosslinking of a 49 kDa apomorphine binding protein from bovine striatum with [^{125}I]-N-(4'-azido-2'-nitrobenzamidyl-propyl)2-amino-6-hydroxy-7-iodo-1,2,3,4-tetrahydronaphthalene. Incorporation of [^{125}I]ANB-AP-ATN is blocked by coincubation with apomorphine or N-propyl-norapomorphine (NPA); (-)NPA is more effective than (+)NPA in blocking incorporation, suggesting stereospecific requirements. Other dopaminergic ligands, including SKF-38393, SCH-23390, spiperone and LY-171555 (all 100 μM) were ineffective in blocking photolabel incorporation; the dopamine uptake blocker GBR 12909 and putative autoreceptor ligands (+)PPP and (-)PPP were also ineffective (100 μM). Other compounds (all at 100 μM) which failed to prevent covalent incorporation include serotonergic (metergoline and 5-HT), adrenergic (phentolamine and isoproterenol) and opiate (dipenorphine and etorphine) ligands. The functional significance of this protein is not currently understood.

Supported by MRC Canada and the Ontario Mental Health Foundation.

165.6

POLYCLONAL ANTISERUM AGAINST THE DOPAMINE AGONIST ADTN AS A PROBE FOR DOPAMINE BINDING PROTEINS. A.J. Keukens*, G.M. Ross, B.E. McCarry*, L.K. Srivastava and R.K. Mishra (SPON:G. Madhavan). Depts. of Psychiatry and Neurosciences and Dept. of Chemistry, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

High affinity antibodies are useful for the isolation of antigens (e.g., ligands) from mixtures. In the case where such ligands are covalently attached to proteins, the use of antibodies against the ligand should facilitate the isolation and identification of that protein. For this study, antibodies against a derivative of the dopamine agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydro-naphthalene (ADTN) were obtained as potential aids for the isolation of proteins crosslinked to photolabile ADTN derivatives. Rabbit polyclonal antiserum was raised against aminopropyl-ADTN (AP-ADTN) conjugated to bovine serum albumin; conjugation was achieved by using glutaraldehyde and $NaBH_4$, resulting in a ratio of 60 mol AP-ADTN per mol BSA. Reactivity of the polyclonal antiserum towards the hapten conjugate was established using an enzyme-linked-immunoabsorbent assay (ELISA). Antibody specificity was tested by measuring the ability of various aminotetralins to compete for AP-ADTN-G-rabbit serum albumin binding sites in the antiserum. High affinity antibodies against conjugated ADTN and its analogues were obtained. These are currently used to probe dopamine binding proteins crosslinked to ADTN derivatives. Supported by MRC Canada & OMHF

165.7

PROPERTIES OF CHOLATE-SOLUBILIZED DOPAMINE D_1 RECEPTOR. L.K. Srivastava, S.B. Bajwa*, G.M. Ross and R.K. Mishra. Neuropharmacology Lab., Depts. of Psychiatry and Neurosciences, McMaster Univ., Hamilton, Ont., Canada L8N 3Z5.

The dopamine D_1 receptor from bovine striatal membranes was solubilized with cholate-NaCl in the presence of phospholipids in a form sensitive to guanine nucleotides. The solubilized receptor was reconstituted into lipid vesicles by gel-filtration. The solubilized and reconstituted preparation binds [^{3}H]SCH-23390 to a homogeneous site in a saturable, stereospecific and reversible manner with a K_d (0.95 nM) and the B_{max} (930 fmol/mg protein) similar to that observed with the membrane-bound receptors. The competition curves of dopaminergic antagonists for [^{3}H]SCH-23390 binding were monophasic and exhibited a rank order of potency (SCH-23390)+ butaclamol)cis-flupenthixol)haloperidol)spiroperidol) which is clearly a D_1 dopaminergic order. The competition curves of agonists vs. antagonist fit best to a two-site model showing the following order of potency: SKF-82526)SKF-38393)NPA)ADTN)DA. The high affinity component of agonist binding showed requirement of Mg^{2+} ions while Gpp(NH)p, at 100 μM concentration, caused full conversion of high affinity agonist binding sites to the low affinity ones, suggesting that the solubilized receptor is coupled to a guanine nucleotide binding protein. The solubilization procedure described will aid in understanding the mechanisms of receptor-G-protein interactions and the purification of the receptor. (Supported by MRC and OMHF.)

165.8

COMPARISON OF MEMBRANE-ASSOCIATED AND DETERGENT-SOLUBILIZED BINDING SITES FOR ^{125}I-SCH-23982. T. Filtz*, R.R. Luedtke* and P.B. Molinoff. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6084.

^{125}I-SCH-23982 is a high-affinity antagonist selective for the D_1 subtype of dopamine receptors. This radioligand has also been shown to label 5-HT_{1C} receptors in the choroid plexus. The rank order of potency for the inhibition of the binding of ^{125}I-SCH-23982 to membrane preparations of canine caudate by a panel of agonists and antagonists is consistent with the pharmacological specificity of the D_1 receptor. Competition curves for antagonists and agonists (in the presence of 100 μM GTP) fit best to a one-site model.

A polyethylene glycol precipitation binding assay was used to characterize binding sites for ^{125}I-SCH-23982 after extraction of membranes with 1% digitonin. Solubilized binding sites adsorbed to heparin-agarose and Q-Sepharose FF when applied in the absence of NaCl. In contrast to results of studies carried out with membranes, the data from inhibition experiments using solubilized preparations and ^{125}I-SCH-23982 consistently fit best to a two-site model for antagonists. A high-affinity site for 5-HT suggests that ^{125}I-SCH-23982 may bind to both D_1 and 5-HT_1 receptors in detergent-solubilized preparations. (Supported by the USPHS NS18591 and the Scottish Rite Research Program)

165.9

PHOTOAFFINITY LABELLING OF HUMAN BRAIN D2 DOPAMINE RECEPTORS IN SCHIZOPHRENIA, ALZHEIMER'S, PARKINSON'S & HUNTINGTON'S DISEASES. N.H. Bzowej*, H.B. Niznik and P. Seeman (Spon: L. Spero). **Department of Pharmacology, University of Toronto, Ontario, Canada, M5S 1A8.**

Considerable evidence exists to suggest abnormal brain dopamine transmission in schizophrenia, Alzheimer's, Parkinson's and Huntington's diseases. The molecular structure of the striatal D2 receptor binding subunit in these diseased brains was identified by photoaffinity labelling with [^{125}I]Azido-NAPS. In all diseased tissues studied, [^{125}I]Azido-NAPS specifically and covalently labelled a broad band of Mr ≅ 94,000, as assessed by autoradiography following sodium dodecyl sulfate polyacrylamide gel electrophoresis, which was similar to human control brain D2 subunits and that observed in canine striatal membranes. Further work will assess the relative electrophoretic mobilities of deglycosylated photo-labelled D2 binding subunits in these tissues.

This work was supported by the MRC of Canada, The Canadian Friends of Schizophrenics, OMHF and NARSAD.

165.10

SOLUBILIZATION AND AFFINITY CHROMATOGRAPHY OF THE DOPAMINE D2 RECEPTOR FROM BOVINE STRIATUM. C. Chung*, M. Dame*, R. Schoenleber* and J. McKelvy (SPON: R. S. Janicki). Neuroscience Research Division, Pharmaceutical Discovery, Abbott Laboratories, Abbott Park, IL 60064.

The dopamine D2 receptor has been purified from bovine striatum using a two step detergent solubilization procedure followed by chromatography on AES-Sepharose, an antagonist affinity resin prepared as described previously (Dame, M., *et al.*, FASEB J., 2:A1400, 1988). [^{3}H]Spiroperidol binding activity was solubilized from membranes using 0.25% cholate/1.5M NaCl, followed by precipitation and subsequent treatment with 0.5% digitonin. This solubilization scheme resulted in a preparation with 1.5 to 2 times the specific activity of the starting membranes. The binding activity is saturable and of high affinity (0.5 nM). Solubilized preparations maintain the ligand binding characteristics of a dopamine D2 receptor as assessed by agonist and antagonist competition for [^{3}H]spiroperidol binding sites. 100% of the soluble [^{3}H]spiroperidol binding activity was retained by the affinity matrix at a receptor to resin ratio (v/v) of 2:1 whereas 55-65% was retained at a ratio of 10:1, the ratio used in affinity experiments. After adsorption at 4°C for 16 hr and extensive washing, 15% of the adsorbed activity was eluted using 50 μM haloperidol. Protein was not measurable by Bradford assay but was estimated at <0.5 μg/ml based on silver stained gels. Using this estimate, >1400 fold purification was achieved. Experiments are underway to determine whether additional receptor activity can be recovered from the affinity column eluate using reconstitution procedures.

165.11

SOLUBILIZATION AND PURIFICATION OF D_2 DOPAMINE (DA) RECEPTORS FROM A RAT ANTERIOR PITUITARY TUMOR. J.Y. Lew and M. Goldstein. N.Y. Univ. Med. Center, Neurochem. Res. Labs, New York, NY 10016.

D_2 DA receptors were characterized in a rat anterior pituitary tumor (APT) (Lin et al., J. Pharm. Exp. Ther. 242:950, 1987). We have investigated whether the APT is a suitable source for purification of D_2 DA receptors. The D_2 DA receptors in the APT were solubilized with 10mM CHAPSO, purified by precipitation of the proteins with 15% polyethylene glycol and chromatography on wheat germ agglutinin (WGA) and on a DEAE-Sepharcyl column or on a Mono Q column. The recovery from the WGA column is only 20-30%, suggesting that the D_2 DA receptor is present as a glycoprotein to a lesser extent in tumor than in brain. Purification on Mono Q or on DEAE-Sepharcyl columns results approximately in a 10-fold increase in specific binding of ^{3}H-spiroperidol. SDS-gel electrophoresis of the [^{125}I] labeled proteins following Mono Q or DEAE-Sepharcyl column chromatography revealed four major peptides with M.W. of 205, 110, 85 and 55 KDa. The association of these peptides with the D_2 DA receptor binding sites is under investigation. Suppported by NIMH 02717 and NINCDS 06801.

165.12

IDENTIFICATION AND CHARACTERIZATION OF D1 AND D2 DOPAMINE RECEPTOR SUBTYPES EXPRESSED IN CULTURED NEUROBLASTOMA AND RETINOBLASTOMA CELL LINES. Frederick J. Monsma, Jr., and David R. Sibley. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20892

The recent availability of high specific activity radiolabeled dopaminergic antagonists with specificity for dopamine receptor subtypes has allowed us to screen a wide variety of cultured mammalian cell lines for the presence of D_1 and D_2 dopamine receptors. Initial screening of cell lines was carried out using both radioiodinated and tritiated dopamine receptor ligands and has resulted in the identification of two cell lines which contain either D_1 or D_2 dopamine receptors.

Specific binding of the D_1 selective antagonists [^{125}I]-SCH 23982 and [^{3}H]-SCH 23390 was detected in membranes prepared from NS20Y cells, a clonal cell line derived from the C1300 murine neuroblastoma cell line. Saturation analysis of [^{125}I]-SCH 23982 binding revealed saturable, high affinity binding with a dissociation constant (K_d) of 630 pM and a receptor density of 16 fMoles/10^6 cells (≈10,000 receptors/cell). Inhibition of [^{125}I]-SCH 23982 binding by a series of dopaminergic antagonists exhibited appropriate stereoselectivity and pharmacological specificity, verifying the D_1 nature of this site. Dopamine inhibition of [^{125}I]-SCH 23982 binding reflected the presence of high and low affinity agonist binding sites, and, in the presence of GppNHp the inhibition curve for dopamine was shifted to the right.

In membranes prepared from the human retinoblastoma cell line WERI 27, specific binding of the D_2 antagonist [^{3}H]-methylspiperone was observed. Saturation analysis revealed the presence of a single class of high affinity binding sites with a K_d of 100 pM and a B_{max} of 5.5 fMole/10^6 cells. [^{125}I]-Sulpride, another D_2 specific antagonist also bound to a single class of sites with high affinity (K_d=600 pM). Inhibition of [^{125}I]-Sulpride binding by dopaminergic antagonists exhibited a rank order of potency consistent with the identification of a D_2 dopamine receptor subtype. In addition, dopamine inhibition curves of [^{125}I]-Sulpride binding exhibited both high and low affinity sites, and were shifted to the right by addition of GppNHp.

These results represent the first direct demonstration of D_1 and D_2 dopamine receptors in cultured mammalian cell lines. These cells should provide useful model systems for investigating the molecular mechanisms involved in dopamine receptor /effector coupling and regulation.

165.13

STABLE EXPRESSION OF PUTATIVE RAT D-2 RECEPTOR IN TRANSFECTED MOUSE L-CELLS. T.S. Khurana[1,*], P. Sajovic[1,*], K. O'Malley[2], and R. D. Todd[3,*] (SPON: E. H. Rubin) Department of Biology[1], City College of New York, New York, NY, 10031 and Departments of Anatomy and Neurobiology[2] and Psychiatry[3], Washington University School of Medicine, St. Louis, MO 63110

Dopamine receptors have been implicated as the site of dysfunction or therapeutic intervention for a variety of neuropsychiatric disorders. To develop model culture systems for the study of D-2 receptors and as a first step in isolating the D-2 receptor gene, we have established a mouse fibroblast line which appears to express rat D-2 receptors.

Rat genomic DNA was partially digested with Msp I, ligated to Msp I digested ϕX174 DNA and co-transfected into TK⁻ mouse L-cells (ATCC CCL.1.3) with an HSV-TK containing plasmid. Transfected cells were selected in HAT media and replica plated onto polyester filters at low density. Subsequently, filter colonies were screened for the ability to binding 0.25 nM ^{125}I-spiperone. Out of one million colonies screened, one colony bound ^{125}I-spiperone. This colony (DA-10) was subcloned by limiting dilution, analysed by Southern blotting, and found to contain integrated ϕX174 hybridizing fragments.

DA-10 cells were grown in quantity, briefly trypsinized and assayed as single cell suspensions for [^{3}H]-spiperone binding. Specific [^{3}H]-spiperone binding was completely blocked by 1 uM eticlopride, but not by 1 uM SCH (+) 23390, ketanserin, or 8-OH-DPAT. The (+) isomer of butaclamol had a six to ten-fold greater affinity for the [^{3}H]-spiperone binding sites than did the (-)isomer. The concentration of [^{3}H]-spiperone binding sites was about 3500 per cell.

These findings suggest that the DA-10 cell line is stably expressing a transfected D-2 like gene. The ϕX174 tagging should allow us to isolate the DNA sequences responsible for this effect.

165.14

ESTABLISHMENT OF A PROLACTIN-SECRETING CELL LINE WITH FUNCTIONAL DOPAMINE D-2 RECEPTORS. K.J. Nicklaus, J.C. Lin* and P.B. Molinoff. Dept. of Pharmacology, Univ. of Penn. School of Medicine, Philadelphia, PA 19104-6084.

Studies of the regulation of dopamine receptors have been impeded by the lack of cell lines which express either D-1 or D-2 receptors. The transplantable pituitary tumor 7315a has been shown to contain D-2 receptors negatively coupled to adenylate cyclase. We have used the 7315a tumor to isolate cell lines that express D-2 receptors. Tumors were dispersed into single cell suspensions with collagenase, and cell lines were established by limiting dilution. These cell lines were screened for the presence of binding sites for ^{125}I-IBZM, a radioligand selective for D-2 receptors. One of the cell lines with binding sites for ^{125}I-IBZM, SUP1, secretes prolactin measurable by RIA. Analysis of the binding of ^{125}I-IBZM to membranes prepared from SUP1 cells is consistent with the existence of a single population of binding sites with a high affinity for ^{125}I-IBZM (K_d = 0.8 nM; B_{max} = 30 fmoles/mg of protein). The order of potency of ligands at this site, domperidone > sulpiride > ketanserin, is consistent with the properties of the D-2 receptor. Furthermore, dopamine inhibits adenylate cyclase activity in membranes prepared from SUP1 cells, and this inhibition is blocked by the D-2 receptor antagonist spiroperidol. These results suggest that SUP1 cells contain D-2 receptors negatively coupled to adenylate cyclase. SUP1 cells will represent a useful model system for future studies of the regulation and function of D-2 receptors *in vitro*. (Supported by USPHS Grant NS 18591 and an NSF Predoctoral Fellowship to K.J.N.)

165.15

FURTHER CHARACTERIZATION OF THE FUNCTIONAL D-2 DOPAMINE RECEPTOR ON THE MMQ CELL. R.G. MacKenzie, M.S. Ackerman*, R.R. Bhatt*, R.M. MacLeod, G.L. Snyder, and J.W. Kebabian. Neuroscience Research Division, Pharmaceutical Discovery, Dept. 47U, Abbott Laboratories, Abbott Park, IL 60064.

The MMQ cell line, derived from the rat anterior pituitary 7315a tumor, is the only cell line expressing functional D-2 dopamine receptors. In the present study, we used radioligand binding and cAMP accumulation assays to further characterize this receptor. Saturation binding with [3H]-spiperone resulted in binding parameters determined by Scatchard analysis of Kd=55pM and Bmax=27 fmoles/mg protein. In the presence of 0.5mM IBMX, dopamine inhibited forskolin(0.1uM)-stimulated cAMP accumulation by 50% (EC50=0.59uM) and this effect was completely reversed by the D-2 antagonist spiperone (1uM). The specific D-2 agonists LY-171555 and N-0437 were also effective inhibitors of cAMP accumulation (EC50s=0.33uM and 0.25 nM respectively). Surprisingly, the specific alpha-2 adrenergic agonist UK 14,304 also inhibited cAMP accumulation in these cells, apparently via an alpha-2 receptor since its maximal effect, achieved at 1uM, was reversed by 0.3uM yohimbine whereas 10uM spiperone was ineffective. The effect of the specific D-2 agonist LY-171555 was clearly mediated via a D-2 receptor since its maximal effect at 10uM was reversed by 0.1uM spiperone and 1 uM raclopride (another D-2 antagonist) while the alpha-2 antagonists yohimbine and phentolamine were both ineffective at 10uM. In summary, MMQ cells express D-2 dopamine receptors which, upon activation, inhibit cAMP accumulation. However, the presence of an alpha-2 adrenergic receptor inhibitory to cAMP accumulation suggests caution before drug effects be attributed to selective activation of the D-2 receptor.

165.16

MODULATION BY CATIONS OF [^{3}H]SPIPERONE BINDING TO DOPAMINE RECEPTORS IN 7315a AND MtTW15 TUMORS. K. Coenen*, D. Cloutier* and T. Di Paolo, (SPON.: M. Fleury). Dept. of Molecular Endocrinology, Laval University Medical Center, Quebec, G1V 4G2 and School of Pharmacy, Laval University, Quebec, G1K 7P4.

The effect of sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) were studied on dopamine (DA) receptors in prolactin (PRL) secreting 7315a and MtTW15 tumors grown in intact female rats. At 25°C in 7315a tumors, Mg^{2+} and Ca^{2+} increase the dissociation constant (Kd) of [^{3}H]spiperone binding compared to the Kd obtained using a buffer without ions while these ions have no effect on the Kd values when the assay was performed at 37°C. Na^+ and K^+, at 25°C, do not change the Kd of [^{3}H]spiperone binding while a decrease is observed at 37°C. In the presence of all cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) [^{3}H]spiperone binding affinity is unchanged at 25°C while it is decreased at 37°C compared to Kd values obtained using a buffer without these cations. At 25°C and 37°C in 7315a tumors choline chloride does not change the Kd of [^{3}H]spiperone binding compared to the Kd obtained using a buffer without ions, indicating that the effect of ions are specific and not due to differences in ionic strength. At 25°C, in MtTW15 tumors as for 7315a tumors, an increase of Kd is observed in presence of bivalent cations. For monovalent cations, by contrast to 7315a tumors, at 25°C in MtTW15 tumors an increase of Kd is seen with K^+ whereas the affinity is unchanged with Na^+. In the presence of all cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) [^{3}H]spiperone binding affinity is unchanged at 25°C compared to values obtained using a buffer without these cations. The density of [^{3}H]spiperone binding to DA receptors in 7315a and MtTW15 tumors assessed at 25°C or at 37°C is unaffected by the cations under study. In the intact anterior pituitary, Na^+ and K^+ decrease the Kd values of [^{3}H]spiperone binding whereas Mg^{2+} increases it. Thus, our results suggest a different modulation by monovalent cations of DA receptors in 7315a and MtTW15 tumors compared with intact tissue.

165.17

CONCURRENT EXAMINATION OF D2 RECEPTOR BINDING AND TH-IMMUNOCYTOCHEMISTRY. K.A. Steece, B.C. Blanchard*, R.H. Roth[1] and J.R. Sladek, Jr. Dept. of Neurobiol. & Anat., Univ. of Rochester Sch. of Med., Rochester, NY and Dept. of Pharmacol.[1], Yale Univ. Sch. of Med., New Haven, CT.

The ability to examine immunocytochemistry (ICC) and receptor binding in adjacent, or nearby, brain sections would provide important information on the relationship between anatomy and biochemistry at the receptor level. Of particular interest to us was the potential to examine dopamine (DA) neuron degeneration morphologically and relate this to D2 receptor regulation during varying degrees of the neurodegeneration found in animal models of Parkinson's disease. A standard protocol for TH-ICC is 4.0% paraformaldehyde fixation of brain tissue. Thus a combination of morphology and receptor chemistry would require determining whether the receptor of interest survives fixation. Our preliminary data suggested that although D2 receptors in rat brain did appear viable following intracardiac perfusion with 4.0% paraformaldehyde, there was an apparent decrease in number of binding sites compared to control tissue. Further characterization of D2 specific [3H]-Spiperone (SP) binding using biochemical 'wipe' techniques, indicates there is no significant effect of 4.0% fixation on receptor binding capacity or affinity compared to controls. In addition, IC50 analysis of D2 specific, sulpiride displacement of SP was not different in 4.0% brain compared to control. However, 4.0% perfusion significantly decreased the affinity of butaclomol displacement of SP. Butaclomol binds to both serotonin (S2) and DA (D2) receptors. The effect of fixation on butaclomol binding may reflect a differential sensitivity of D2 and S2 receptors to 4.0% fixation. Studies are underway to elucidate this finding. D2 binding was also examined using autoradiography. Computer-assisted analysis of autoradiograms revealed similar D2 specific striatal binding of SP in 4.0% and control brains. In addition to perfusion, other processing of brain tissue often is utilized in ICC. Data from 'wipe' studies indicate that while 1 hour of immersion post-fixation is compatible with D2 binding, longer duration post-fixation, and storage in ethylene glycol-based cryoprotectant is not. A demonstration of concurrent ICC and receptor binding will be presented in intact and 6-OHDA lesioned animals. (Supported by PHS grant NS24032 and MH18260-02)

165.18

[^{125}I]-SPECTRAMIDE: A NOVEL BENZAMIDE LIGAND DISPLAYING POTENT AND SELECTIVE EFFECTS AT THE D_2 DOPAMINE RECEPTOR. P.M. Sanchez-Roa*, J.Sharkey*, A.A. Wilson*, D.E. Grigoriadis, R.F. Dannals*, D.F. Wong*, and M.J. Kuhar. (SPON: A. Goldberg). NIDA Addiction Research Center, Baltimore, MD 21224 and Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Spectramide, (N-[2-[4-iodobenzyl-N-methylamino]-2-methoxy-4-ethyl]-5-chloro-methylamine]benzamide), is a novel benzamide derivative. In the present study we show that it can be used as a potent and highly selective ligand for the dopamine D_2 receptor. In competition studies, spectramide potently inhibited [^{3}H]-N-methylspiperone (100pM) binding from the dopamine D2 receptor with a K_i of 400pM. Spectramide also exhibited a weak affinity for the a_1-adrenergic receptor (K_i of 62nM). By contrast, spectramide did not significantly inhibit binding at the D_1, S_2 receptors or to the dopamine transporter, at concentrations up to 1uM. Kinetic and saturation studies in rat striatal homogenates, revealed that [^{125}I]-spectramide exhibits reversible, saturable and high affinity binding characteristics. Preliminary kinetic analysis showed a K_{off} = 0.0567min^{-1}, a K_{on} = 0.71 min^{-1}.nM^{-1} with the calculated K_D from the off/on rates of 80pM. The estimated K_D from saturation analysis was 24.4pM, with a Bmax = 173fmol/mg wet weight tissue. These data indicate that spectramide is a potent and highly selective D_2 ligand, which may prove useful for imaging D_2 receptors in brain using either PET or SPECT.

165.19

CNS DOPAMINE RECEPTORS MODULATION BY CONFORMATIONALLY CONSTRAINED ANALOGUES OF L-PRO-L-LEU-GLYCINAMIDE (PLG). R.K. Mishra, C. Barlas*, L.K. Srivastava and R.L. Johnson* Depts. of Psychiatry and Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

The tripeptide L-prolyl-L-leucyl-glycinamide (PLG) has been shown to possess a variety of pharmacological activities in the CNS including modulation of dopamine receptors. Several conformationally constrained analogues of Pro-leu-gly-NH_2 (PLG) have been synthesized. In one series of analogues, the Leu-gly-NH_2 dipeptide segment of PLG was replaced with the δ-lactam residues 3(S)- and 3(R)-amino-2-oxo-pyrrolidineacetamide and the δ-lactam residue 3(S)-amino-2-oxo-piperidineacetamide. The corresponding δ-lactam analogues of <Glu-leu-gly-NH_2 were also synthesized. In another series Gly-NH_2 was replaced with 2-keto-piperazine, 3(S)-amino-2-pyrrolidone. Of all the conformationally constrained analogues of PLG synthesized in the study, only the δ-lactam analogue 3(R)-N(L-prolyl)amino-2-oxo-1-pyrrolidineacetamide was found to posses significant activity under preincubation conditions, this analogue was 1000-5000 times more potent than PLG. The binding of ADTN was significantly increased at concentrations of 10^{-10} - 10^{-9} M. Furthermore, the conversion of high affinity state of D_2 receptor to low affinity state by GTP was also partially prevented by this compound.

Supported by MRC, OMHF, and NIH.

ADRENERGIC RECEPTORS I

166.1

DIFFERENTIAL EXPRESSION OF IMIDAZOLE AND α_2-ADRENERGIC RECEPTORS AND CLONIDINE-DISPLACING SUBSTANCE (CDS) IN NG108-15, GLIAL, AND CHROMAFFIN CELLS. *G. Feinland*, P. Ernsberger, M.P. Meeley, M.J. Evinger, and D.J. Reis* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Clonidine binds to imidazole as well as α_2-adrenergic receptors in brain (Ernsberger et al., *Eur J Pharmacol* 134:1 '87). We sought to determine whether (a) imidazole and α_2 receptors coexist on single cells, (b) these receptors necessarily coexist, and (c) cells with imidazole receptors also contain CDS, the putative endogenous ligand at these sites (Ernsberger et al., *Brain Res* 441:309 '88). Membranes (P_2) were prepared from cultured NG108-15, glial, and chromaffin cells and incubated with 1 nM ^{3}H-*p*-aminoclonidine (^{3}H-PAC) for 40 min at 25°C. Nonspecific binding was defined by 10μM phentolamine. In NG108-15 clonal cells, the non-imidazoles epinephrine (100μM) and guanabenz (10μM) displaced 48±3% and 49±2%, respectively, of total specific ^{3}H-PAC binding. In glia, however, guanabenz inhibited 109±4% of specific ^{3}H-PAC binding as defined by phentolamine. In contrast, the imidazole cimetidine (10μM) inhibited ^{3}H-PAC binding in NG108-15's (40±1%), but not in glia (-12±8%). Thus, in glial membranes, ^{3}H-PAC labeled α_2-receptors exclusively. In chromaffin cells, epinephrine inhibited only 15±12% of total specific ^{3}H-PAC binding, while cimetidine inhibited 68±25%, indicating that imidazole but not α_2 receptors were labeled. Specific ^{3}H-PAC sites were enriched in chromaffin cells relative to P_2 membranes from the source tissue, adrenal medulla (B_{max}= 1600±300 and 63±17 fmol/mg protein, respectively) but exhibited similar affinity (K_d=4±1 and 2±1nM) and ligand specificity. CDS was isolated (Meeley et al., *Neurosci Lett* 84:84 '88) from osmotically shocked P_2 fractions of NG108-15's. CDS from NG108-15's was active in ^{3}H-PAC and ^{3}H-cimetidine radioreceptor and smooth muscle bioassays (yield=10-18 Units from 7 x 10^7 cells). Thus, although imidazole and α_2 receptors coexist in a 1:1 ratio in NG108-15's, these receptors occur independently in glia (α_2 only) and in chromaffin cells (imidazole only). NG108-15 cells may serve as a model system for both the imidazole receptor and its endogenous ligand, CDS.

166.2

A NOVEL ALPHA-2 ADRENERGIC RECEPTOR IS EXPRESSED IN THE OK CELL LINE. T.J. Murphy, J.C. Baker*, C. Ray-Prenger* and D.B. Bylund, University of Missouri, Columbia, MO 65212.

OK cells, a cell line derived from an American opossum kidney, contain alpha-2 adrenergic receptors (A2AR). We wished to determine which subtype of A2AR these receptors represented. The pharmacological properties of the OK cell A2AR were compared to those in tissues expressing a single A2AR subtype. Human platelet and HT29 cell line membranes served as a source for the A2AR-A subtype, whereas neonatal rat lung and NG108 cell line membranes were used to supply the A2AR-B subtype. K_i values were obtained for several drugs in each tissue by competition binding analysis using either [^{3}H]yohimbine or [^{3}H]rauwolscine. Comparisons were made by linear regression of plots of log [K_i] values to assess correlations of drug affinities. As is shown below, the OK cell A2AR correlates poorly with both the A2AR-A or A2AR-B subtype. We conclude that OK cells express neither the A2AR-A nor A2AR-B subtypes.

	r	Slope	n
OK Cells vs. Rat Lung (2B)	0.87	0.69	30
OK Cells vs. HT29 Cells (2A)	0.84	0.85	30
Rat Lung (2B) vs. HT29 Cells (2A)	0.71	0.91	30
Rat Lung (2B) vs. NG108 Cells (2B)	0.99	1.02	13
Human Platelet (2A) vs. HT29 Cells (2A)	0.98	0.97	19

166.3

EVIDENCE FOR ALPHA$_2$-ADRENORECEPTOR HETEROGENEITY IN RAT CORTEX. RK Zolfoski and CE Dunlap, III. Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC 27103.

Previous studies by others have provided pharmacological and anatomical evidence for alpha$_2$-adrenoreceptor heterogeneity within rat brain. In an attempt to further elucidate the extent of this heterogeneity, a series of experiments utilizing [^{3}H]-Guanabenz (^{3}H-GB) have been undertaken in our laboratory.

Initial studies with ^{3}H-GB in rat cortex were conducted to determine the kinetics of GB binding. Association and dissociation experiments yielded two binding sites; a high affinity site with association rate constant (k_1) of 7.67±6.86x10^7 min^{-1} and dissociation rate constant (k_{-1}) of 1.34±4.50x10^{-2} min^{-1} and a low affinity site with k_1 of 6.70±4.03x10^8 min^{-1} and k_{-1} of 0.24±0.08 min^{-1}. K_d values calculated from these rate constants were 3.76±1.72 and 0.9±0.65 nM, respectively. Saturation experiments yielded a K_d of 5.83±1.01 nM and B_{max} of 266.40±67.43 fmoles/mg protein. Competition studies were then used to determine the displacement of ^{3}H-GB. GB displayed a two-site displacement; a high affinity site with K_i of 2.43±1.58nM and a low affinity site with K_i of 271.47±141.98 nM. Various other adrenergic agents were also tested, and the following K_i values (nM) were obtained:

Clonidine	24.28±1.58	UK-14304	9.98±0.19
Epinephrine	17.32±10.70	Propranolol	no displacement
Idazoxan	24.33±10.59	Yohimbine	no displacement

Studies by others indicate that idazoxan labels a heterogeneous population of alpha$_2$-adrenoreceptor sites; one population of these sites are selectively labeled by raulwolscine. Also parallel competition studies indicate that clonidine and GB have separate high affinity binding sites. From these studies, we hypothesize that there may be a large pharmacological heterogeneity of alpha$_2$-adrenoreceptors in rat brain, a select population of which binds GB.

This research was supported by NIH grant HL34460.

166.4

PHARMACOLOGICAL CHARACTERIZATION OF [^{3}H]GUANFACINE BINDING. T.D. Smith, and F.M. Leslie, University of California, Irvine, CA. 92717, USA.

Guanfacine is an α_2 agonist which is used clinically as a potent antihypertensive. Recently it has also been shown to selectively improve cognitive function in aged monkeys (Arnsten et al. J.Neurosci., in press). Previous work in our laboratory has provided evidence for two α_2-adrenoceptor subtypes in rat brain and kidney which exhibit differential affinity for guanfacine. We have currently used radioligand binding and autoradiographic techniques to characterize the pharmacological properties and anatomical localization of [^{3}H]guanfacine binding sites in these tissues. Specific binding was defined as the difference in binding in the absence and presence of unlabeled guanfacine (10μM). In brain, radioligand binding data provided evidence that [^{3}H]guan labels a single high affinity binding site. Clonidine and several other selective α-adrenoceptor ligands yielded greater than eighty percent displacement of specific binding. Autoradiographic distribution of [^{3}H]guan binding paralleled that of [^{3}H]clonidine and was completely displaced by excess unlabeled clonidine (1μM). In kidney, radioligand binding provided evidence that [^{3}H]guan labels two sites with high affinity. A number of selective alpha adrenergic ligands failed to displace more than thirty percent of [^{3}H]guan specific binding. The autoradiographic distribution of [^{3}H]guan was also distinct from that of [^{3}H]clon. Whereas [^{3}H]guan densely labeled the renal cortex, the inner medulla and the outer zone of the outer medulla, [^{3}H]clon labeled only the inner medulla and the outer zone of the outer medulla. Clonidine failed to displace [^{3}H]guan labeling in the renal cortex. These data suggest that in peripheral tissues, guanfacine may bind with high affinity to a site other than the α_2-adrenoceptor.

This work was supported by NIH grant NS 19319.

166.5

HETEROGENEITY OF [3H]PRAZOSIN BINDING SITES IN CANINE BRAIN AND LIVER S.S. Bowersox, J. Mignot*, E. Maddaluno*, W. C. Dement, and R. D. Ciaranello Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, California 94305.

We have identified two classes of putative alpha-1 adrenoceptors in canine brain and liver using conventional radioligand binding methods. Scatchard plots of specific [3H]prazosin (Pz) binding to brain and liver homogenates prepared from 100 day old Doberman pinscher dogs were consistently curvilinear and best fit a two-site binding model [Brain: Kd1= 57.7± 10.0 pM, Bmax1 = 64.6 ± 17.1 fmol/mg protein, Kd2 = 1.5 ± 0.5 nM, Bmax2= 159.5±37.6 fmol/mg protein; Liver: Kd1= 82.6±36 pM, Bmax1= 7.0±5.1 fmol/mg protein, Kd2= 0.8±0.2 nM, Bmax2= 62.1±8.7 fmol/mg protein]. Kinetically-derived affinity constants from association and dissociation experiments agreed with those obtained by Scatchard analysis of equilibrium binding data. Binding sites were saturable, heat labile, bound ligand reversibly, and were appropriately distributed in relation to endogenous neurotransmitter. Saturation studies performed on brain tissues from dogs killed on the same day as the binding experiments and without any intervening freezing steps, yielded findings similar to those obtained using frozen tissue preparations. Competitions for [3H]Pz binding to canine frontal cortex membranes were conducted with WB-4101, corynanthine, benoxathian, phenoxybenzamine, chlorethylclonidine, thymoxamine, prazosin, and yohimbine. All ligands but prazosin competed for specific [3H]Pz binding in a statistically significant, biphasic manner. Benoxathian and WB-4101 displayed highest affinity (benox: Ki1= 0.26nM, WB-4101: Ki1=0.20nM) and selectivity (benox: Ki1/Ki2= 0.11, WB-4101: Ki1/Ki2= 0.007) for the high affinity [3H]Pz binding site; chlorethylclonidine and phenoxybenzamine showed highest affinity (CEC: Ki2= 91nM, phenox: Ki2= 3.4nM) and selectivity (CEC: Ki1/Ki2= 375, phenox: Ki1/Ki2= 225) for the lower affinity [3H]Pz binding site. Yohimbine competed with nanomolar affinity (Ki = 16.1nM) for the highest affinity [3H]Pz binding site. These findings raise new questions about the validity of current adrenoceptor classification schemes which posit a primary alpha-1 and alpha-2 adrenoceptor subdivision on the basis of presumed differential binding profiles of prazosin (alpha-1) and yohimbine (alpha-2). The fact that Pz possesses higher affinity for both classes of binding site than does yohimbine favors identification of these sites as alpha-1 adrenoceptors.

166.6

ALPHA$_2$ ADRENERGIC BINDING FOLLOWING BENZODIAZEPINE TREATMENT IN BORDERLINE PERSONALITY DISORDER. R. Yehuda*[1], B.D. Perry[2], S.M. Southwick*[1], and E.L. Giller[1]. Psychiatry, WHVA-Yale, West Haven, CT 06516. [2]Harris Center, Chicago.

We investigated the effect of benzodiazepines (BZ) on platelet alpha$_2$ adrenergic receptor regulation in patients with both Major Depression and Borderline Personality Disorder (n=17), and in normal controls (n=17). Extended saturation radioligand binding assays (0.1-35nM ^{3}H-rauwolscine) and epinephrine competition studies assessed both high and low affinity states of the receptor. The number of alpha$_2$ adrenergic receptor sites in platelets from nonmedicated patients (n=10) was significantly reduced compared to controls, owing to a decreased number of high affinity ("coupled") sites. BZ-treated patients were comparable to controls on this measure. Both patient groups showed a higher degree of receptor "uncoupling" compared with controls. Clinically, BZ-treated patients reported significantly less anxiety than nontreated patients, but Hamilton Depression Scores were similar in both groups. Platelets obtained from a subgroup of patients (n=5) before and during BZ administration revealed that the number of alpha$_2$ adrenergic receptor sites doubled following this treatment to a level comparable to controls. In contrast, repeat determinations in control platelets (n=5) revealed little variation in receptor number over time.

166.7

REGULATION OF PINEALOCYTE INTRACELLULAR pH BY α_1-ADRENERGIC RECEPTOR. A.K. Ho*, C.L. Chik*, and D.C. Klein* (SPON: A.S. Basile). Section on Neuroendocrinology, Lab. of Developmental Neurobiology, and Developmental Endocrinology Branch, NICHD, NIH, Bethesda, MD 20892.

The purpose of the present study was to investigate the regulation of intracellular pH (pHi) in pinealocytes. The fluorescent pH indicator 2'7'-bis(carboxyethyl)-5(6)carboxyfluorescein (BCECF) was used. With an extracellular pH of 7.2, the pHi was 7.09. Treatment of pinealocytes with the physiological regulator of pineal function, norepinephrine (NE) resulted in a dose-dependent increase in pHi. NE (1μM) or phenylephrine (PE) (10μM) treatment increased pHi by about 0.07; isoproterenol (10μM) had no effect. The response of NE (1μM) was almost completely blocked by prazosin (1μM), but only slightly inhibited by propranolol (1μM), indicating that NE is acting via an α_1-adrenoceptor to influence pHi. In rat pinealocytes, α_1-adrenergic receptor activation translocates calcium-phospholipid dependent protein kinase (PKC). One possible consequence of PKC translocation is activation of the Na^+/H^+ antiport resulting in an increase in pHi. This was examined using activators of protein kinase C. Phorbol esters which directly activate PKC increased pHi by 0.06. An inactive analog had no effect. K^+, which activates PKC in the pinealocyte, increased pHi in a dose-dependent manner. An inhibitor of the Na^+/H^+ antiport, amiloride (50 μM), reduced pHi by 0.08 suggesting that the Na^+/H^+ antiport is involved in maintaining pHi in the pinealocyte. These findings indicate that α_1-adrenoceptor activation may increase pHi through PKC-dependent activation of the Na^+/H^+ antiport.

166.8

REGULATION OF CHICK PINEAL N-ACETYLTRANSFERASE ACTIVITY BY LIGHT AND NOREPINEPHRINE: INVOLVEMENT OF ALPHA-2 ADRENERGIC RECEPTORS. J.A. Creighton*, P.K. Rudeen and D.B. Bylund. Depts. of Anatomy and Pharmacology, University of Missouri School of Medicine, Columbia, MO 65212.

Cultured chick pineal glands show a diurnal oscillation in the activity of serotonin N-acetyltransferase activity (NAT). Light exposure of the gland leads to a rapid decline in activity. Norepinephrine (NE) treatment also inhibits NAT activity, indicating an adrenergic inhibition which is mediated through alpha-2 adrenergic receptors.

We demonstrate that light and NE inhibition of NAT is preceded by a transient increase in activity, evident at 5 min after light exposure or NE administration. The effects of NE are biphasic and dose-dependent. Stimulation of NAT by NE occurs at a concentration between 1-3uM with inhibition of enzyme activity occurring within 30 min. Propranolol given immediately prior to NE did not block the NAT increase by 3uM NE. Treatment of chick pineal glands with UK 14,304, an alpha-2 agonist, resulted in a similar stimulation at a dose of 0.1-1.0uM. These data further demonstrate that chick pineal melatonin biosynthesis is regulated by alpha-2 adrenergic receptors. The unanticipated transient stimulation of NAT by light and NE indicate a complex mechanism whereby these agents regulate NAT activity.

166.9

EFFECT OF LONG-TERM DESIPRAMINE ON α_2-ADRENOCEPTORS AND NOREPINEPHRINE UPTAKE REGULATION: AN ELECTROPHYSIOLOGICAL STUDY IN THE RAT HIPPOCAMPUS. D. Lacroix, O. Curet and C. de Montigny. Dept. of Psychiatry, McGill University, Montreal, Quebec, Canada H3A 1A1.

Sprague-Dawley rats were treated for 2 weeks with desipramine (DMI) (5 or 10 mg/kg, i.p.). Experiments were carried out 24 h after the last dose.

The responsiveness of CA$_3$ dorsal hippocampus pyramidal neurons to microiontophoretic application of norepinephrine (NE) was unchanged, indicating that the sensitivity of postsynaptic α_2-adrenoceptors was not modified.

The effectiveness of the electrical stimulation of the locus coeruleus in suppressing firing activity of dorsal hippocampus pyramidal neurons was not modified. However, clonidine (2 µg/kg, i.v.) reduced to a much smaller extent the effectiveness of the locus stimulation in DMI rats than in controls. These results indicate that long-term DMI treatment reduces the function of terminal α_2-autoreceptors.

The time required for pyramidal neurons to recover by 50% (RT_{50}) from microiontophoretic application of NE was similar in DMI rats and controls. However, the acute administration of high doses of DMI (6 and 8 mg/kg, i.v.) produced a smaller increase of RT_{50} values in DMI rats then in controls. This suggests that long-term DMI treatment reduces the number of [^{3}H]DMI sites without affecting the number of NE transport sites.

166.10

MEDIATING MECHANISM FOR THE ANESTHETIC ACTION OF ALPHA-2 ADRENERGIC AGONISTS. M.Maze* V.A.Doze*, B-X. Chen*, Z. Li* (SPON: D.L.Tanelian) Department of Anesthesia, Stanford University, Stanford, CA 94305

This study examined central alpha$_2$(A2) adrenergic receptor mediated loss of righting (LOR) reflex in rats. Neurotoxins specific for the functional components of the A2 adrenoceptor-effector mechanism, were used to determine if: i) a postsynaptic site, ii) a G_i/G_o protein, and iii)a K^+ conductance are involved in A2 receptor mediated hypnosis. Dexmedetomidine (DEX), a highly-selective A2 agonist, dose-dependently increased the duration of LOR reflex or sleep-time(ST). Centrally-active (idazoxan, MPV-1248) but not peripherally active (DG-5128) A2 antagonists blocked the hypnotic action of DEX. After depletion of monoamine stores with alpha-methyl-para-tyrosine and reserpine, the [DEX]-ST response curve was shifted to the left. Pertussis toxin (PT) pretreatment completely attenuated the hypnotic action of DEX. In both naive and monamine-depleted rats, 4-aminopyridine (4-AP) dose-dependently reduced DEX-induced ST while the peripherally acting 3,4-DAP was without effect. These data suggest that DEX-induced hypnosis is mediated *via*: i) a postsynaptic as well as a presynaptic A2 adrenoceptor ii) a PT-sensitive G protein; and iii) a 4-AP sensitive effector mechanism. Thus, activation of central A2 adrenergic receptors produces anesthesia in rats. This represents the first definitive example of a specific receptor-mediated anesthetic action.

166.11

RECOVERY OF POSTSYNAPTIC SENSITIVITY TO METHOXAMINE AFTER SPINAL CORD TRANSECTION IN THE CAT. S.L. Stoddard, L.K. Harstad*, S.W. Carmichael and T.L. Yaksh*. Dept. of Anatomy, Indiana Univ. Sch. of Med., Fort Wayne, IN 46805 and Depts. of Anatomy and Neurosurgery, Mayo Clinic, Rochester, MN 55905.

We previously reported that the adrenal medulla becomes hyperreactive to visceral and somatic stimuli over time following high spinal transection in the cat (Soc. Neurosci. Abstr. 13:271, 1987). Such increased reactivity might be related to generalized postsynaptic hypersensitivity, as occurs following denervation. To test this we determined the changes in mean arterial blood pressure (MAP) following iv administration of methoxamine, an alpha-adrenergic agonist, in non-transected cats (control; N=4) and as a function of time following surgical transection of the spinal cord at T3. Cats were tested acutely (4 hr post-transection, N=6), and at 4-5 days (N=5), 14-19 days (N=7) and 61-69 days (N=6) with methoxamine at 5, 10, 20, 40 and 80 ug/kg. The sensitivity to methoxamine (slope of the regression line of the log of methoxamine dose vs. change MAP) was significantly decreased from control values in both the 4-5 and 14-19 day groups. Sensitivity to methoxamine returned between 19 and 61 days; the response at 61-69 days was significantly greater than that seen at 14-19 days, and was not different from control values. Since the decreases in alpha-adrenergic receptor activity return to non-transected levels by 9 wk, these data do not support the concept that adrenal medullary hyperreactivity is due to a generalized denervation hypersensitivity occurring after spinal cord transection.

166.12

MEDETOMIDINE AND LOCUS CERULEUS NEURON FIRING. A. L. Curtis and J. Marwah. Wayne State University, Detroit, MI 48202.

In the current study, the effects of medetomidine (ME), d-medetomidine (d-ME), l-medetomidine (l-ME) and clonidine (CLD) on locus ceruleus (LC) neuron firing were compared in urethane anesthetized rats. Single, spontaneously firing noradrenergic LC neurons were invariably inhibited by the systemic administration of ME; d-ME and CLD. L-ME had no effect on neuron firing at doses up to 1 mg/kg, i.v. The inhibitory effects of ME; d-ME and CLD on LC neuron firing were antagonized by the alpha$_2$ adrenoceptor antagonist yohimbine. The rank order for inhibition was d-ME > ME > CLD. It is concluded that medetomidine inhibits noradrenergic neurons in the LC by specifically interacting with alpha$_2$ adenoceptors.
(Supported in part by a grant from NIDA DA 04158 and the Farmos Group.)

166.13

THE PUTATIVE ANTIPSYCHOTIC AGENT AMPEROZIDE PRODUCES BEHAVIORAL STIMULATION IN RATS. K. Svensson* N. Waters* G. Pettersson*[1] and L. Löfberg* (SPON: J. Engel). Dept of Pharmacol., Univ. of Göteborg, Sweden. [1] Dept. of Pharmacol., Ferrosan AB, Malmö, Sweden.

Amperozide (which is currently being evaluated as an antipsychotic in humans) has been shown to possess strong antiaggressive properties in various species (e.g. rats and pigs). The compound also antagonizes d-amphetamine-induced hyperlocomotion (at about 1 mg/kg), but not stereotypies, in mice. Furthermore, amperozide fails to induce catalepsy in rodents. This suggests that amperozide has a limbic profile of action (A. Björk et al., Proceedings VIIIth Internat. Med. Chem. Meeting, Uppsala, Sweden, 1984). In vitro binding studies indicate a high affinity for brain 5-HT2 receptors (Ki = 16 nM) whereas the affinity for central D2 and α1 receptors is lower (Ki = 540 and 172 nM, respectively).
Recently we found that amperozide dose-dependently (5-80 mg/kg) reverses reserpine (5 mg/kg,18h)-induced hypokinesia. The behavioral stimulation consisted of periodic locomotion (backward and forward locomotion), weak sniffing, and stereotyped movements with the forelegs. This behavioral syndrome was not blocked by high doses of raclopride (D2 antagonist), SCH 23390 (D1 antagonist), propranolol (β-receptor blocker), idazoxan (α2-receptor antagonist) or ritanserin (5-HT2 antagonist). However, pretreatment with the catecholamine synthesis inhibitor α-MT (250 mg/kg, 1 h) or the dopamine-β-hydroxylase inhibitor FLA 63 (40 mg/kg, 2.5 h) partially blocked the amperozide-induced behavioral stimulation. These results suggest that amperozide produces behavioral stimulation via an indirect effect on central α-receptors. We recently found that amperozide can induce a similar behavioral syndrome in habituated, non-pretreated rats. Behavioral and biochemical data will be presented and discussed in relation to the clinical effects of amperozide.

BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT I

167.1

NMDA CURRENT IN HIPPOCAMPAL SLICES FROM NEWBORN RATS. Ben-Ari, Y., Cherubini, E. and Krnjević, K., INSERM U-29, 123 Blvd. Port-Royal 75014 , Paris, France. (SPON:G. Mandl)

NMDA (5-10 μM)-evoked inward currents (I_{NMDA}) were compared in single-electrode voltage-clamped CA3 neurons in slices from adult Wistar rats (>120 g) and from pups at post-natal days 1-8. In most cases, recording was with 3M CsCl microelectrodes and fast Na currents were routinely blocked with TTX. Similar peak I_{NMDA}'s were evoked at various stages of development. In mature neurons, I_{NMDA} consistently showed the characteristic negative slope in the region between -60 and -30 mV (-12.4 ± 2.35 pA/mV, n=13) and had a reversal potential at -21.1 ± 3.29 mV (n=5). In immature neurons (1-8 days), the voltage-dependence was much more variable, giving a *positive* slope in the -60 to -30 mV region in 6 cells out of 17 and an overall mean slope of -0.89 ± 3.17 pA/mV (for n=17); moreover, the reversal potential was at a less negative level, -8.3 ± 2.08 mV (n=11). In addition, NMDA tended to trigger sharp, periodic inward currents - superimposed on the slow I_{NMDA} - much more frequently in immature neurons. These observations suggest that endogenous NMDA-agonists may activate Ca^{2+} influx particularly readily in the young hippocampus, perhaps thus facilitating maturational and other "plastic" changes.

167.2

CHANGES IN MESSENGER RNAs CODING FOR NEUROTRANSMITTER RECEPTORS IN THE DEVELOPING RAT CEREBRAL CORTEX. M.K. Carpenter*, I. Parker*, and R. Miledi (SPON: K.Sumikawa). Laboratory of Cellular and Molecular Neurobiology, Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

The developmental changes in the expression of mRNAs coding for several neurotransmitter receptors (kainate, glutamate, ACh, and serotonin) were examined by injecting mRNA isolated from the cerebral cortex into Xenopus oocytes. The oocytes translate the foreign mRNA and incorporate functional receptor/ion channel complexes into the cell membrane (Gundersen, C., Miledi, R., and Parker, I. Nature, 308:421, 1984). Thus, recordings of agonist-evoked membrane currents in voltage-clamped oocytes give a measure of the relative amounts of different messengers. In these experiments poly (A)$^+$ mRNA was isolated from rats of different embryonic (E) and postnatal (P) ages, and 50 ng was injected into each oocyte. Responses induced by kainate (10^{-4} M), glutamate (10^{-3} M), ACh (10^{-4} M), and serotonin (10^{-5} M) all increased with age reaching a maximum in oocytes injected with mRNA from adult cortex. The earliest ages examined, E15 and E18, gave little or no response to kainate, glutamate, or ACh. Responses to these agonists increased with age, reaching 50-70% of the adult response by P10. In contrast, the serotonin-induced response was relatively large (16% of adult) in oocytes injected with E15 mRNA and increased postnatally to adult levels.

167.3

Flow cytometric analysis of membrane potential responses in the embryonic chick spinal cord. A. Prasad*, E.A. Novotny*, G.D. Lange and J.L. Barker. Laboratory of Neurophysiology; NINCDS, NIH; Bethesda MD 20892.

The development of receptor and ion channel responses in the embryonic chick spinal cord was examined using a combination of flow cytometry and a fluorescent voltage-sensitive dye. This approach has been successfully used in developmental studies of the mammalian nervous system (Mandler et al., (1988) J. Neurosci. Methods; 22:203). Results from the current studies indicate that these techniques are also applicable to the developing avian nervous system.

Acutely dissociated spinal cord cells from chick embryos ranging in age from E5 - E9 (St. 26 - St. 35) were incubated in the fluorescent, voltage-sensitive dye oxonol. It was found that muscimol (a $GABA_A$ agonist) and GABA depolarized (increased oxonol staining) spinal cord cells in a dose-dependent manner. These effects were blocked by the $GABA_A$ antagonist bicuculline and were evident as early as E5 (St. 26, 27) in a subpopulation of spinal cord cells. However, glycine, another inhibitory neurotransmitter, had no apparent effect at the ages examined. The sodium channel blocker TTX effectively blocked the massive depolarization caused by veratridine. Again, these responses were evident at E5. Experiments designed to examine the onset of GABA receptor modulatory responses and the development of other amino acid responses in the embryonic chick spinal cord are currently in progress.

167.4

RETINAL CULTURES AS A MODEL FOR EXCITATORY AMINO ACID RECEPTOR DIFFERENTIATION. F. Somohano* and A.M. López-Colomé. Instituto de Fisiología Celular, U.N.A.M., 04510 México, D.F., México.

In vitro differentiation in primary cultures has been used to study the mechanisms regulating the characteristics of membrane proteins; among these, uptake- and synaptic-receptors for neurotransmitter substances. During synaptogenesis, the establishment of adequate synaptic contacts depends on feedback mechanisms mediated, at least in part, through neurotransmitter receptors with certain characteristics at a particular moment. We have studied the maturation of receptors to excitatory amino acids (EAAR) in primary cultures of neurons from chick retina. Assays were performed at 1,5,8, and 12 days in vitro (DIV). Uptake receptors were assayed through the incorporation of ^{3}H-D-Asp; synaptic receptors were measured by binding of ^{3}H-AMPA, ^{3}H-Glu, ^{3}H-Asp or ^{3}H-KA to frozen membranes or whole cells. Na^+ and temperature-dependent uptake of ^{3}H-D-Asp showed a single saturable system at all ages studied ($Km \simeq 10$ uM); B_{max} increased 10 times from DIV 1 to 8. Synaptic EAAR were enriched in cultures versus membranes from embryonic retina, indicating preferential localization in neurons. EAAR subtypes vary in time of expression: ^{3}H-Glu binding increases between 5 and 8 DIV, and then decreases; ^{3}H-AMPA binding remains constant; ^{3}H-KA binding could not be detected at any stage; ^{3}H-Asp binding increases at day 8 in vitro. Data suggest a trophic role for EAAR during differentiation.

167.5

DEVELOPMENTAL REGULATION OF NEUROTRANSMITTER RECEPTORS ON CHICK PREGANGLIONIC NEURONS. B. Clendening and R.I.Hume. Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109

We have studied the expression of neurotransmitter receptors on chick sympathetic preganglionic neurons (SPN) in dissociated cell culture. The sensitivity of SPN to GABA, glycine and glutamate was measured by making whole cell voltage clamp recordings. The time of appearance of sensitivity to these three substances differed. At 24 hr after plating, more than 90% of the SPN gave detectable responses to 100 uM GABA, and most of these responses were easily detectable (> 50 pA with a 50 mV driving force). In contrast, less than 40% of SPN gave detectable responses to 100 uM glycine or glutamate at 24 hours, and when currents were evoked they were quite small (< 20 pA with a 50 mV driving force). Over the first 7 days *in vitro* there was a gradual threefold rise in the amplitude of GABA evoked currents. In contrast, glycine and glutamate evoked currents did not begin to increase until day three, and the relative increase in current at day 7 was more dramatic. Mean glycine sensitivity and mean glutamate sensitivity were both increased by more than tenfold over their values at 24 hours.

It is unlikely that the low level of responsiveness to glycine and glutamate at early times was due to the selective removal of receptors for these transmitters by enzyme treatment during dissociation. Treatment of 5-7 day cultures with enzyme did not reduce the sensitivity to either transmitter. It also seemed possible that receptors to these two transmitters might be down-regulated or desensitized by glutamate and glycine in the culture medium. However, we found no difference in the amplitude of the peak currents evoked by glutamate and glycine, or in the time of onset of sensitivity, when these amino acids were absent from the medium.

These data are consistent with the idea that independent mechanisms regulate the expression of multiple neurotransmitter receptors.

167.6

HETEROGENEITY OF TARGET SPECIFIC POPULATIONS OF SYMPATHETIC NEURONS IN THE RAT SUPERIOR CERVICAL SYMPATHETIC GANGLION. A.J. Smolen Dept. of Anatomy, The Medical College of Pa., Philadelphia, PA 19129.

We have begun to investigate morphological and biochemical differences among sympathetic neurons in the superior cervical sympathetic ganglion (SCG) of the rat. Two target specific populations of neurons were studied: one that projects to the submandibular gland (SMG), and the other that innervates the iris.

The perikarya of sympathetic neurons that project to the SMG are significantly larger than those that project to the iris, and their dendritic trees are more highly elaborated. In addition the rate of turnover of the neurotransmitter, norepinephrine (NE), is much greater in the SMG than in the iris.

These two groups of neurons also differ in their responses to altered synaptic input. Neonatally denervated iris neurons do not release significant amounts of NE at their terminals, while release of NE is normal from terminals of denervated SMG neurons. Denervation results in a decrease in the size of the neuron soma in both groups, but dendritic maturation is unaffected. Neonatally hyperinnervated SMG neurons respond by an enhanced release of NE from their terminals, and a significant increase in both perikaryal size and dendritic arborization.

Studies are in progress to relate these morphological and biochemical variations to differences in the level of activity of these two populations of neurons.

(Supported by NIH Grants NS15952 and NS21822)

167.7

THE EFFECTS OF $MgCl_2$ INFUSION ON THE PERIODICITIES IN PHRENIC (PHR) AND SYMPATHETIC (SYMP) ACTIVITY OF NEONATAL SWINE. P.M. Gootman, H.L. Cohen, B.W. Hundley*, M. Brust*, L.P. Eberle*, G. Condemi*, B.T. Altura,* B.M. Altura*. Dept. Physiol., SUNY-Hlth Sci. Ctr. Bklyn, Bklyn,NY 11203

The effects of changes in plasma concentration of magnesium on efferent PHR, cervical sympathetic (CS) and splanchnic (SPL) activity were examined in piglets <1 day to 5 weeks of age, lightly anesthetized with Saffan, paralyzed, tracheotomized and artificially ventilated on 100% O_2; pO_2,pCO_2 and pH were monitored. Infusion of $MgCl_2$ for 15 min at rates ranging (depending upon age) from 0.191 to 0.76 mmoles/min increased arterial plasma levels of Mg from 1.82 mg/dl to 4.61 mg/dl. Neuronal activity was analyzed by averaging, correlation and power spectral techniques. Spectral densities of SYMP activity demonstrated peaks in the range of 5-30 Hz; locking to both the cardiac and respiratory cycle was present. Following $MgCl_2$ infusion, there was a significant increase in the respiratory modulation of SYMP activity; CS power appeared to increase in > 1 month old piglets. While there was no systematic change in PHR high frequency oscillations, there were age-related changes in inspiratory and expiratory durations. The results suggest that changes in serum Mg can alter both the sympathetic rhythm generating systems and phase switching in the respiratory rhythm generator in the brainstem of neonatal swine. (Supported by NIH grant HL-20864.)

167.8

ALTERED BRANCHING OF 5HT-NEURONS IN *Drosophila* MUTANTS UNABLE TO SYNTHESIZE SEROTONIN AND DOPAMINE. V. Budnik, C.-F. Wu, and K. White. Biophysics program, Dept. of Biology, Brandeis University, Waltham, MA 02254, and Dept. of Biology, University of Iowa, Iowa City, IA 52242.

The neurotransmitters serotonin (5HT) and dopamine (DA) have been implicated in the regulation of developmental processes in a number of animals. In the fruit fly *Drosophila melanogaster*, genetic manipulation of the gene *dopa decarboxylase* (*Ddc*), allows the generation of flies which are completely deficient in 5HT and DA (*DfDdc*). Previous studies demonstrated that in the absence of serotonin and dopamine, 5HT-neurons in the CNS appear to develop normally, and express normal selective 5HT uptake properties. In this study, we have examined the pattern of branching of 5HT-neurons in a peripheral target, the proventriculus and midgut of *Drosophila* larvae, by immunofluorescent techniques. Developmental studies showed that most of these peripheral 5HT-projections are present, and contain 5HT before the larvae hatch. We found that *DfDdc* larvae show a dramatic increase in the number of ramifications of 5HT-fibers in the proventriculus and midgut, as revealed by 5HT uptake experiments. Mutant flies that have low, but detectable levels of 5HT and DA throughout embryogenesis (Ddc^{ts2}, 30°C) did not show this mutant phenotype. Feeding *DfDdc* larvae with 5HT-containing medium resulted in the incorporation of 5HT into appropriate neurons and fibers in the nervous system. However, this postembryonic increase in the levels of 5HT did not rescue the aberrant branching in the mutant. The nature of this phenomenon is being further analyzed by additional genetic and physiological manipulations.

167.9

THE APPEARANCE OF SEROTONIN AND PROCTOLIN DURING DEVELOPMENT IN THE LOBSTER NERVOUS SYSTEM. B. S. Beltz, Department of Biological Sciences, Wellesley College, Wellesley, MA 02181, & M. C. Pontes*, and E. A. Kravitz, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115. The form and behavior of lobsters changes dramatically in the transition from the 1st-4th larval stages (L1-L4). Serotonin (5-HT) and proctolin, neurohormones widely distributed in the lobster nervous system, have been implicated in various behaviors. Using immunocytochemical and biochemical methods, we have screened for the appearance of these substances during development. In mid-stage embryos, the adult complement of 5-HT-staining cells is already present. Embryonic serotonin cells are proportionally very large and more well developed than other neurons, suggesting possible developmental roles. In one case, an unpaired 5-HT-containing cell is very prominent in embryos, L1, and L2, but stains weakly in juveniles. The development of proctolinergic neurons is very different. Only 2% of the proctolin-staining neurons of juvenile animals are seen in mid-stage embryos. The number of immunoreactive cells gradually increases, but even by L6 only half the number of cells that will eventually stain for proctolin are observed. 5-HT and proctolin coexist in large pairs of neurons in the adult A1 and T5 ganglia. These cells already contain 5-HT in mid-stage embryos. Proctolin appears in these cells only after L1. Thus in the same cells, proctolin and 5-HT first appear at very different times in development. Quantitative measurements for 5-HT in lobster larvae were performed by HPLC with dual electrochemical detection, and for proctolin were performed using RIA. A gradual growth-related increase in the amount of 5-HT was seen during larval development. This is consistent with the immunocytochemical data showing that no new 5-HT cells appear during this period. Proctolin levels during development are currently being quantified. (Supported by NIH and Klingenstein Foundation).

167.10

SEROTONERGIC PROPERTIES OF GROWTH CONES ISOLATED FROM FETAL RAT BRAIN. N. Ivgy-May*, H. Tamir, and M.D. Gershon, (SPON: D. Colman) Dept. Anat. and Cell Biol. Columbia Univ. P&S, New York, N.Y. 10032.

High affinity ^{3}H-imipramine binding sites are associated with the 5-HT transporter in neuronal plasma membranes. The ontogeny of ^{3}H-imipramine binding sites (displaceable by 1μM norzimelidine) in subcellular fractions derived from developing rat brain was studied. Analysis of saturation isotherms indicated that ^{3}H-imipramine bound to a single class of sites. Specific high affinity ^{3}H-imipramine binding ($K_D \cong 3.2 \pm 0.6$ nM [adult $K_D \cong 2.8 \pm 0.1$ nM]) was first detected at day E15 at which time the B_{max} was ~20% of the adult level (adult $B_{max} \cong 298 \pm 33$ fmol/mg). The B_{max} increased with age and reached ~70% of the adult value by day P9. Previous studies have shown that ^{3}H-imipramine binding sites in the adult rat brain are mainly located on the membranes of serotonergic axon terminals. Our observation that high affinity ^{3}H-imipramine binding sites are present at day E15, before serotonergic synapses have begun to form, implies that the binding sites may be present on the growth cones of serotonergic axons. To test this possibility, isolated growth cones (IGC) were prepared from developing rat brain homogenates. High affinity ^{3}H-imipramine binding sites were significantly ($p < 0.05$) enriched in the IGC fraction, 4.5-fold, at day E15, and 1.4-fold at day E 20. No enrichment was found in the IGC fraction at later ages, when ^{3}H-imipramine binding was found in synaptosomes. Specific ^{3}H-imipramine binding sites were radioautographically detected in the cerebral cortex of fetal rats at day E20. At this age, serotonergic axons are found in the cortex, but they have not yet formed synapses; therefore, since serotonergic growth cones are present, while synapses are not, the binding of ^{3}H-imipramine found in cortical radioautographs is probably to serotonergic growth cones. Fractions containing intact IGC had detectable levels of endogenous 5-HT as early as day E15. These studies suggest that the growth cones of serotonergic axons have at least some of the properties of mature serotonergic axon terminals. Supported by NIH grants NS 12969, NS 07062 and NIMH 37575

167.11

POSTNATAL EFFECTS OF PRENATAL ADMINISTRATION OF NEUROPEPTIDES ON ^{3}H-5HT UPTAKE BY SEROTONERGIC NEURONS. Davila-Garcia M. I., Hlibczuk V., Akbari H., Alves, S., and Azmitia E.C. Washington Square Center for Neuroscience, New York University, NY. NY. 10003.

Based on tissue culture experiments, neuropeptides have been shown to affect serotonergic neurons during development as endogenous regulatory factors. In this study we examined the postnatal effects of prenatally administered neuropeptides on high affinity ^{3}H-5HT uptake by brainstem, hippocampus and spinal cord.

Pregnant rats were injected daily from 7 to 21 days of gestation (7-21DG) with the ACTH 4-9 analog Organon 2766 (10ug/kg), leu-enkephalin (1ug/kg), or saline (9% NaCl). A group of untreated pregnant rats was included as control. At 26 (DPN) the pups were weighed, sacrificed, and the brainstem, hippocampus and spinal cord were dissected. A synaptosomal preparation was obtained to measure 3H-5HT uptake by serotonergic neurons during a 20 min incubation.

The Organon 2766 group showed a significant increase in ^{3}H-5HT uptake in brainstem and hippocampus suggesting the ascending serotonergic system is affected by prenatal administration of this peptide. Leu-enkephalin, however, showed an increase in ^{3}H-5HT uptake in spinal cord suggesting this peptide affects the descending serotonergic system.

This research was supported by NSF BNS-8607796

167.12

POST-NATAL DEVELOPMENT OF SUPRAEPENDYMAL 5-HT FIBERS: IMPLICATIONS FOR 5-HT1c RECEPTOR ONTOGENY. J. Giordano*[1], G.A. Barr[2] and P. Hartig[1]; (SPON: R. Thompson) (1) Environ. Hlth. Sci., The Johns Hopkins Med. Inst., Baltimore, MD. and (2) Biopsychology Program, Hunter College, CUNY, New York, NY.

Supraependymal (SE) 5-HT fibers are the probable source of CSF-borne 5-HT that activates and regulates the 5-HT1c receptor in the choroid plexus. Using antibodies to serotonin, this study examined whether the normal postnatal maturation of these projections correlates with the previously reported pattern of 5-HT1c receptor ontogeny (Zilles et al., Brain Res. 380, 1986). Serotonin immunohistochemistry was accomplished using Sternberger PAP method on coronal and horizontal brain section of rat pups aged 0 to 21 days and of adults. Serotonin-like immunoreactivity was discernable at birth; individual varicose fibers were sparsely arranged along the SE surface. In 3-5 day olds, staining was somewhat more intense than in the newborn and fibers were more aggregated, notably on the dorsomedial ventricular walls. By 8 days postnatal, dense staining was observed and fibers formed networks along the SE surface. This pattern of staining persisted in the 14 and 21 day olds as well as the adult. We were unable to detect 5-HT hyperinnervation at any age. These results indicate that maturation of SE fibers parallels the development of 5-HT1c receptors from days 1-21.

167.13

CHANGING LEVELS OF CSF 5-HT PARALLEL DEVELOPMENT OF SEROTONIN 5-HT1c RECEPTORS IN CHOROID PLEXUS. C. Eastman*, J. Giordano*, T. Guilarte* and P. Hartig. (SPON: Z. Annau) Dept. of Environ. Hlth. Sci., The Johns Hopkins Medical Institutions. Baltimore, MD.

Levels of serotonin 5-HT1c receptor in choroid plexus undergo rapid down regulation immediately after birth; followed by a linear increase in sites to adult levels by 2 weeks postnatal (Zilles et al., Brain Res. 380:1986). CSF-borne 5-HT from supraependymal (SE) fibers appears to activate and regulate this receptor. The present study examined CSF indole levels in the developing rat using HPLC-EC.

CSF 5-HT levels were high at birth, followed by a precipitous drop at day 1 and increased from day 3 to 14. A significant rise in CSF 5-HT levels was measured between 14-21 days, at which point levels were comparable to the adult. 5-HIAA levels followed a similar ontogenetic pattern. Taken together with the pattern of SE fiber maturation (Giordano et al., this volume), these findings suggest that 1) a prenatal 5-HT surge may subserve rapid changes in 5-HT1c receptor levels immediately following birth, and 2) the gradual postnatal development of SE fibers parallels the developmental expression of 5-HT1c receptors from days 1-21 postnatally.

167.14

AMPHETAMINE-INDUCED ALTERATIONS IN DOPAMINE RELEASE AND METABOLISM IN THE NEOSTRIATUM OF DEVELOPING AND ADULT RATS. R.A. Gazzara, X.Y. Xuan* and S.G. Howard. MRRC, BRI and Dept. of Pharmacology, UCLA, Los Angeles, CA 90024.

Amphetamine (AMPH) has been shown to produce several effects on the dopaminergic neurotransmitter system in the CNS, including: facilitation of dopamine (DA) release; inhibition of DA re-uptake; and the inhibition of MAO.

The study reported here analyzed the effect of AMPH on the extracellular levels of DA and the DA metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the neostriatum of urethane-anesthetized adult rats, and 21-22-day-old and 35-36-day-old rat pups, using in-vivo microdialysis. Samples were collected every 10 min and levels of DA, DOPAC and HVA were measured by HPLC-EC. Rats were injected s.c. with 0.1, 1.0 or 5.0 mg/kg d-AMPH, or saline vehicle. In the adult group, AMPH produced an increase in DA release, in a dose-dependent manner, that peaked at 20-30 min postinjection. Conversely, AMPH produced a decrease in levels of DOPAC and HVA also in a dose-dependent manner. Minimum levels were reached at various times postinjection depending on dose. Preliminary data from the 21-22 day and 35-36 day groups suggest that the effect of AMPH on DA release is generally similar to that of the adult group. However, levels of DOPAC and HVA were generally higher in the 21-22 day and 35-36 day groups especially at the lower doses. These data suggest that AMPH may have a lesser effect on DA re-uptake in immature animals. Supported by USPHS HD 22548.

167.15

SIGNIFICANCE OF THE DIFFERENCE IN TIME OF APPEARANCE DURING DEVELOPMENT OF THE A AND B FORMS OF MONOAMINE OXIDASE IN THE RAT STRIATUM. G.S. Carter and A.J. Azzaro. Departments of Neurology and Pharmacology/Toxicology and Psychiatry West Virginia University Health Science Center, Morgantown, WV 26506.

The development of the MAO subtypes in the striatum of Sprague-Dawley rats was studied from birth to maturity. The development of the glial cell enzyme, glutamine synthetase, the monoaminergic neuron enzymes, tyrosine hydroxylase and tryptophan hydroxylase, and the intrinsic striatal neuron marker enzymes, choline acetyltransferase and glutamic acid decarboxylase were also studied and correlated with the MAO subtypes. Rats were lesioned at birth with the selective neurotoxins 6-hydroxydopamine or 5 7-dihydroxytryptamine and the development of the MAO subtypes studied. The postnatal studies showed that MAO type A activity preceded MAO type B activity in early development. The early development of both subtypes, however, was highly correlated with astrocytic development as demonstrated by glutamine synthetase activity. Neonatal lesioning of the monaminergic afferent neuronal terminals caused no decrement of the activity of either MAO subtype at the peak of astrocytic enzyme activity on the 15th postnatal day. It would thus appear that the difference in development of the two subtypes of MAO represents a phenomena of astroglial mitochondrial development in which MAO type B is a later addition to the mitochondrial membrane.

167.16

ISOLATED HYPOTHALAMIC NEURONS IN CULTURE RAPIDLY DISPLAY IMMUNOCYTOCHEMICALLY-DETECTABLE TYROSINE HYDROXYLASE AND VASOPRESSIN. C.A. Dudley, R.L. Moss, & P.W. Coates. Dept. Physiol., Univ. Tex. SW Med. Ctr., Dallas, TX 75235 & Cell Bio. & Anat., TTUHSC Sch Med., Lubbock, TX 79410.

Hypothalamic neurons cultured on three-dimensional (3-D) matrices rapidly express characteristic neuronal morphologies as single cells (Coates, SN Abs.12:1504,'86). To determine if such individual neurons contain vasopressin (VP) or tyrosine hydroxylase (TH), a series of immunocytochemical studies was performed. Hypothalamic neurons from late gestation fetal rats were plated onto 3-D matrices at low cell densities to assure wide separation of cells. After 1, 2 or 3 days, cultures were fixed with aldehydes in phosphate or cacodylate buffer. A modified version of Sternberger's peroxidase anti-peroxidase technique (Immunocytochemistry, Prentice Hall, Inc.'74) was used to detect immunostaining for antibodies (Ab) to VP and TH. When examined with light microscopy, control cultures (no exposure to primary Ab) contained no accumulation of reaction product. In neurons exposed to Ab, some positive staining was observed as early as 1 day after plating. In these and later cultures, two basic staining patterns were observed: 1) Smooth brown reaction product evenly distributed throughout the cytoplasm of the soma, and 2) Punctate deposits of reaction product localized to one side of the cell body. Some neurons showed intensely stained beaded fibers characteristic of intact tissue. Results demonstrate that isolated hypothalamic neurons quickly display TH and VP immunoreactivity. The data suggest that hypothalamic neurons, not in contact with cells of any other kind, are inherently capable of rapid functional as well as morphological differentiation. Supported by HD09988-V and HD22806.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY I

168.1

GLUTAMATE BLOCKS KAINATE NEUROTOXICITY IN THE CHICK EMBRYO RETINA. J.L. Mosinger, M. T. Price, J. Labruyere, M. E. Mueller, E. Silverman and J.W. Olney. Washington University Sch. of Med., St. Louis, MO 63110.

N-methyl aspartate (N), quisqualate (Q) and kainate (K) are excitatory amino acid (EAA) analogs of glutamate (G) which mimic the excitotoxic action of G in the chick embryo retina. We postulate that each of these three analogs acts at a different subset of EAA receptors since certain agents which antagonize the neurotoxicity of N do not antagonize Q or K and the quinoxaline CNQX (FG 9065) antagonizes the neurotoxicity of K more potently than that of N or Q. In addition, although retinal neurons in the inner half of the inner nuclear layer (INL) are affected by all three agents (implying that such cells are endowed with all three receptor subtypes), somata in the position of horizontal cells are damaged only by Q and K while others in the outer half of the INL are damaged only by K. The cells that are damaged only by K are of particular interest in that the toxic action of K on these cells is blocked by G or Q.

At least two possible models might explain the protective effect of G/Q against K toxicity, one based on 3 EAA receptor subtypes and the other based on 4. In either case, they can be designated as G receptor subtypes since G appears to interact at all sites. In the triadic model G-1 = the N receptor through which either N or G exerts excitotoxic action and various N receptor ligands selectively block N toxicity; G-2 = the Q receptor through which either Q or G exerts excitotoxic action and Q toxicity is blocked by CNQX (50 μM) but not by N receptor ligands; G-3 = the K receptor through which only K exerts excitotoxic action and this is blocked by CNQX at 15 μM or by G or Q at concentrations which are excitotoxic to other cells. The other model postulates the same three receptor subtypes but divides the G-3 receptor into two subsets (Ka and Kb) each of which is sensitive to K agonist action but only one of which (Kb) is sensitive to Q antagonism. Regardless which of these taxonomic models best describes the true situation, possible mechanisms which might explain the antagonist properties of G/Q at K sites include receptor blockade, agonist-induced ion channel block and/or the promotion of desensitization. Q is known to exert a very rapid desensitizing action in some systems; thus, if Q were to displace K as agonist at the G-3 site but not express agonist excitotoxic action because of rapid receptor desensitization, the net effect would be a blockade of K toxicity. However, since Q expresses excitotoxicity at G-2 sites, it apparently does not desensitize so rapidly at this receptor. Supported by RSA MH 38894 (JWO), HD 24237 and DA 05072.

168.2

ROLE OF THE N-METHYL ASPARTATE RECEPTOR IN DEVELOPMENTAL PSYCHONEUROPATHOLOGY. J. W. Olney, C. Ikonomidou*, J. Mosinger, K. Shahid Salles and G. Frierdich*, Washington University Sch. of Med., St. Louis, MO 63110.

Accumulating evidence suggests that the endogenous excitotoxins, glutamate (Glu) and aspartate, acting at excitatory amino acid (EAA) receptors may be responsible for hypoxic/ischemic brain damage. Presumably such damage is mediated either by N-methyl aspartate (NMA) or kainic acid (KA) receptors (or both) since they are the most abundant EAA receptor subtypes in brain. We have observed that hypobaric/ischemic (H/I) conditions cause a Glu-like neurodegenerative reaction in the 10 day old rat brain and that there is a period in early neonatal life when rat CNS neurons are hypersensitive to both H/I degeneration and the toxic action of NMA (but not KA). This suggests that NMA receptors may play a special role in perinatal H/I brain damage. In support of this hypothesis, we now report that systemic administration of MK-801, a potent NMA antagonist, protects the immature rat brain against H/I damage.

Following unilateral carotid ligation, 10 day old rats were subjected to hypobaric conditions for 75 min (vacuum chamber maintained at a pressure of 225 mm Hg and temperature 38° C). MK 801 (1 mg/kg sc) or saline was administered to experimental or control pups respectively 15 min prior to hypobaric exposure. In each experiment, 10 pups (5 experimental and 5 control) were placed in the chamber together so that each experienced the same conditions. After hypobaric exposure, the pups were observed for 2 hrs, during which body temperatures were maintained at 37 ± 0.5° C ; then they were sacrificed by perfusion fixation under halothane anesthesia for histopathological evaluation of the brains. Amount of brain damage was rated as 0, mild, moderate or severe based on the size of lesions and number of brain regions involved. Among 49 control pups, the majority qualified for a severe rating, whereas among 47 experimental pups, only 1 qualified for a severe rating and the majority had 0 brain damage.

Based on these and other recent findings, we suspect that the NMA receptor-ionophore complex may play an important role in developmental psychoneuropathology; specifically, we propose that it may be a characteristic of immature NMA receptors to be hypersensitive to excitatory (excitotoxic) stimulation, in which case CNS neurons bearing such receptors might be destroyed by relatively minor degrees of hypoxic/ischemic exposure. Conditions for which this concept could conceivably have relevance include cerebral palsy, schizophrenia and mental retardation. Supported by Research Scientist Award MH 38894, HD 24237, DA 05072 and ES 00875.

168.3

ZINC CENTRAL NEUROTOXICITY MAY REQUIRE OPEN NMDA CHANNELS. D.W. Choi and J. Koh. Stanford Univ. Med. Sch., Stanford, CA 94305.

Large amounts of Zn can be released from excitatory synaptic terminals with nerve stimulation, and may contribute to neurotoxic injury in certain disease states. We have previously reported that a 15 min exposure to 1 mM Zn is sufficient to produce widespread neuronal injury in murine cortical cell cultures. We report here that this Zn neurotoxicity can be largely blocked by both competitive (APV) or non-competitive (MK-801) NMDA antagonists. However, Zn neurotoxicity is probably not simply mediated through the toxicity of endogenous excitotoxins (such as glutamate): 1) removing extracellular Na and Ca blocks the neurotoxicity of NMDA or glutamate, but enhances Zn toxicity; 2) the antagonism of Zn neurotoxicity by MK-801, but not APV, can be overcome by increasing Zn concentration - a situation reversed from that seen in the antagonism of NMDA neurotoxicity; and 3) the concentration of MK-801 required to block Zn neurotoxicity (IC_{50} 5 - 10 μM) is higher than the concentration required to block NMDA neurotoxicity (IC_{50} 0.3 μM).

We speculate that Zn may require open NMDA channels to injure neurons. Zn may pass through this channel (perhaps interacting at or near the PCP binding site) to accumulate intracellularly and disrupt cellular processes, leading to neuronal damage.

168.4

BETA-N-METHYLAMINO-L-ALANINE (BMAA) NEUROTOXICITY ON MURINE CORTICAL NEURONS. J.H. Weiss, J. Koh and D.W. Choi Stanford Univ. Med. Sch., Stanford, CA 94305.

BMAA is a cycad plant excitotoxin recently implicated (Spencer et al., Science 237: 517) in the pathogenesis of Guam amyotrophic lateral sclerosis - Parkinsonism - dementia (ALS-PD). The basis for BMAA excitotoxicity is unclear, as it lacks the omega-acidic moiety characteristic of other excitatory amino acids. We have investigated the neurotoxicity of BMAA in murine cortical cell cultures, and report three observations:

1) BMAA, unique among excitotoxins we have tested (including BOAA), required the presence of physiological concentrations of bicarbonate to produce either neurotoxicity or neuroexcitation. We suggest bicarbonate interacts non-covalently with the positively charged beta amino group of BMAA to produce in combination a configuration appropriate for activation of glutamate receptors.

2) Even in the presence of bicarbonate, BMAA is a weak neurotoxin (toxic EC_{50} with 24 h exposure about 1 mM).

3) The widespread neuronal injury produced by intense BMAA exposure was largely blocked by APV, consistent with mediation by NMDA receptors. However, at the lower exposures likely relevant to ALS-PD, BMAA selectively damaged the small subpopulation of cortical neurons staining for NADPH-diaphorase, a finding suggesting predominant non-NMDA receptor-mediated toxicity.

168.5

EXCITOTOXICITY IN CHICK RETINA CAUSED BY THE UNUSUAL AMINO ACIDS BOAA AND BMAA:EFFECTS OF MK-801 AND DIDS. G. Zeevalk, S. Olynyk* and W. Nicklas. UMDNJ-Robert Wood Johnson Medical School, Dept. of Neurology, Piscataway, N.J. 08854

β-N-oxalylamino-L-alanine (BOAA) and β-N-methylamino-L-alanine (BMAA) have been shown to cause a glutamate (GLU)-like lesion in CNS. Since the retina is sensitive to GLU toxicity, we have examined the effects of these amino acids on retinal histology and amino acid release. Retina from E13 chicks was incubated with various amounts of agonists: BOAA (0.025-0.5mM), BMAA (0.75-3mM), NMDA (0.002-0.2mM), kainate (KA 0.002-0.2mM) with or without the NMDA antagonist, MK-801 (1μM) or the Cl^-/bicarbonate channel blocker, DIDS (0.6mM). All agonists produced an excitotoxic lesion. BOAA was only slightly less potent than KA; BMAA had a potency similar to GLU. BOAA, like KA, caused a large release of GABA and taurine. NMDA-induced release was similar to the other agonists except that serine efflux was also increased. MK-801 completely blocked the lesion caused by 0.05mM NMDA or 2mM BMAA, but was ineffective against KA (0.05mM) or BOAA (0.1mM). Consistent with histological findings, MK-801 prevented amino acid release caused by NMDA but not that caused by KA or BOAA. DIDS was effective in blocking all agonist induced lesions and in returning amino acids back to basal levels. These studies demonstrate that BOAA and BMAA produce retinal damage that is most likely mediated via an excitotoxic mechanism; BOAA appears to induce toxicity through a nonNMDA type GLU receptor and BMAA through the NMDA receptor.

168.6

PROTECTION OF HIPPOCAMPAL NEURONS FROM A PLANT NEUROTOXIN-INDUCED CELL DEATH BY ACIDIC AMINO ACID ANTAGONISTS IN VITRO. S. Rapp*, E. Sun* and D. Roufa (SPON :P. Manning) G. D. Searle/CNSDR, St Louis, MO 63198.

A neurotoxic plant "uncommon" amino acid β-N-methyl amino-L-alanine (L-BMAA) isolated from the seed of the plant Cycas circinalis has been implicated as an environmental factor mediating Guam Amyotrophic Lateral Sclerosis-Parkinsonism-Dementia (ALS-PD) (Spencer et al., Science 237:517, 1987). The same authors reported that L-BMAA-induced excitotoxicity is attenuated by N-methyl-D-aspartate (NMDA) receptor antagonists. Cultures of dissociated rat hippocampal neurons were used to investigate L-BMAA toxicity and its inhibition by competitive and noncompetitive NMDA receptor antagonists. The addition of L-BMAA to the culture medium resulted in concentration (10^{-3}-10^{-6}M) and time (1- 24 hr)- dependent selective neuronal cytotoxicity. Co-administration of 2-amino-7-phosphonoheptanoate (AP7), 3-((±)-2-carboxypiprazin-4-yl) propyl-1-phosphonic acid (CPP), phencyclidine (PCP) or MK801 with L-BMAA afforded the neurons protection from cytotoxicity. The rank order of antagonist potency corresponded to the relative binding affinities of these compounds for NMDA and PCP receptors. These results are consistent with the hypothesis that the accumulation of L-BMAA, or L-BMAA metabolite, within the brain can affect hippocampal neurons and lead to the dementia component of ALS-PD.

168.7

ANTAGONISM OF STRIATAL QUINOLINIC ACID TOXICITY BY VARIOUS POSSIBLE NMDA ANTAGONISTS S.-G. Zhu*, E.G. McGeer and P.L. McGeer (SPON: P.C.K. Leung). Kinsmen Lab, Dept Psychiatry, Univ of British Columbia, Vancouver, B.C., Canada, V6T 1W5

The effect of systemic pretreatment of rats with a variety of agents given once (MK-801, dextrorphan, dextromethorphan and tetrahydroaminoacridine (THA)) or repeatedly (dextrorphan, glycine, 4-acetylpyridine, and kynurenic acid) on the neurotoxicity of intrastriatal injections of quinolinic acid (QUIN) in rats was assessed by measuring choline acetyltransferase (ChAT) and glutamate decarboxylase (GAD) in the striata. The results with MK-801 confirmed and extended the report of Woodruff et al. (Neuropharmacology 26:903, 1987) since doses as low as 4 mg/kg were totally protective against 75-150 nmoles QUIN. The other agents were not protective at the doses used except for a mild effect of kynurenate at 75 nmoles QUIN. THA inhibited binding of [^{3}H]TCP to rat hippocampal membranes with a $K_i \cong 18$ μM but had no significant effect on QUIN toxicity. Measurements of γ-glutamyl transferase (γGT) activity indicated significant, but minimal, effects of both toxin and pretreatment with no significant interaction between the two. Overall, γGT did not correlate with either ChAT or GAD, indicating the small group differences seen were not a reflection of extent of neuronal loss.

Sponsored by grants from the MRC of Canada and the B.C. Medical Services Foundation. Dr. Zhu is a visiting scholar from The People's Republic of China.

168.8

EXCITOTOXIC BRAIN INJURY ENHANCES QUISQUALIC ACID (QA) STIMULATED PHOSPHOINOSITIDE(PPI) TURNOVER IN DEVELOPING BRAIN. D. Statman, C.K. Chen, M.V. Johnston, S.K.Fisher, F.S. Silverstein. University of Michigan, Ann Arbor, MI

QA, a potent excitotoxin in 7 d.o. rats, stimulates PPI hydrolysis at this developmental stage. Inositol phosphate(IP) release is a sensitive measure of PPI hydrolysis. We examined QA stimulated IP release after intra-striatal QA injections to learn more about the pathogenesis of excitotoxic injury in immature brain. Stereotaxic intra-striatal injections of QA (100 nmol) were done in 7 d.o. rats;pups were sacrificed 1,2, or 5 days later (9 experiments; in each, n=16 QA, 8 controls). After incubation with 3H-myoinositol and lithium, in vitro QA stimulated 3H-IP release was measured in lesioned brain & control striatal tissue slices. To assess the specificity of observed lesion-induced effects, cholinergic (carbachol) stimulated 3H-IP release was also assayed. IP release was expressed as % of basal in controls. 1,2, or 5 days post-lesion, incubation of lesioned brain tissue with QA (10^{-5} M) resulted in a paradoxical enhanced stimulation of IP release (mean ± SEM at 2 days, 1153±207% vs 576±118% in controls, $p<.01$, t-test). In contrast, there was no difference in carbachol stimulated IP release between lesioned & control striatum. These data suggest altered coupling of EAA recognition sites with phospholipase C; this may represent an adaptive synaptic response to injury.

168.9

CNQX POTENTLY AND SELECTIVELY BLOCKS KAINATE EXCITOTOXICITY IN THE CHICK EMBRYO RETINA. M.T. Price, T. Honore*, M.E. Mueller*, J. Labruyere*, E. Silverman* and J.W. Olney. Washington University Sch. of Med., St. Louis, MO and Ferrosan Research Division, Denmark.

The excitatory amino acid (EAA) agonists, N methyl-D-aspartate (NMDA), quisqualate (Quis) and kainate (KA), induce distinctive excitotoxic lesions in the ex vivo chick embryo retina. The toxic action of NMDA is selectively blocked by various antagonists which are ineffective against Quis or KA. Mixed EAA antagonists, such as kynurenic acid and cis-2,3-piperidine dicarboxylic acid, block the toxic actions of all three agonists but are of low potency, especially against KA and Quis. Recently, Honore et al (Neurosci. Abstr. 13, 383, 1987) described CNQX as a selective antagonist of EAA receptors of the non-NMDA type based on electrophysiological experiments conducted in rat spinal cord.

We have studied the anti-excitotoxic properties of CNQX in the chick embryo retina and found that at 15 uM concentration it totally prevents the neurotoxic action of KA, and at 50 uM prevents either NMDA or Quis toxicity. This identifies CNQX as the most selective and potent antagonist of KA excitotoxicity yet described. While CNQX also qualifies as the most potent known antagonist of Quis excitotoxicity, it is not selective against Quis compared to NMDA toxicity in this retinal preparation.

Previously we described MK-801 as the most potent known antagonist of NMDA toxicity, but we found MK-801 relatively impotent in preventing ischemic damage in the chick retina; for example, it blocked NMDA toxicity at 100 nM concentration but did not block ischemic damage at >100 uM. Here we report that concentrations of CNQX up to 100 uM are ineffective in blocking ischemic damage but a combination of MK-801 (500 nM) plus CNQX (100 uM) does effectively prevent ischemic damage. This supports the interpretation that endogenous EAA acting at both NMDA and non-NMDA receptors mediate ischemic neuronal degeneration in the chick retina, and that both classes of receptor must be blocked for optimal protection against retinal ischemic damage. Supported in part by DA 05072, HD 24237, DAMD 17-86-C6010 and Research Scientist Award MH 38894 (JWO).

168.10

GLUTAMATE IS TOXIC AT THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR WHEN ENERGY LEVELS ARE COMPROMISED. M.A. Vigano*, A. Novelli*, J.A. Reilly*, P.G. Lysko* and R.C. Henneberry*, (Sponsor: J.P. Bressler.) NINCDS, NIH Bethesda, MD. 20892.

We have found that excitatory amino acids (EAAs) are neurotoxic for cerebellar granule cells in primary culture when neuronal energy metabolism is compromised. Glutamate stimulated cGMP synthesis in metabolically competent granule cells, but killed the cells when ATP levels were reduced or ion pumps inhibited. Glucose starvation, oxygen deprivation, or oxidative phosphorylation inhibition enabled NMDA agonists to become lethal. Toxicity was blocked by both competitive antagonists such as AP5, AP7, and CPP and by noncompetitive antagonists such as MK-801, ketamine, and phencylidine (which are thought to block the NMDA receptor channel). Results indicate that compromised energy levels in the neuron are reflected in decreased ion pump activity leading to sufficient depolarization to relieve the voltage-dependent Mg^{++} block of the NMDA receptor, which permits the opening of large numbers of NMDA receptor channels. Again due to reduced availability of ATP, ion pumps are unable to keep pace with the influx of Na^+ and Ca^{++}, and neuronal death follows. This mechanism may be involved in the death of neurons following ischemia due to stroke or cardiac arrest, or in a variety of neurodegenerative disorders in which neuronal energy is compromised.

168.11

MODULATION OF GLUTAMATE-INDUCED NEUROTOXICITY BY THE PUTATIVE NEUROTOXIN β-N-OXALYLAMINO-L-ALANINE. P.G. Lysko* and R.C. Henneberry* (SPON: J.W. Thomas). Molecular Neurobiology Section, Lab. of Molecular Biology, NINCDS, National Institutes of Health, Bethesda, MD 20892.

The unusual amino acids β-N-methylamino-L-alanine (BMAA) and β-N-oxalylamino-L-alanine (BOAA) have been implicated as environmental neurotoxins when consumed in food. We have tested these putative neurotoxins for interaction with the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor found in our model system of cultured rat cerebellar granule cells. Neither BMAA nor BOAA (10 mM) was itself toxic to glucose-depleted cells, as shown by fluorescein diacetate staining for viability. However, the toxicity of glutamate at normally sublethal levels (10 μM) was potentiated by as little as 10 μM BOAA, resulting in complete killing; 1 mM BMAA did not potentiate. The lethal effects of BOAA with 10 μM glutamate were completely abolished by 1 mM 2-amino-5-phosphonopentanoate (AP5), 1 μM MK-801, or 5.6 mM glucose. The modulation by BOAA of glutamate-induced neurotoxicity may be mediated via membrane depolarization. When Ca^{++}-dependent cGMP synthesis under energy-compromised conditions was monitored as an indicator of ion channel activity, either 100 μM BOAA or 10 μM glutamate alone stimulated low levels of intracellular cGMP production; BOAA together with glutamate resulted in a strongly synergistic stimulation of cGMP synthesis which was blocked by AP5 and MK-801.

168.12

NMDA RECEPTOR LOSSES IN HUNTINGTON'S DISEASE PUTAMEN SUPPORT A NEUROTOXIC HYPOTHESIS. A.B. Young, J.T. Greenamyre, Z. Hollingsworth, R. Albin, C.J. D'Amato, I. Shoulson, and J.B. Penney. Depts. of Neurology and Pathology, Univ. of Michigan, Ann Arbor, MI 48109 and Dept. of Neurology, Univ. of Rochester, Rochester, NY 14642.

N-Methyl-D-aspartate (NMDA)-and phencyclidine (PCP) receptor binding were compared to quisqualate, benzodiazepine, GABA and muscarinic cholinergic receptor binding in the putamen and cerebral cortex of Huntington's disease (HD) brains and control brains. NMDA and quisqualate receptors were measured with [^{3}H]glutamate; PCP receptors with [^{3}H]N-(1-[2-thienyl]cyclohexyl)-3,4-piperidine (TCP); muscarinic cholinergic receptors with [^{3}H]quinuclidinyl benzilate; GABA receptors with [^{3}H]muscimol and benzodiazepine receptors with [^{3}H]flunitrazepam. NMDA and PCP receptor binding were reduced 93% and 68%, respectively, in HD putamen compared to binding in controls. Quisqualate receptors were reduced 67% and the binding to other receptors was reduced 55% or less. Cerebral cortex receptor binding was unchanged in HD compared to controls. The prominent loss of NMDA receptors supports the hypothesis that NMDA receptor mediated neurotoxicity plays a role in the pathophysiology of HD.

Supported by USPHS grants NS 19613, 17978 and 15655 and the Hereditary Disease Foundation.

168.13

MONOSODIUM-L-GLUTAMATE(MSG)- AND KAINIC ACID(KA)-INDUCED NEUROCHEMICAL CHANGES IN ADULT AND AGED RATS. D.R. Wallace and R. Dawson Jr. , Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, Fl. 32610.

The neurochemical effects of the exogenous excitotoxins, KA and MSG, were evaluated in adult(6 month) and old(20-30 month) rats. Female Long Evans rats received KA at a dose of 15mg/kg (i.p.) and were sacrificed two hours later. Male Fischer-344 rats received MSG at a dose of 500mg/kg (i.p.) and were sacrificed 30 minutes later.

KA treatment resulted in significant ($p<0.001$) decreases in norepinephrine (NE) levels in the entorhinal cortex [ECX], frontal cortex [FCX], and striatum[STR] in the old rats. Levels of 5-HIAA and HVA however, increased in the ECX and FCX of old rats. KA decreased levels of aspartate and glutamate in the ECX, but only aspartate was lowered in the FCX of old rats. KA treatment in adult rats produced trends similar to the old rats, although these effects were not statistically significant.

NE levels were decreased by MSG administration in the cerebellum and hypothalamus of adult rats, but NE content was increased in the CER of old rats. MSG elevated ($p<0.05$) glutamate in the posterior cortex of old rats.

These data point to important in vivo relationships between excitatory amino acids and central noradrenergic systems. Age-induced alterations in NE-excitatory amino acid interactions may contribute to cognitive and memory deficits associated with aging. This work was supported by the American Federation for Aging Research.

168.14

EXCITATORY AMINO ACID-EVOKED RELEASE OF [^{3}H]GABA FROM STRIATAL NEURONS IN PRIMARY CULTURE. Dorothy E. Kemp* and Samuel Weiss (SPON: J. Bevan). Dept. of Pathology, University of Calgary, Calgary, Alta., Canada T2N 4N1

The actions of excitatory amino acids (EAAs) on the release of previously incorporated [^{3}H]GABA was examined in purified (>93%) striatal neurons in primary culture. Glutamate, KCl and veratrine evoked a dose-dependent, saturable and reversible release of [^{3}H]GABA. Glutamate actions were not reduced in the absence of calcium, and were insensitive to tetrodotoxin. The dose-response relationships of EAAs demonstrated the following rank order of potency: glutamate > aspartate = N-methyl-D-aspartate > kainate >> quisqualate. Kainate was the most effective agonist, evoking an 8-fold increase over baseline levels of [^{3}H]GABA release. Aspartate- and N-methyl-D-aspartate-evoked release was abolished in the presence of either APV or γ-DGG. Release due to glutamate and kainate was ineffectively attenuated by these agents. Glutamate-, aspartate- and N-methyl-D-aspartate-evoked GABA release were augmented when calcium was omitted and reduced when sodium was replaced with choline or lithium. Kainate-evoked release was unaffected when calcium was omitted, unchanged when choline replaced sodium, and markedly potentiated when lithium substituted for sodium. These findings suggest that two distinct receptor systems for EAAs mediate the evoked release of [^{3}H]GABA from striatal neurons.

168.15

EVALUATION OF THE ANTI-EXCITOTOXIC ACTION OF MK-801 IN THE RAT STRIATUM BY MEANS OF APOMORPHINE-INDUCED ROTATIONAL BEHAVIOR: COMPARISON WITH NEUROCHEMICAL MEASUREMENTS. Z. Susel*, T.M. Engber and T.N. Chase. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20892.

MK-801 can prevent the production of excitotoxic lesions caused by excessive stimulation of the N-methyl-D-aspartate (NMDA) receptor. Previous reports have demonstrated this effect *in vivo* by means of histological examination of brain tissue or by measurement of neurochemical markers. In the present study, the ability of MK-801 to preserve the functional integrity of the rat striatum following intrastriatal injection of the NMDA agonist quinolinic acid was assessed by measurement of apomorphine-induced rotational behavior. In addition, choline acetyltransferase (CAT) activity was determined in the striata of these rats. Apomorphine-induced rotations were recorded six days after injection of quinolinic acid (300 nmoles) into the left striatum. MK-801 (3, 5 or 10 mg/kg, i.p.) or saline was administered 30 minutes before injection of quinolinic acid. One day after rotation tests, rats were sacrificed and the striata dissected for measurement of CAT activity. Pretreatment with MK-801 at a dose of 10 mg/kg virtually eliminated ipsilateral rotational behavior but had no effect compared to saline-pretreated controls at doses of 3 and 5 mg/kg. CAT acitivity in the lesioned striata of MK-801-pretreated rats (which ranged from 48% of the unlesioned striatum at 3 mg/kg to 93% at 10 mg/kg) was significantly different from that of saline controls (10-12% of unlesioned striatum) at all three doses of MK-801. Thus apomorphine-induced rotational behavior can be used to assess the functional integrity of the rat striatum following excitotoxic lesion. The relationship between rotational behavior and other neurochemical markers in the striatum remains to be investigated.

168.16

EXCITATORY AMINO ACIDS IN EXPERIMENTAL SPINAL CORD TRAUMA: INJURY-INDUCED CHANGES IN TOTAL TISSUE LEVELS AND PHARMACOLOGICAL INTERVENTION WITH THE COMPETITIVE ANTAGONIST CPP. P. Demediuk* and A.I. Faden. Center for Neural Injury, VA Medical Center, San Francisco, and Neurology Department, University of California, San Francisco, CA 94121.

Changes in total spinal cord tissue content of amino acids were followed over time after 25, 50, and 100 g-cm impact injury (Allen method) to the T9 segment of pentobarbital-anesthetized rats. Samples were frozen *in situ* with liquid nitrogen, and amino acids were quantitated electrochemically as their O-pthalaldehyde, 2-propanethiol derivatives after HPLC separation. Trauma resulted in time-dependent, injury-dependent alterations among the various measured amino acids. Glu and asp were significantly reduced at all postinjury sampling times, whereas GABA, gly and tau were decreased at 5 min, with partial recovery at 4 hr and 24 hr. For pharmacological studies, intrathecal perfusion with 10 and 100 μg of the competitive inhibitor CPP [(±-4-(3-phosphonopropyl)-2 piperazinecarboxylic acid] at 15 min postinjury (50 g-cm) significantly improved both motor function and ability to remain on an inclined plane. The outcome measures were affected in a dose-dependent manner when compared with saline-treated injury controls. These studies provide evidence for the role of "excitotoxins" in delayed injury following spinal cord trauma.

168.17

PICOLINIC ACID PROTECTS AGAINST QUINOLINIC ACID-INDUCED CORTICAL CHOLINERGIC DAMAGE. K. Jhamandas, R.J. Boegman, R.J. Beninger†, and M. Bialik* (SPON: J.V. Milligan). Departments of Pharmacology and Toxicology, and Psychology†, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Quinolinic (QUIN) injections into the nucleus basalis magnocellularis (nbM) destroy cortical neurons, and kynurenic acid (KYA) prevents this action. This study examined whether picolinic acid (PIC), and several other products of the kynurenine pathway of tryptophan metabolism, share these actions. PIC injection into the nbM did not produce cholinergic damage, but fully protected against the excitotoxic action of QUIN or kainic acid. Histological examination of nbM confirmed the antagonistic action of PIC and protected neurons showed normal release of ^{3}H-acetylcholine when stimulated with potassium (35 mM). While PIC was less potent than KYA, it was more potent than quinaldic acid, hydroxy-quinaldic acid or anthranilic acid. None of these agents were excitotoxic when injected singly into the nbM. It is concluded that to KYA, PIC and several other tryptophan metabolites can antagonize the toxic action of QUIN on basal forebrain cholinergic neurons. (Supported by the Ontario Mental Health Foundation)

168.18

INHIBITION OF CYSTINE UPTAKE MEDIATES QUISQUALATE TYPE GLUTAMATE TOXICITY IN A NEURONAL CELL LINE. T.H. Murphy, M. Miyamoto *, A. Sastre, R.L. Schnaar and J.T. Coyle. Depts. of Pharmacology, Physiology and Neuroscience, The Johns Hopkins School of Medicine, Balto. MD, 21205.

Quisqualate (Quis) type glutamate (Glu) toxicity in N18-RE-105 cells is delayed in onset, calcium dependent, not caused by depolarization, not prevented by putative Quis receptor antagonists, nor produced by the agonist AMPA. The pharmacologic specificity of the cytotoxic effect exhibits similarities to that of cystine (Cys) sensitive Cl^- dependent Glu transport, prompting an examination of the role of Cys in cytotoxicity. Increasing the Cys concentration of the culture medium from the usual 0.2 mM to 1.0 mM prevented the toxic effects of both 1.0 mM Quis and 10 mM Glu, whereas medium containing 0.005 mM Cys caused cell death itself, with a cytopathology, time course, Ca^{++} dependence, and protection by antioxidants similar to 10 mM Glu. [35S]Cys and [3H]Glu are transported into the cells with apparent K_ts of 9 and 45 μM , respectively. The observed K_is for inhibition of [35S]Cys or [3H]Glu uptake by Glu, Cys, and Quis were nearly identical, suggesting that Cys and Glu compete at the same uptake site. Alterations in media Cys concentrations (5-200 μM) affected the toxic potency of Glu and Quis in a manner which could be predicted from their apparent affinities for the Cys/Glu transporter; the correlation between cell death and occupancy of the Cys transporter was linear with a slope of 1. Glu treatment reduced glutathione (GSH) levels to less than 50% of control in 4 h. Consistent with the reduction in GSH levels being the critical factor in Glu toxicity, the GSH synthesis inhibitor buthionine sulfoximine was also toxic. These data suggest that the Quis sensitive, Cl^- dependent Glu transport is a mechanism for Cys sequestration for maintenance of redox homeostasis by GSH synthesis. Furthermore, inhibition of this process by extracellular Glu or Quis kills these neuron-derived cells by oxidative stress (Miyamoto *et al.* and De Long *et al.*, these proceedings) through GSH depletion.

168.19

ANTIOXIDANTS PROTECT AGAINST GLUTAMATE CYTOTOXICITY IN A NEURONAL CELL LINE. M. Miyamoto*, T. H. Murphy, R. L. Schnaar and J. T. Coyle . Depts. of Neuroscience and Pharmacology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

L-Glutamate (Glu) and quisqualate (Quis) cause a delayed Ca^{2+}-dependent cytotoxicity in the N18-RE-105 cell line between 8-24 hours of exposure (Murphy *et al.*, Brain Res. 444:325, 1988). Since the competitive inhibition of cystine uptake by Glu and Quis correlates precisely with their cytotoxic potency, cystine deprivation likely accounts for the cell lysis (Murphy *et al.*, this meeting). In this study, the effects of antioxidants and reducing agents on Glu cytotoxicity were examined in the N18-RE-105 cell line.

Reducing agents, cysteine (10-1000 μM), dithiothreitol (DTT, 10-250 μM) and glutathione (GSH, 10-1000 μM) protected the cells against the cytotoxicity of 10 mM Glu in a concentration dependent manner. Antioxidants, α-tocopherol (10-100 μM), idebenone (0.1-3 μM) and vinpocetine (30-300 μM) also provided marked protection. Furthermore, these agents prevented delayed cell death caused by lowering the concentration of cystine in the medium from 200 μM to 5 μM. Incubation with 10 mM Glu caused a marked decrease in the GSH levels of the cells. Whereas cysteine and DTT prevented the GSH reduction caused by Glu, the antioxidants were ineffective. The cellular levels of oxidants were assessed using 2,7-dichlorofluorescin, a probe that accumulates within cells and is converted to a fluorescent product by oxidation. α-Tocopherol and idebenone markedly reduced the number of fluorescent cells caused by 10 mM Glu. These results indicate that oxidative stress due to loss of cellular levels of GSH is one mechanism whereby Glu/Quis exert cytotoxicity and suggest that centrally active antioxidants may reduce neuronal damage in pathologic conditions associated with excessive Glu release.

168.20

NAD(P)H:QUINONE REDUCTASE PROTECTS AGAINST GLUTAMATE TOXICITY IN A NEURONAL CELL LINE. M.J. DeLong *, T.H. Murphy, M. Miyamoto* and J.T. Coyle (SPON: L. Fechter) Depts. of Environmental Health Science and Psychiatry, The Johns Hopkins University School of Hygiene and School of Medicine, Baltimore MD 21205.

A select population of surviving neurons in the neostriatum of Huntington's disease (HD) patients stain intensely for NAD(P)H-diaphorase, a histochemically defined group of enzymes postulated to be 70-80% NAD(P)H:quinone reductase (QR) in the brain. Glutamate (Glu) toxicity of neurons may involve oxygen radical reactions. QR (also known as DT diaphorase) is uniquely capable of eliminating oxygen free radicals, thus protecting cells from oxidative stress. We have investigated the role of QR in the delayed quisqualate-sensitive Glu toxicity in N18-RE-105 cells.

Exposure of these cells to 10 mM Glu causes a 50-70% loss of QR in 2-3 hr similar to decreases observed with menadione and paraquat, oxidative stress producing compounds. Glu exposure of these cells is associated with a greater than 50% loss of glutathione (GSH) in 4 hr due to inhibition of cystine uptake which is associated with oxygen radical formation (Murphy *et al.* and Miyamoto *et al.*, these proceedings). Induction of QR levels to 2 to 4-fold by treatment with tert-butyl hydroquinone (3-20 μM) prior to Glu exposure protects against cell death by 50 to 100% over control cells. Dicumarol, a specific inhibitor of QR, potentiates cell death by 30-60% in a dose-dependent manner with 1 mM Glu. We speculate that the initial rapid loss of both QR and GSH potentiates cell death by perturbation of the redox homeostasis which they regulate. These *in vitro* data demonstrate QR protection against Glu toxicity and suggest a possible protective role of QR against neuronal degeneration as in HD.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY II

169.1

PHENCYCLIDINE (PCP) REDUCES INFARCT SIZE IN A MODEL OF FOCAL CEREBRAL ISCHEMIA: L.F. Copeland, P.A. Boxer, F.W. Marcoux. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

NMDA antagonists have been shown to prevent global ischemic injury to the hippocampus. Phencyclidine (PCP) is a potent noncompetitive NMDA antagonist. We have evaluated PCP in a model of focal brain ischemia induced by permanent combined common carotid and ipsilateral middle cerebral artery (MCA) occlusion in male Fisher 344 rats. PCP was administered IV via a femoral catheter 30 min and 24 hrs post MCA occlusion. The areas and estimated total infarct volume were determined by measuring Evan's Blue extravasation in three cross-sectional slices. PCP, at 3 mg/kg (N=10), produced disruptive behavioral effects and a 35% reduction in infarct volume compared to saline controls ($p<0.05$, ANOVA). At 10 mg/kg (N=10), PCP produced anesthesia and a 24% decrease in infarct volume that was not statistically significant. These results suggest that PCP can reduce infarct volume in focal brain ischemia. The NMDA antagonist actions of PCP may contribute to its neuroprotective and disruptive behavioral effects.

169.2

QUISQUALIC ACID(QA) AND ITS ANALOGUE AMPA ENHANCE PHOSPHOINOSITODE(PPI) TURNOVER IN N-METHYL-D-ASPARTATE (NMDA) LESIONED BRAIN OF PERINATAL RATS. C.-K.Chen, D.Statman, F.S.Silverstein, M.V.Johnston, Univ. of Michigan, Ann Arbor, MI 48104

Excitatory amino acid(EAA) mediated receptor responses may be one of the factors which trigger a cascade of neurotoxicity in ischemic-hypoxic brain damage. We previously reported that QA mediated PPI turnover was increased after ischemic-hypoxic brain insult in perinatal rats(*J. Neurochem.*,in press), suggesting altered coupling of receptors and phospholipase C. We examined this QA mediated PPI turnover in an excitotoxic model which replicates several important features of ischemic-hypoxic injury in the immature brain.

NMDA, 17 nmole in 0.5 uL, was injected into the right posterior corpus striatum of ether anesthetized 7 day old rats and the pups were sacrificed 3 days later. Hippocampal or striatal tissue slices were prepared from both ipsilateral and contralateral side of the injected brain as well as uninjected controls. Slices were incubated with ^{3}H-myoinositol plus QA(10^{-5}M) or AMPA(10^{-3}M) in the presence of lithium and inositol monophosphate(IP1) accumulation was measured. Both QA(7 experiments) and AMPA(2 experiments) incubation enhanced IP1 release(expressed as % of basal in controls) in tissue ipsilateral to the NMDA injection(Mean±SEM, Hippocampus: 1237±334 vs. 510±178 in contralateral, $p<0.05$, paired *t* test; Striatum: 1381±576 vs. 562±279 in contralateral, $p<0.05$). AMPA had a similar though weaker effect(Hippocampus: 260±79 vs. 176±84, $p<0.05$; Striatum: 319±46 vs. 117±31, $p<0.05$), whereas there were no differences between contralateral and controls in both cases.

These results suggest that both the ischemic-hypoxic insults and NMDA lesion in 7 day old rats have the similar effects to enhance QA receptor coupled PPI turnover.

169.3

3H-TCP BINDING IN HYPOXIC-ISCHEMIC (HI) BRAIN. F.S. Silverstein, J.W. Mcdonald, M.V. Johnston. (Sponsored by G.W. Goldstein). Univ. of Michigan, Ann Arbor, Mi.

Recently, we found that MK-801, a non-competitive antagonist of N-methyl-D-aspartate (NMDA), protects the neonatal brain from HI damage (Eur J Pharm 140:359). MK-801 binds to a phencyclidine (PCP) site associated with the NMDA receptor. 3H-N-(1-[2-thienyl]-cyclohexyl)-3,4-piperidine (3H-TCP), a PCP derivative, binds to the channel associated with the NMDA receptor. We used in vitro autoradiography to examine 3H-TCP binding in HI brain and in MK-801 pre-treated animals that underwent the same HI insult. 8 d.o. rat pups were sacrificed 24h after right carotid ligation followed by a 2.5h exposure to 8%02 [n=7 saline treated, n=7 MK-801 (1mg/kg) treated]. To prepare ARG's, frozen sections were incubated with 3H-TCP; binding density was quantitated bilaterally in 10 brain regions. In regions susceptible to irreversible neuronal damage e.g. hippocampus, 3H-TCP binding declined ipsilateral to ligation in HI brain [e.g. dentate gyrus (DG) -35+5%, $p<.001$ ANOVA vs control]. In ligates treated with MK-801, inter-hemispheric asymmetries in 3H-TCP binding were reduced (for DG, $p< .01$, ANOVA, HI vs HI + MK-801); however, diffuse suppression of 3H-TCP binding in MK-801 treated pups contributed to this result. Acute disruption of 3H-TCP binding appears to be a sensitive marker for brain regions susceptible to HI injury.

169.4

GLUTAMATE-INDUCED INCREASE OF Ca^{2+} IN NEURONAL CEREBELLAR GRANULE CULTURES STUDIED AT THE ULTRA-STRUCTURAL LEVEL. P. Putzolu and H. Alho. FGIN, Georgetown Univ., Medical School, Washington, D.C. 20007.

Glutamate receptors, sensitive to NMDA stimulation and Mg^{2+} inhibition, are members of a family of ionotropic and metabolotropic receptors that included allosteric regulatory centers. Stimulation of the primary transmitter recognition sites is transduced into transient cationic (Ca^{2+}) influxes followed by an activation of Ca^{2+}-regulated enzymes which, can cause neuronal death. Hence it is presumed that glutamate neurotoxicity is due to a sustained increase of the cytosolic concentrations of Ca^{2+} followed by H_2O influx and activation of Ca^{2+}-dependent enzymes. In this study, the presence of Ca^{2+} was demonstrated by electron spectroscopic element imaging using a Zeiss-902 transmission electron microscope. Prefixation with glutaraldehyde followed by postfixation with osmium-$K_2Cr_2O_7$ was used to precipitate and visualize endogenous Ca^{2+} without the addition of external Ca^{2+}. In cerebellar granular neurons, electron dense precipitates containing Ca^{2+} were found in intracellular compartments. In the glutamate-treated neurons, the intracellular signal for Ca^{2+} was considerably increased. Using the present method, it is possible to obtain ultrastructural information concerning the molecular mechanism of glutamate neurotoxicity.

169.5

EXCITATORY AMINO ACID MECHANISMS OF ZERO-CALCIUM INDUCED DEATH OF CEREBELLAR GRANULE CELLS P. P. McCaslin Dept. Pharmacol.& Toxicol., UMC, Jackson, Ms, 39216

Primary cultures of cerebellar rat neurons were grown for 18-22 days *in vitro*. After this time, growth medium was washed from slides with a balanced salt solution containing the following mM concentrations: 154 NaCl, 5.6 KCl, 2.5 $CaCl_2$, 10 glucose and 5 HEPES (pH 7.4; r.t.). Cells were left in this buffer for 15 minutes and then rinsed and placed in the above buffer lacking calcium (no EGTA was added). Changes in the cells were evident within 2 min and included an apparent cellular granulation with a loss of cell body birefringence at 10x magnification with complete cell death by 30 min. Cells were protected from zero-calcium induced death by the excitatory amino acid (EAA) antagonist, 2-amino-7-phosphonoheptanoic acid (APH; 200 *u*M). Also, the addition of 2 mM $MgCl_2$ to the buffer protected against death. Evidence that zero-calcium was inducing the secretion of a toxic compound was obtained by the following experiments. Cells were protected from death by continuously washing zero-calcium buffer over the cells, thus washing away substances released by the cells. This was accomplished by placing slides in 50 ml of zero calcium buffer and gently swirling the dish. After 30 min the swirling could be stopped and the cells would survive in zero-calcium buffer. A 0.2 ml drop of zero-calcium buffer placed onto fresh cells and swirled for 3 min resulted in the secretion of amino acids into the buffer. Adding $MgCl_2$ to this amino acid-containing buffer placed onto fresh cells resulted in cell death. Examination of select amino acids secreted into 0.2 ml of zero-calcium buffer revealed a decrease in GABA (2- to 5-fold) and increases in glutamate (10-fold) and glycine (5-fold) when compared to amino acids secreted into calcium buffer under the same conditions. No change was seen in the secretion of threonine between these 2 groups. Collectively, the data suggest that zero-calcium induces the release of glutamate and glycine by unknown mechanisms, since vesicle fusion with the membrane would not be expected in the absence of calcium. The released glutamate then activates the EAA receptor which ultimately leads to cellular death. Death is prevented by 1] washing glutamate from the cells, 2] preventing the release of glutamate with magnesium or 3] blocking the EAA receptor with APH. Under the non-physiologic condition of zero-calcium, cerebellar neurons can release a compound which results in the auto-stimulation of an EAA receptor, ultimately leading to cell death.
Supported by grant BRSG S07RR05386.

169.6

DEVELOPMENTAL SUSCEPTIBILITY TO EXCITATORY AMINO ACIDS IN RAT STRIATUM. L. Arcavi*, D.M. Ferriero, S.M. Sagar and R.P. Simon. Dept. of Neurology, Univ. of Calif., San Francisco, CA 94143

In immature rats, there is selective sparing of NADPH diaphorase-reactive (NADPHd+) neurons after forebrain hypoxic-ischemic injury. This pattern is mimicked by intrastriatal injection of the endogenous excitotoxin, quinolinic acid (QUIN). The vulnerability of these NADPHd+ neurons seems to change during development. To test this hypothesis, we examined the effects of intrastriatal injection of either QUIN (80 nm), quisqualate (QA; 100 nm) or kainate (KA; 15 nm) at postnatal day (P) 7, P14, and in the adult Sprague Dawley rat. After 1 week survival, the brains were processed for NADPHd histochemistry. Despite significant loss of striatal tissue, there was a striking preservation of NADPHd+ neurons in the striatum of P7 rats after QUIN infusion. P14 and adult animals, however, lacked NADPHd+ cells in the area of damage. With QA infusion, severe neuronal damage without sparing was seen at all ages. Intrastriatal injection of KA did not affect the P7 brains, whereas total cell death was seen after P14. These findings show that, early in development, NADPHd+ neurons are resistant to NMDA and KA; QA susceptibility appears early and does not seem to change.

169.7

LESION-INDUCED CHANGES IN GLUTAMATE UPTAKE SITES. R.J. Bridges, K.J. Anderson, A.L. Tavoularis*, D. Bhatt* and C.W. Cotman. Department of Neurology and Psychobiology , Univ. California, Irvine, CA 92717.

The termination of the excitatory action of L-glutamate and the prevention of subsequent excitotoxic neuronal damage is dependent upon the activity of high affinity transport systems. In the present investigation we have used *in vitro* autoradiography to examine the response of glutamate uptake sites in the hippocampal formation to a lesion of the entorhinal cortex. Binding to the Na^+-dependent site was studied using [^{3}H] D-aspartate and binding to the Cl^--dependent site was visualized using [^{3}H] L-glutamate. Animals were subjected to a complete unilateral lesion of the entorhinal cortex and killed at various times postlesion.

Both the Na^+- and Cl^--dependent transport sites were found to transiently increase in the dentate gyrus molecular layer in response to entorhinal ablation. An approximate 25% increase in binding was seen at 5 and 7 days postlesion. The levels of binding returned to control values by 12 days postlesion. No significant changes in binding to these uptake sites was seen at long postlesion times (3-6 months). Enhancement in binding was seen to parallel the increase in GFAP immunoreactivity, supporting the hypothesis that these uptake sites are present on reactive astroglia. An increased ability to transport glutamate may represent a mechanism by which the molecular layer is protected, at least in part, from possible excitotoxic damage mediated by L-glutamate.
Supported by MH19691, AG00096 and AG00538

169.8

AUTORADIOGRAPHIC COMPARISON OF NA^+ AND CL^--DEPENDENT EXCITATORY AMINO ACID UPTAKE SITES. K.J. Anderson, R.J. Bridges, A.L. Tavoularis*, D. Bhatt* and C.W. Cotman. Department of Psychobiology and Neurology, Univ. California, Irvine, CA 92717.

The density, distribution and pharmacology of binding to the two major excitatory amino acid uptake carriers was studied in rat brain using *in vitro* autoradiographic techniques. Binding to the Na^+-dependent site was studied using [^{3}H] D-aspartate and binding to the Cl^--dependent site was visualized using [^{3}H] L-glutamate. Both were studied on fresh-frozen 6 µm tissue sections. We found that in fresh tissue sections, up to 50% of the total pool of bound radioactivity appeared to be sequestered in membranes. This pool was decreased by 1) stimulation of homoexchange, 2) the additon of cellobiose to the incubation buffer and, 3) by rinsing the tissue in H_2O. Uptake into the transported pool was reduced by incubating the tissue sections in fresh xylenes for 10 minutes prior to incubation with radiolabeled substrate. Xylene pre-treatment did not affect the pharmacology of binding to either site. Autoradiographic analysis of the binding sites showed a complimentary distribution; the Na^+-dependent binding was highest in the cerebellar molecular layer, lateral septum, entorhinal cortex and hippocampus while Cl^--dependent binding was highest in thalamic nuclei, striatum, outer cortical layers and dentate gyrus molecular layer. These sites also differed by their pharmacology. Na^+-dependent binding was most potently inhibited by D,L-threo-β-hydroxyasparate>cysteine sulfinate>quisqualate. Cl^--dependent binding was most potently inhibited by α-aminoadipate>quisqualate>ibotenate. The density of uptake sites was compared between 10 day-old, 3 month-old and 26 month-old rats. A reduction in binding was seen to occur in select regions of the aged brain, indicating that these circuits may be more vulnerable to excitotoxic damage.
Supported by MH19691, AG00096 and AG00538

169.9

CORRELATION BETWEEN EXCITATORY AMINO ACID INDUCED ELECTROPHYSIOLOGICAL RESPONSES AND NEUROTOXICITY.
W.J.Koroshetz, A.Freese, M.DiFiglia and D.P. Corey. Department of Neurology and Howard Hughes Med Inst., Mass General Hospital, Boston, Ma.

Evidence suggests that activation of excitatory amino acid receptors mediates glutamate-induced neurotoxicity (Rothman and Olney, 1987). In animal models of stroke and Huntington's disease, EAA toxicity is selective for specific neuron types. As a first inquiry, we asked whether *in vitro* selectivity was simply a function of the presence of responses to glu or NMDA

Freese et al.(abstr., this meeting) have shown that *in vitro* glutamate is weakly toxic in 6 d old cultures of P0 neurons but a potent neurotoxin in older cultures. In cultures prepared as in Freese et al., cells were patch clamped and held at -60mv. First, responses were recorded to 2.5 sec pressure applications of 100uM NMDA. Next, after a delay (>1 min), responses to 10uM glu were obtained in the same cell. Peak responses (picoAmps) to 100uM NMDA and 10uM glu in 6-7 d old cells varied widely (N=19; NMDA:60$\pm$154; glu:57$\pm$123). Frequency histograms of responses in cells>12 d old (N=23; NMDA:29.4$\pm$33.5; glu:31.7$\pm$21.2) showed considerable overlap with those from younger cells. However, cells from 6-7 day old cultures were more likely to give a small or absent response to 100uM NMDA: 31% had no response and 11% had <10pA response. In >12 d old cultures, all cells had NMDA responses; only 17% were <10pA. Peak responses were also recorded in 8 cells(>11 d old, different culture conditions) within 12 hours of a 3 hour, 1mM glu treatment. All had NMDA responses (19$\pm$14 pA). **In summary**, younger cultures which are less responsive morphologically to glu's toxic effects have a greater proportion of cells with small or absent NMDA responses than do older cultures in which glu toxicity is pronounced. Results also suggest that additional mechanisms influence which cells with glutamate activated channels survive glu exposure. Supported by the Huntington's Society of America.

169.10

CHARACTERIZATION AND MECHANISM OF GLUTAMATE NEUROTOXICITY IN PRIMARY STRIATAL CULTURES.
A. Freese, M. DiFiglia, M.F. Beal, and J.B. Martin. Dept. Neurology, Mass General Hospital and Harvard Medical School, Boston, MA 02114

Excitatory amino acids may play a role in the toxicity associated with many neurodegenerative diseases, including Huntington's Disease (HD). In an attempt to develop a tissue culture model for HD, the toxicity of glutamate (GLU) was examined in striatal cultures derived from newborn rats. Morphological criteria were used to determine the toxic effects of GLU exposure to 6, 12, and 18 day old cultures. In all experiments, the same groups of neurons were photographed and analyzed before and after GLU introduction. Neuronal susceptibility to GLU was age dependent, with older cultures showing significantly greater cell death than younger cultures ($p<.001$). Toxicity was dose-dependent (ED50 $\simeq$ 300uM) and maximal within 3 hr after GLU exposure. Affected cells demonstrated somal swelling w/in 1 hr and disruption of somal integrity and neuritic processes w/in 2-3 hr. GLU-induced toxicity was increased in the presence of high extracellular Ca++ concentrations ($p<.001$) and could be diminished by the addition of Mg++ to the media ($p<.001$). The NMDA receptor agonist, quinolinate, achieved a similar toxicity profile as GLU and the NMDA antagonist, APV, significantly reduced the toxic effects of GLU ($p<.005$). These results provide evidence that GLU can be toxic to striatal neurons in vitro and that the toxicity is largely mediated by the NMDA receptor. (Supported by NIH grant NS16367 to MD and an MIT-HST Fellowship to AF).

SENSORY SYSTEMS I

170.1

TRANSIENT AChE IN VB THALAMUS: DEVELOPMENTAL AND EMHISTOCHEMICAL EVIDENCE FOR RELATION TO NEURITIC GROWTH.
D.A.Kristt. Univ. of Md. Medical System/Hospital, Dept. of Pathol., Balto., MD.

Ultrastructural observations were made on VB from fetal-neonatal rats during the initial phase of neuritic outgrowth, when AChE reactivity is maximal. AChE was localized to the nuclear envelope, RER, Golgi stacks and vesicles, as well as vesicles and tubules in distal processes. On the cell surface, AChE occurred in small patches on distal processes. It is proposed that the AChE is transported to **growing neurites,** intracellularly, in association with membranous structures. At these distal sites, **AChE-associated membrane would be inserted** into the surface membranes. With maximum expression between 18 dg - 4 dpn, the following conclusions are suggested: AChE does not play a role in **migration.** Ultrastructurally, onset of AChE reactivity preceded ingrowth of most afferents. Therefore, AChE is unlikely to result from a transient **cholinergic input.** Since AChE disappears as synaptogenesis proceeds, it is unlikely that AChE is part of the synaptic complex. On the other hand, AChE could play a role in defining sites suitable for synaptogenesis.
Support: NIH, Bressler Foundation.

170.2

DEVELOPMENT OF THE SENSORY INNERVATION IN THE RAT HINDLIMB.
W.J.T. Wessels*, H.K.P. Feierabend* and E. Marani*. (SPON: P.G.M. Luiten). Anatomisch Embryologisch Laboratorium, University of Leiden, Wassenaarseweg 62, NL-2300 AL Leiden, The Nederlands.

This study was undertaken to describe the development of the sensory innervation of the rat hindlimb with special attention to the dorsal root ganglia (DRG) and the plexus lumbosacralis. Injections of WGA-HRP were made into the hindlimb of rat embryos of gestational ages ranging from E15 to E19. After a survival time of approximately 24 hours the embryos were perfusion fixed, partially eviscerated and then embedded in gelatine blocks of which serial sagittal 40 um frozen sections were cut. The sections were further processed according to Mesulam (1978, J. Histochem. Cytochem. 26:106). Camera lucida drawings of the sections were used for computerized 3D-reconstruction. Circumscribed injections of the tracer into the hindlimb of the embryos give rise to completely and partially labelled DRG. In case of partially labeling this always concerns a rostral or caudal part of the DRG. The associated dorsal roots of partially labelled DRG were also partially labelled in a rostro-caudal fashion. These observations indicate that there is, at least during development, a topographical relationship between the sensory innervation of the hindlimb and the rostro-caudally organized parts of the DRG. Such a somatotopic organization within the DRG has not been reported so far.

170.3

DEVELOPMENT OF SPECTRAL SENSITIVITY IN GOLDFISH.
De-Mao Chen and Maureen K. Powers. Department of Psychology, Vanderbilt University, Nashville, TN 37240

The rod:cone ratio changes throughout life in goldfish. Both rods and cones contribute to dark-adapted spectral sensitivity in adult fish. We asked whether this was also true of juvenile fish, and if so whether spectral sensitivity at any given age reflected the relative numbers of rods and cones present at that age. Electroretinograms were recorded intravitreally from anesthetized and paralyzed fish 3-10 cm standard body length (approx. 2-5 yrs old), and b-wave thresholds were measured as a function of wavelength. We found that dark-adapted spectral sensitivity changed shape with body length: larger fish were more sensitive in the mid-wavelength region of the spectrum than were smaller fish, and they tended to be more sensitive at 400 nm as well. The heightened sensitivity to mid-spectrum lights can be explained by the relatively greater influence of rods compared to cones in larger fish. The heightened sensitivity at 400 nm cannot easily be explained by neural factors or by pre-retinal absorption factors (which would predict the opposite result with growth), but may be explained by fluorescence in the lens. We conclude that dark-adapted spectral sensitivity is mediated by rods and cones in both juvenile and adult fish, and that absolute threshold is determined by the rod:cone ratio over most of the visible spectrum.

170.4

DEVELOPMENTAL ALTERATIONS IN OLFACTORY BULB AND SENSORY EPITHELIUM IN GOLDFISH.
J. Silveira* and P.C. Brunjes. Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22903.

Goldfish exhibit central neurogenesis during most of their lifespan, making them an excellent model for examining both normal developmental processes and plasticity in an adult animal. While the goldfish visual system has been extensively examined from such a perspective, no similar study of the goldfish olfactory system exists. Our data indicate considerable growth occurs within the olfactory system. For example, fish length correlates extremely well with rosette area ($r = .94$), a pattern nearly identical to that described by Johns & Easter (1977) for the retina. Rosettes appear to grow via two processes: elongation of existing lamellae and addition of new lamellae (number of lamellae correlates .84 with fish length, although none appear to be added once a certain body length is attained). ^{3}H-thymidine autoradiography is being used to identify regions of mitotic activity in bulbs and rosettes. Olfactory bulbs are being examined to determine how patterns of innervation by receptors change during development. (Supported by NS 23154, ONR N00014-86-K-0342 and MH 18411)

170.5

INDUCTION AND STABILIZATION OF OLFACTORY GLOMERULI IN THE DEVELOPING INSECT BRAIN. Peter A. Sirianni & Leslie P. Tolbert, Dept. of Molecular and Cellular Biology & ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Sensory axons are required for the development of olfactory glomeruli in the CNS of the moth Manduca sexta. In our ongoing effort to understand the nature of the instructive influence exerted by the sensory axons, we have asked: how many axons must be present, and for how long? In the first set of experiments, different lengths of antenna (i.e. different amounts of the olfactory epithelium) were allowed to innervate the antennal lobe (AL) of the brain of developing moths, and brains were examined histologically at a semi-mature stage. The AL neuropil was scored from 1 to 5 for degree of glomerular formation: control ALs (with normal glomeruli) averaged a score of 1.6, and ALs that developed in the total absence of olfactory axons (and thus lacked glomeruli) scored 4.7. Innervation by up to 11% of the normal antenna produced aglomerular or "protoglomerular" ALs with an average score of 3.8. However, 12.5%-antennae were sufficient to produce normal-looking, but smaller, glomeruli; ALs in these animals averaged a score of 2.1 0.5. In experiments designed to test how long the axons must be present, developing antennae were removed various days after axons begin to grow into the AL. Preliminary results indicate that axons present for only 2 days (the 2 days during which glomeruli form) can sometimes produce stable glomeruli; axons present for 3 days always do. Our results indicate that glomeruli can be induced to form by a small proportion of the normal olfactory input, and that glomeruli are stable after they have formed. (NIH grant #NS 20040)

170.6

GLIAL CHANGES DURING FORMATION OF OLFACTORY GLOMERULI IN AN INSECT BRAIN. LA Oland & LP Tolbert. ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Partitioning of the first-order olfactory neuropil into glomeruli in the developing CNS of the moth Manduca sexta appears to be mediated by changes in glial cells (Oland et al., J. Neurosci., 1988). The arrival of sensory axons in the brain (beginning early in stage 4 of the 18 stages of metamorphosis) triggers changes in glial-cell shape and position that result (by late stage 6) in the formation of glial envelopes for the glomeruli. Our previous studies indicated that the emergence of glomerular walls depends also upon proliferation of glial cells. To determine whether glial proliferation is induced by olfactory axons, we have used ^{3}H-thymidine to label dividing cells starting at stage 3. Comparison of the patterns of proliferation in normal and chronically unafferented olfactory neuropils showed no significant differences in mitotic indices through stage 6, indicating that the proliferation required for initial glomerular formation is not under afferent control.

As a first step in determining the mechanisms for the glial changes that are induced by olfactory axons, we have examined in the electron microscope regions of interface between ingrowing axons and glia during stages critical in glomerular formation (stages 3-7). Before axons arrive, glial cells are interconnected by numerous close appositions which may be gap junctions and by scalariform-like junctions (SJs; Tolbert et al., J. Neurosci., 1983). During stages 5 & 6, however, as glial cells extend processes and migrate into the neuropil to form septa, the glia lose their close appositions and the SJs lack their differentiated structure. At no stage did we observe specializations between afferent axons and glial cells, suggesting that the inductive signal is not mediated via intercellular junctions. (NIH #NS07602 and NS20040.)

170.7

UNILATERAL ODOR DEPRIVATION: RAPID EFFECTS ON PROTEIN SYNTHESIS. D.L. Korol and P.C. Brunjes, Neuroscience Program and Dept. of Psych., Univ. of Virginia, Charlottesville, VA 22903.

Unilateral naris occlusion in neonatal rats leads to a 25% decrease in the volume of ipsilateral olfactory bulbs by Day 30. Large alterations such as these undoubtedly represent secondary consequences of more basic cellular regulatory events. We examined the possibility that deprivation has rapid effects on protein synthetic activity. Pups underwent either right nares closure or sham surgery on Day 1 and were injected with ^{3}H-leucine 1, 12, 24, or 48 hrs later. After 30 min pups were sacrificed and bulbs processed for emulsion autoradiography. Amino acid incorporation was quantified by grain counts. Rapid changes were observed: reduced labelling in deprived bulbs was evident 24 hrs post-occlusion. Interestingly, after 48 hrs of deprivation, higher grain densities were observed in certain regions of deprived bulbs compared to contralateral controls. Sham operated subjects demonstrated no laterality at any time examined. The data indicate that olfactory bulb cellular regulatory processes change rapidly after naris occlusion. Supported by NS-23154, ONR N00014-86-K-0342, and HD 07323.

170.8

NEURONS THAT SURVIVE FETAL TRANSECTION OF THE INFRAORBITAL NERVE RESPOND LIKE NORMAL GANGLION CELLS TO TRANSECTION OF THEIR AXONS ON THE DAY OF BIRTH. N.L. Chiaia and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

We have previously demonstrated (Chiaia, N.L. et al. Dev. Brain Res. in submission) that transection of the rat's infraorbital nerve (ION) on embryonic day 17 (E-17) results in much more extensive reinnervation of the brainstem by regenerate ION axons than is the case after similar lesions carried out on the day of birth (P-1). This difference occurred even though the number of cells that survived fetal ION transection was no greater than that observed after neonatal nerve cuts. To test whether the differential response to transection in the fetal animals may have been due to some special characteristic of the surviving cells, we cut the ION on E-17 and transected it again on P-1. In neonatally nerve damaged, adult rats (N=9), 5,001 ± 1,287 ganglion cells send axons into the regenerate ION; in fetally nerve damaged rats (N=6), this value is 5,476 ± 3,056. In the rats that sustained ION transections on E-17 and P-1, this value was 1,799 ± 1,050. In the last group of rats, reinnervation of the brainstem was almost completely restricted to layers I and II of subnucleus caudalis, as is the case in rats that sustain only neonatal ION transections. Supported by DE 07734 and BNS 17537.

170.9

NORMAL DEVELOPMENT OF, AND EFFECTS OF INFRAORBITAL NERVE DAMAGE UPON, SUBSTANCE P AND FLUORIDE RESISTANT ACID PHOSPHATASE IN THE RAT'S MEDULLARY DORSAL HORN. P.R. Hess*, N.L. Chiaia, H.L. Enfiejian and R.W. Rhoades (SPON: A.M. Golub). Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

We have used immunocytochemistry and histochemistry to determine the distributions of substance P-like immunoreactivity (SPLI) and fluoride resistant acid phosphatase (FRAP) from 0-10 days of age in rats that sustained transection of the infraorbital nerve (ION) on the day of birth. Neonatal transection of the ION produced no reduction in the density of SPLI in the ipsilateral brainstem at any time between 6 hrs and 10 days after the nerve cuts. However, there was a naturally occurring reduction in the distribution of SPLI in the developing brainstem. At birth, SPLI was heaviest in subnucleus caudalis (SpC) and subnucleus oralis (SpO), but it was also present in nucleus principalis (PrV) and subnucleus interpolaris (SpVi). By day 10, the density of the labelling in PrV and SpVi was much reduced and its distribution in SpO was also restricted. SPLI remained heavy in SpC. FRAP staining in the V brainstem complex was also heavy on the day of birth. Neonatal ION damage caused a reduction in the density of FRAP staining that was apparent within 10 hrs and remained into adulthood. Supported by DE 07734 and BNS 85-17537.

170.10

EXPRESSION OF CHOLINERGIC PROPERTIES BY SENSORY NEURONS OF THE CHICK EMBRYO. S.V. Bhave* and A.R. Wakade (Spon.: F. Kao), Dept. of Pharmacology, SUNY-HSC at Brooklyn, 450 Clarkson Ave., Brooklyn, NY 11203.

The cholinergic properties of sensory neurons of the chick embryos were studied by measuring the choline acetyltransferase (ChAT) activity and ^{3}H-choline uptake. Dorsal root ganglia of 8-day-old chick embryos showed significant amounts of ChAT activity (24 ± 2.52 pmol ACh/min/mg protein), which increased to 45.4 ± 9.69 pmol ACh/min/mg protein in the ganglia of 12-day-old embryos. Sensory neurons derived from dorsal root ganglia of 10-day-old embryos and maintained in a serum-free culture medium supplemented with nerve growth factor (NGF) also showed significant amounts of ChAT (21.9 pmol ACh/min/mg protein). The cultures maintained in absence of NGF had a negligible amount of ChAT activity. A dose-dependent inhibition of ChAT activity was observed after the addition of hydroxyethyl naphthylvinyl pyridine, a specific inhibitor of ChAT, to the assay mix. Cultured sensory neurons incubated with ^{3}H-choline followed by repeated washouts retained significant amounts of ^{3}H-choline. Replacement of NaCl with LiCl from incubation medium reduced ^{3}H-choline uptake by about 45%, whereas in presence of a specific inhibitor of choline uptake, hemicholinium-3, it was inhibited by about 75%. These results suggest that sensory neurons of the chick dorsal root ganglia can express the classical cholinergic properties during development.

170.11

INFLUENCE OF SEGREGATED AFFERENT PATTERNS ON LOCAL CIRCUITS IN RODENT SOMATOSENSORY CORTEX. A. Peinado, T. Murakoshi*, and L.C. Katz. Lab. of Neurobiology, The Rockefeller Univ. NY, NY.

The role of thalamocortical afferents in shaping intrinsic cortical circuits remains largely unknown. Although earlier studies in the rodent somatosensory "barrelfield" cortex have shown that dendrites of stellate cells in layer 4 were confined primarily to one barrel (e.g. Harris & Woolsey, Br. Res. 161:143, 1979), the use of Golgi staining and relatively thin sections made distinguishing stellate and pyramidal cells, or visualizing the intrinsic axons of cells difficult. We have approached this question by using intracellular staining to reveal the dendritic and axonal arborizations of layer 4 neurons in rat barrelfield cortex in relation to the segregated pattern of afferent axons. Thalamic afferents were labelled by anterograde transport of rhodamine (TRITC) injected into the thalamus. Tangential cortical brain slices (350 µm thick) were prepared, and layer 4 neurons in the barrels were intracellularly stained with Lucifer Yellow while simultaneously visualizing the TRITC-labelled barrels. The dendrites and intrinsic axons of three classes of stained cells show distinct relationships to the segregated afferent pattern. Both the axons and dendrites of spiny stellate cells invariably remain strictly confined to one barrel as defined by labelled afferents, frequently making sharp turns in order to do so. The axons of non-spiny stellate cells also remain largely confined to one barrel. Their dendrites, however, can project into the sides and septal region separating barrels. In contrast, the axons of pyramidal cells freely cross barrel boundaries. The dendrites of pyramidal cells located within a barrel remain primarily, although not exclusively within that barrel.

Each whisker termination zone (barrel) represents a region of highly correlated afferent activity. Our observations reveal that the construction of axonal and dendritic arbors of cortical neurons can be greatly influenced by these patterns in a fashion that is cell-type specific and which distinguishes between dendrites and intrinsic axons. Supported by the L.P. Markey Charitable Trust (to L.C.K.).

170.12

EARLY DEVELOPMENT OF FORELIMB REPRESENTATION IN SI CORTEX: BARREL SUBFIELD IN NEONATAL RATS AS DEMONSTRATED WITH PEANUT AGGLUTININ BINDING: EVIDENCE FOR DIFFERENTIAL DEVELOPMENT OF FORELIMB AND FACE. R.S. Waters, C. McCandlish,* and N.G.F. Cooper. Dept. Anatomy & Neurobiology, Univ. of Tennessee, Memphis, Sch. of Med., Memphis, TN 38163.

Physiological studies have demonstrated a highly organized somatotopic representation of the body surface in SI cortex of rat. This representation is correlated morphologically with the presence of barrel-shaped structures in layer IV. Conventional staining techniques reveal barrels in the second postnatal week. Recently, the peroxidase conjugate of peanut lectin (PNA), which recognizes glycosylated molecules, has been used to study barrel field formation. PNA binds primarily to prospective barrel sides and septae as early as postnatal day 4 (PND–4). To date, investigations of SI cortex using PNA have been confined to the study of the barrel field representation of the face and mystacial vibrissae. In the present study we extend these findings to the development of the forelimb representation. Rats ranging from PND–1 (first 24 hrs after birth) to PND–11 were anesthetized with Nembutal and perfused with buffered saline followed by 4% paraformaldehyde and 2% glutaraldehyde in 0.2 M sodium cacodylate buffer. Brains were removed, flattened tangentially, and sectioned on a vibrotome at 30–120 micron thickness. Sections were blocked in 2% bovine serum albumin and incubated in peanut lectin at 4° C. Following incubation, sections were washed with TBS and processed using peroxidase histochemistry.

Lectin binding in the prospective forelimb representation was apparent between PND–7 and PND–10 whereas PNA binding to the prospective face-mystacial vibrissae representation occurred before PND–6 and extended through PND–11. Unlike previous reports in mouse, barrel field labeling in rat was not seen before PND–4. These results suggest that body part representations show individual variations during early pattern formation. In rat, limb formation may lag behind the development of the face–mystacial vibrissae during early postnatal development.
Supported by NSF Grant BNS 85-16076.

170.13

MYSTACIAL PELAGE INFLUENCES VIBRISSA DENERVATION-PRODUCED METABOLIC PLASTICITY IN RAT SI CORTEX. S-C Sheu[1] and P. Hand[2]. [1]Dept. Anat., Nat. Yang-Ming Med. Col., Taiwan, R.O.C.; [2]Dept's Animal Biol. and Neurol., Sch's. Vet. Med. and Med., Univ. Penna., Phila., PA 19104

Neonatal vibrissa denervation produced 2-deoxyglucose (2DG) labeling differences in rodent SI may be partly due to surgical procedures used. Under ice anesthesia, 2 different procedures were used in 2 day old rats to produce identical unilateral vibrissa follicle removals, sparing C3 (SC3): #1 (skin flap removal method; Kossut, J. Neurophys., 1988; N=6) spared row C pelage and #2 (row A-E skin incisions method of Rice; N=6) spared the entire mystacial pelage. 90 days later, SC3 and control C3 (CC3) vibrissae were stroked (4-5hz, rostro-caudally) during the ^{14}C-2DG exp't. Qualitative 2DG changes in lamina IV of SI are reported. SC3 labeling (CC3 comparison) was en-larged, oval-shaped, and diffusely overlayed barrel rows B-D, edges of rows A&E, and involved interrow septa in #1. SC3 labeling was comparably enlarged, primarily overlayed row C (C1-C4), less densely involved a part of row D (typically D3) and occasionally B3, and largely spared interrow septa in #2. Thus the presence of different amounts of mystacial pelage markedly alters neonatal vibrissa denervation produced SI metabolic plasticity. [Grant's NS-22283 (USPHS); NSC77-0412-B010-12 (NSC, Taiwan, R.O.C.)]

170.14

LEFT-RIGHT INTERACTIONS DETERMINE THE CENTRAL EFFECT OF WHISKER CLIPPING IN THE ADULT MOUSE; A DEOXYGLUCOSE (DG) STUDY.

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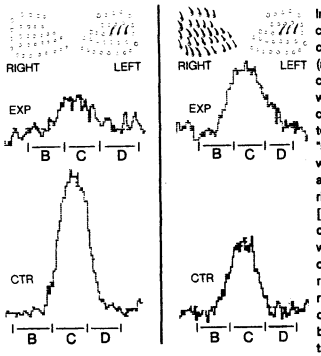

In adult mice (n=8) all whiskers were clipped, except left whiskers C1-3 (left column in Fig); in another set of mice (n=4), all whiskers on the left side were clipped, except C1-3, while the right side was kept intact (right column). The clipped mice (EXP) and a number of untouched mice (CTR) were left in a "stimulus-rich" environment for 5 days; whiskers of all mice were checked daily and clipped when regrown. After this period the animals were injected with [^{14}C]2-DG and left freely-moving in their cage for 45 minutes. Prior to injection the whiskers of CTRs were clipped in either one of the two configurations. In autoradiograms of tangential sections of the right hemisphere grain densities were determined in a strip of cortex across barrels B2, C2 and D2 by the transmittance of light as measured by a tv-camera densitometer (inversely plotted in Fig). In EXP, left column, DG uptake was considerably lower than CTR. In EXP, right column, the maximal DG uptake remained equal to CTR, but in B and D, DG uptake was increased. In CTR of left column DG uptake is higher than that of CTR in right column. We conclude that DG-uptake in the cortex depends not only on the number but also on bilateral distribution of whiskers over a given period of time. Support: Swiss NSF grant 3.158.

170.15

IMPROVEMENT OF TACTILE ROUGHNESS DISCRIMINATION BY EXERCISE IS NOT RESTRICTED TO THE AREA OF TRAINING STIMULATION. M.Pause*, M.Halmanns*, T.Nöcker*, K.H.Mauritz. Rehabilitation Clinic, University of Cologne, D-5000 Köln, FRG.

With the stimulation method described by Lamb(J.Physiol. 338:551,1983) we investigated 1. if tactile roughness discrimination can be improved by exercise and 2. if an improvement is generalized or observed only at the trained area.

Patterns of raised nylon dots (fixed on a rotating disc, velocity 20cm/s) with dot diameters of 1mm (height 0.98mm) and a period of 3.0mm (0%),3.06mm (2%),3.09mm (3%),3.15mm (5%),3.21mm (7%) and 3.3mm (10%) were used. Four subjects were tested for all pattern variants (forced choice for 40 stimulus pairs, 20 identical (0%/0%), 20 different (0%/2,3,5,7,10%) in random order) first at the left index finger pad then at the left little finger pad (stimulated area about 11x11mm) within one session. During the next 10 days all patterns which could not be discriminated better than the 75% level were presented daily in 40 exercise trials only at the index finger pad with confirmation of right decisions and then tested again without feedback. Thereafter the initial test was repeated. In all four subjects there was a clear learning effect: initially discrimination above the 75% level was observed only for the 10% (versus 0%) pattern, after exercise for the 5% or even 2% pattern. In all subjects the learning effect was in the same range at the non-trained little finger. A localized improvement which might have been expected by a plasticity effect of the somatosensory cortex could not be demonstrated.

170.16

AGE DEPENDENT CHANGES IN ACETYLCHOLINESTERASE ACTIVITY IN CAT PRIMARY SOMATOSENSORY CORTEX. C.S.Heck, S.L. Lui*, H.L. Davis & P.A. McKinley. Department of Anatomy, and School of Physical and Occupational Therapy, McGill University, Montreal, Quebec H3A 2B2.

Total acetylcholinesterase (AChE) activity and activity in membrane bound and soluble fractions were measured in cat somatosensory cortex using the method of Ellman et al. (Biochem. Pharmac. 7: 88, 1961). Cats were killed on postnatal days 4, 10, 12, 14, 17 and 28 days by drug overdose. The isolated somatosensory cortex was removed, homogenized and centrifuged and the three fractions were prepared for biochemical assays (electrophoretic analyses and esterase activity). From birth to 10 days, total AChE activity was low (5 moles of substrate hydrolyzed/min/mg protein), increased dramatically by day 12 (20 moles/min/mg), and reached a plateau by day 14 (30 moles/min/mg). Activity in the soluble fraction was low throughout the 28 days studied (2 moles/min/mg), while the activity of the membrane bound fraction increased significantly at day 12 (14 moles/min/mg). It is concluded that the increase in membrane bound activity is responsible for the dramatic increase in total AChE activity by day 12. This change in activity is coeval with other maturational changes in the central nervous system (McKinley, P.A. et al., Dev. Brain Res. 31: 136, 1987), and may have important ramifications for changes in cortical plasticity. (Supported by MRC and J.W. McConnell Fellowship to CSH).

170.17

SEQUENTIAL WAVES OF DIFFERENTIATION IN THE COCHLEA OF MONODELPHIS DOMESTICUS F.H. Willard and B.L. Munger*, Depts. of Anatomy, Univ. of New England, Biddeford, ME, and The Milton S. Hershey Med. Ctr., The Penn. State Univ., Hershey, PA.

Complex interactions exist between the development of the receptor organ, peripheral nerve and brainstem nuclei in the auditory system. We have begun investigating these interactions using an age-graded series of Monodelphis domesticus pups prepared with a silver stain for axons. Sequential sections through the temporal bone were recorded. The cochlear duct developed from 0.75 turn at birth to approximately 2.25 turns by 8 postnatal days. Subsequently, successive waves of differentiation were observed sweeping from base to apex in the cochlea. In order, the waves consisted of 1.) differentiation of stainable axons, 2.) appearance of identifiable hair cells, and 3.) opening of the tunnel of Corti. Movement for all three waves was fastest in the basal and middle turns, requiring only 3 days to accomplish each turn; whereas 4-5 additional days were required to complete the apical turn. These observations agree with the notion that sensorineural differentiation precedes that of the transducer cells.

170.18

EFFECTS OF UNILATERAL COCHLEAR ABLATION IN NEONATAL AND MATURE FERRETS ON NEURON SIZE AND NUMBER IN THE COCHLEAR NUCLEUS. David R. Moore, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

Perinatal cochlear ablation in chicks and rodents results in neuron loss and shrinkage in all divisions of the cochlear nucleus (CN), whereas cochlear ablation in adults produces less severe transneuronal atrophy. Similar results have been suggested in the cat, although no study has provided a quantitative analysis of the age dependency of these phenomena in any carnivore or primate. I have examined the effects on anteroventral CN (AVCN) neuron size and number of rearing ferrets with a unilateral cochlear ablation from postnatal day (P)5, P24, P90 or P180 for 90 days. Ablations were confirmed histologically by the total absence of cochlear ganglion cells. Brain tissue was paraffin-embedded, sectioned at 10μm in the frontal plane and Nissl-stained. Ablations at all ages produced similar mean neuron size reductions [9%(P5), 12%(P24), 13%(P90) and 12%(P180)]. In contrast, neuron loss in the AVCN was only observed after cochlear ablation in the two youngest age groups [57%(P5), 13%(P24)]. Detailed analysis of the whole CN in three ferrets ablated at P24 showed a slight gradient of increasing neuron shrinkage and loss towards the rostral CN. These results confirm earlier observations on adult cats and show that, unlike chicks, neuron loss and shrinkage do not occur over the same time course in ferrets.

170.19

THE EFFECT OF AGE AND EXPERIENCE ON INHIBITION OF THE ACOUSTIC STARTLE RESPONSE (ASR) BY GAPS IN BACKGROUND NOISE. K.F. DEAN,* L.P. SHEETS,* K.M. CROFTON, AND L.W. REITER (SPON:R. JANSSEN). U.S. EPA, Research Triangle Park, NC 27711.

The acoustic startle response (ASR) is inhibited when the eliciting stimulus is preceded by a brief gap in background noise (gap inhibition)(Ison, 1982). We have observed that adult rats show increased inhibition over the first three test sessions. This improvement in inhibition over sessions may explain a previous report in which gap inhibition was absent in rats repeatedly tested on postnatal days (PND) 21 and 25, but present on PND 28 (Kellogg et al., 1983). The present experiment examined the ontogeny of ASR gap inhibition in the rat and the role of experience on its development. Independent groups of Long-Evans rats were tested for three consecutive days, at eight ages ranging from PND 14-16 to PND 98-100. Gaps (0, 2, 4, 8, 16, 32, or 64 msec) in broad-band background noise (80 dB ON/35 dB OFF) were presented 190 msec prior to the eliciting stimulus (13-kHz, 120-dB, 40-msec tone with 2.5 msec rise/decay). Results show that longer gaps (16-64 msec) inhibited the ASR at all ages; however, the magnitude of inhibition increased with age and experience. The efficacy of shorter gaps (8-4 msec) in the inhibition of the ASR improved until PND 35-37, when the adult pattern was achieved. These findings indicate that the magnitude of ASR gap inhibition and the efficacy of gaps < 16 msec are dependent on the age and experience of the animal.

170.20

MASSETER INHIBITORY PERIODS AND SENSATION TO ELECTRICAL TOOTH-PULP STIMULATION IN CHILDREN AND ADULTS. Y. Sharav* & M. Tal. School of Dental Medicine, Jerusalem, Israel

Sensory detection and masseter inhibitory periods (MIP) to electrical tooth-pulp stimulation were recorded in 10 children and 5 adults. The MIP thus induced is normally copmosed of a two-phase EMG activity with early and late components.

In the adult group sensation was always detected and usually associated with a two-phase MIP configuration. Sensory detection in 1/2- and 3/4-formed roots of teeth in the younger children was absent or markedly reduced and was accompanied by the absence of the late component of the MIP. In the older children with fully-formed roots (10-12 year-old) sensation did not differ from that of the adults, but the late MIP component occured significantly less ($p<0.01$).

It is suggested that the following developmental stages of response to electrical tooth-pulp stimulation occured, and in that order: 1)the early, oligosynaptic, component of the MIP, 2) detection of sensation, and 3) the late, polysynaptic, component of the MIP. It is concluded that the development of sensory detection and MIP activity to electrical tooth-pulp stimulation are two distinct processes.

Supported by the David and Hedy Epelbaum Fund for Pain Research.

DIFFERENTIATION AND DEVELOPMENT III

171.1

MYELINATION OF AXONS BY SCHWANN CELLS IS F-ACTIN DEPENDENT, AS DETERMINED BY THE USE OF CYTOCHALASIN. D.S. Gorman* and M.B. Bunge. Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Linkage between Schwann cell (SC) basal lamina formation and myelination has been well-established in the dorsal root ganglion culture system; SCs that are unable to form basal lamina are also unable to ensheathe and myelinate axons (Bunge et al., 1986, Ann. Rev. Neurosci. 9:305). Could the basal lamina initiate SC elongation and myelination by causing SC surface polarization and/or modification of the cytoskeleton? To determine the dependence of SC differentiation (elongation, ensheathment and myelination) on actin, neuron + SC cultures were initially grown in serum-free defined medium which does not promote SC differentiation and then switched to differentiation-supporting medium containing cytochalasin D (CD, 0.25 - 1.0 ug/ml). During the two subsequent weeks, cultures were processed for light and electron microscopy. The effects of CD were visible as early as 5 hr; SCs retained a more rounded appearance compared with SCs in control cultures. After 1 wk, SCs were abnormally balled-up on axon fascicle surfaces. The severity of effects was more prominent as CD concentrations increased. Sudan black staining revealed small amounts of early myelin at the lowest concentration and none at the highest concentration. Electron microscopy showed 3-4 partially compacted turns of myelin at the lowest concentration and no myelin and a paucity of SC elongation and ensheathment at the highest concentration. These experiments indicate the dependence of SC elongation, ensheathment, and myelination on a functional actin system. (Supported by NIH NS09923)

171.2

SCHWANN CELLS ARE IMMUNOREACTIVE FOR ALBUMIN DURING NERVE DEVELOPMENT. M. Mata and D.J. Fink, Neurology Research Laboratory, University of Michigan and VA Medical Center, Ann Arbor, MI 48105.

We have demonstrated immunocytochemically that albumin is found extracellularly but not intracellularly in normal nerve endoneurium (J Neuropathol Exp Neurol, 1987), and that during regeneration after a nerve crush injury Schwann cells demonstrate albumin immunoreactivity, but lose that immunoreactivity as myelination of regenerating sprouts proceeds (J Neuropathol Exp Neurol, in press). In order to better define the role of albumin uptake by Schwann cells during myelination, we studied albumin immunoreactivity in developing sciatic nerve.

Neonatal Sprague Dawley rats from 1 to 14 days of age were perfused with 0.5% glutaraldehyde and 4% paraformaldehyde and the sciatic nerves embedded in LR White. Ultrathin sections were exposed to goat anti-rat serum albumin antibody at dilutions of 1:1500 or 1:2000 for 1.5-2 hrs, followed by rabbit anti-goat IgG bound to 15 nm colloidal gold (1:10) for 1 hour. The specificity of the anti-RSA antibody was tested by immunodiffusion and by Western blot.

At 1 d albumin immunoreactivity was confined to the endoneurium, Schwann cells and axons were not immunoreactive. From 3 d to 7 d the Schwann cells showed albumin immunoreactivity, though the axons did not. By 14 d the the immunoreactivity was again limited to the extracellular space of the endoneurium. Neither the Schwann cells nor the axons were immunoreactive.

In vitro studies recently have shown that a serum factor (for which albumin may be substituted) is required in the medium for Schwann cells to myelinate axons in tissue culture. Our results suggests that albumin uptake by Schwann cells may be necessary in vivo as well. Albumin may serve as a carrier for substances required by the Schwann cell.

Supported by VA Merit Review Grants to Dr. Mata and Dr. Fink.

171.3

SCHWANN CELL BASAL LAMINA FORMATION AND ENSHEATHMENT OF UNMYELINATED NEURITES ARE INCREASED IN CULTURES CONTAINING FIBROBLASTS. V.J. Obremski*, M.I. Johnson+, and M.B. Bunge (SPON: M. Luskin). Depts. Anatomy and +Pediatrics, Washington Univ. Sch. Med., St. Louis, MO 63110.

The ability to culture and combine relatively pure populations of nerve cells (NCs), Schwann cells (SCs), and fibroblasts (FBs) has enabled us to examine the contribution of FBs to SC ensheathment of unmyelinated rat superior cervical ganglion (SCG) neurites in vitro. Purified perinatal SCG neurons were grown in culture with SCs alone or with SCs and FBs derived from SCG capsule or periosteum. These cells were maintained for 10-12 weeks on a collagen substratum in a medium known to promote differentiation of SCs with dorsal root ganglion neurites (Eldridge et al., JCB 105:1023, '87). The extent of ensheathment was determined by assessing the proportion of neurites enclosed individually by SC cytoplasm in electron micrographs of cross sections of neurite outgrowth. In all NC/SC cultures without FBs, SCs achieved only a moderate degree of ensheathment; also SC basal lamina was often discontinuous, and extracellular collagen fibrils were not abundant. The addition of FBs to NC/SC cultures enhanced the neurite-ensheathing ability of the SC. Moreover, basal lamina surrounding SC/neurite units was continuous and collagen fibril content was increased. Additional secretion by cells in FB supplemented cultures was suggested by the presence of dense fibrous extracellular material near the FBs. We conclude that the presence of FBs in cultures of SCG neurons increases the ability of the SC to ensheathe neurites, possibly through an augmentation of specific extracellular matrix components. (NIH NS15070, NS21771, NS07071; NSFBNS8508148)

171.4

NEURON-GLIA INTERACTIONS IN HIPPOCAMPAL CULTURES. U.E. Gasser* and M.E. Hatten. Dept. of Pathology, College of Physicians & Surgeons of Columbia University, New York, NY 10032

In previous studies, our laboratory has used a microculture system to analyze neuron-glia interactions of cerebellar cells. Here we have adapted this system to study neuron-glia interactions and astroglial differentiation of developing hippocampus. Hippocampal tissue, harvested from neonatal rats, was dissociated into a single cell suspension and plated in glass coverslip microcultures coated with poly-lysine (PLYS), poly-ornithine (PORN), Concanavalin A (Con A), Matrigel (MAT) or wheat germ agglutinin (WGA). In some experiments, neurons were washed off 45 min after the cells were plated, leaving a pure glial population. After 24h *in vitro*, the cells were immunostained with antibodies against the glial filament protein (GFP) to visualize glial cells and measure the distribution of neurons relative to astrocytes.

In the absence of neurons, purified astroglia had flat, undifferentiated profiles on all substrates tested. In co-culture with neurons, the extent of neuronal association with glia and of glial process extension varied with the culture substrate. On WGA, Con A and MAT, neurons exhibited a marked affinity for astrocytes, and astroglial cells expressed short (WGA, Con A) or elongated (MAT) processes which were intensely stained with GFP. On PLYS and PORN, however, the neurons were randomly distributed on the culture substrate, and astrocytes expressed flat, undifferentiated shapes. These results suggest that the extent of neuron-glia association *in vitro* varies with the culture substrate, and that, as previously shown for cerebellar cultures, the morphological differentiation of hippocampal astroglia is dependent upon contact with neurons.

Supported by NIH grant NS 21097 and the American Paralysis Association.

171.5

DUAL POPULATIONS OF RADIAL GLIAL CELLS IN THE DEVELOPING OPOSSUM SUPERIOR COLLICULUS. P.C. Barradas*, R. Mendez-Otero, A. Madeira-Vieira* and L.A. Cavalcante*(SPON:C.Timo-Iaria),IBCCF,Univ. Fed. Rio de Janeiro and Inst. Biology, UERJ, 21941,Rio de Janeiro, Brazil.

Uptake of HRP following an eye injection reveals radial glia-like cells, the processes of which course through the developing opossum superior colliculus(SC). Such cells persist for long periods and are consistently absent from median and paramedian regions. We report here the results of an analysis of radial glial cells in the opossum SC by GFAP and vimentin(VIM) immunoreactivities and by glycogen histochemistry. We have used pouch youngs of ages ranging from postnatal days 12 to 60 (PND12 to PND60), i.e., developmental stages ranging from the end of collicular neurogenesis to the onset of myelination in the optic layer. Weanlings(PND90) and adult animals were also used to test for long-term radial glia. GFAP and glycogen reactions revealed a median system of tortuous processes(median ventricular formation-MVF) in all but adult animals. Lateral to MVF, GFAP reaction is weak or absent except in rare sub-pial endfeet. GFAP+ astrocytes of the optic layer appear in a medial-to-lateral sequence from PND40 to PND60. VIM reaction shows both MVL and a lateral system (LS) of straight radial processes from PND 12 to PND40 with progressive reduction of LS thereafter and occasional staining of widely dispersed astrocytes. VIM reaction is limited to MVF in PND60 and PND90 and is absent in adults. The results suggest a dual population of radial glia which differ in the degree of interactions with the neuropil as well as in the GFAP expression and glycogen accumulation. Supported by CNPq, FINEP and CEPG/UFRJ.

171.6

OLIGODENDROCYTE DIFFERENTIATION IS ACCELERATED BY A PULSE OF CYCLIC AMP. David W. Raible* and F. Arthur McMorris (Spon.: B. Wolfe). TheWistar Institute, Philadelphia, PA 19104.

Oligodendrocytes develop in primary cultures established from 1-day-old rat brain on a specific schedule, similar to that seen *in vivo*. We have shown previously that this differentiation schedule is accelerated by the addition of the cyclic AMP analog sdibutyryl cAMP (dbcAMP) and 8-bromo cAMP, which accelerate the expression of the myelin components 2',3'-cyclic nucleotide 3'-phosphohydrolase, myelin basic protein and galacto-cerebroside. Dibutyryl cAMP also inhibited DNA synthesis in A2B5-positive oligodendrocyte-type 2 astrocyte (O2A) progenitor cells, consistent with the relationship between cessation of proliferation and onset of differentiation seen *in vivo* and *in vitro*, but had no effect on the proportion of O2A progenitors that became oligodendrocytes rather than type 2 astrocytes. We now report that a pulse of 1 mM dbcAMP as short as five minutes has the same effect as treatment with dbcAMP for two days, implying that oligodendrocyte differentiation can be accelerated by a trigger mechanism. Pulses of dbcAMP obviate the problem of cell detachment that occurs after prolonged dbcAMP exposure, allowing us to study the effects of dbcAMP on oligodendrocyte differentiation over longer periods of time. As observed in continuously treated cultures, a pulse of dbcAMP initially accelerated of the appearance of oligoden-drocytes. Several days after treatment, the number of oligodendrocytes in dbcAMP-pulsed cultures decreased compared to controls. This decrease in oligodendrocyte number may be due to the inhibition of progenitor proliferation reported previously, or to early cell death after accelerated differentiation. Oligodendrocyte numbers eventually return to normal in cultures pulsed with dbcAMP. Supported by NSF BNS 85-18023, NMSS RG1767-A-1 and NIH CA 09171 and NS 11036.

171.7

STRIATAL ASTROGLIA DOPAMINE RECEPTORS ARE AFFECTED BY NEURONS FROM SUBSTANTIA NIGRA. E.Hansson, Institute of Neurobiology, University of Göteborg, Göteborg , Sweden.

Using selective D1 agonists the presence of a dopamine receptor with cyclic AMP as second messenger was demonstrated on astroglial cells in primary culture from striatum. The D1 receptors were not identified in cultures from cerebral cortex, hippocampus or brain stem. In a co-cultivation system neurons from substantia nigra affected the striatal astroglia to shift the dose-effect curve of the D1 cyclic AMP system to the left and thus increase the potency and efficacy of the second messenger of the receptor. The specificity of the results were ensured in two ways: co-cultivating the astrocytes with hippocampal neurons or with another astrocyte culture had no effect on the D1 receptor cyclic AMP system. In addition there was no effect on the β-adrenoceptor cyclic AMP system on astroglia after co-cultivation with neurons from substantia nigra. The results provide evidence for a heterogeneity among astroglia with respect to the expression of receptors. Furthermore, astrocytes in one brain region were influenced upon by neurons from a natural projection area to increase the receptor activated second messenger stimulation.

171.8

GROWTH CHARACTERISTICS OF PRIMARY HUMAN FETAL ASTROCYTE (HFA) STRAINS. J.J. Chao*, R.W. Keane, and W.P. Parks* (SPON: L. Glaser). Depts. of Micro. Immuno., Physiol. Biophys., and Pediatrics, University of Miami School of Medicine, Miami, FL 33136.

Human cell strains consisting predominantly of glial fibrillary acidic protein (GFAP)-positive astrocytes were established from abortuses of 8 - 18 weeks gestation. Several media and substrates were evaluated to determine optimal plating efficiency and growth. Media were supplemented with 5% horse serum; N5, a medium free of selected amino acids and Dulbecco's modified Eagle's medium were comparable as measured by growth rates whereas RPMI 1640 supported only limited growth. Plating efficiency was evaluated using a rat tail collagen preparation, commercial collagens, and poly-L-lysine to pretreat tissue culture flasks. A combination of rat tail collagen and poly-L-lysine treatment yielded the highest plating efficiency (70% ± 5). Under optimal conditions in the logarithmic phase of culture growth, HFA have a doubling time of 30 hours (±6). Cell growth occurs as multilayered foci even in sparsely plated cultures. Over time, cells radiate from foci until confluence is established at cell densities of $2.5 \times 10^5/cm^2$. GFAP-positive HFA can be maintained in culture for up to 9 months with over 25 passages without obvious changes in morphology or growth characteristics. Thus, it is possible to establish and to define growth requirements and characteristics of in vitro HFA cultures for long-term studies.

171.9

SUBSTRATUM EFFECTS ON MELANOGENESIS IN NEURAL CREST CULTURES. S.L. Rogers, K.S. Vogel*, J. Leyba*, A. Waite*, and J.A. Weston*. Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131 and Univ. of Oregon, Eugene, OR 97403.

The role of the extracellular enviroment in neural crest differentiation remains unresolved. In neural crest cultures, the extent of cell-cell association appears to influence both neuronal and melanocyte differentiation (Vogel and Weston, J. Cell Biol. 103: 232a). Clusters of quail neural crest cells were isolated from explanted neural tubes at 24 or 48 hours of culture and then subcultured on fibronectin (FN) and laminin (LM). In cultures of 24 hour clusters, cells plated on FN disperse evenly and approximately 20% of the population differentiates into melanocytes. On LM and untreated substrata, cell dispersal is not as extensive and, consequently, many cells remain in close contact with one another. Close to 40% of cells in these cultures pigment. 48 hour clusters plated on untreated substrata pigment to a much greater extent than do 24 hour clusters (Glimelius and Weston, Cell Diff. 10: 57). When cultured on FN or LAM, dispersal of cells in 48 hour clusters exceeds that of controls, and 20-50% fewer cells pigment. Therefore, crest-derived cells in the two culture paradigms differ in their response to the three substrata. Differences in selectivity and responsiveness to these substrata appear to alter the patterns of cell-cell interaction, which may lead to differences in phenotypic expression. (Supported by NIH grants NS23368 to S.L.R. and DE04316 to J.A.W.)

171.10

EXPRESSION OF MUSCARINIC CHOLINERGIC RECEPTORS ON RAT SWEAT GLANDS. M.P. Grant* and S.C. Landis. (SPON: S.W. Jones) Center for Neuroscience and Dept. of Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

The mechanisms which regulate the expression of neurotransmitter receptors on target cells are poorly understood. Sweat glands provide an interesting model to explore the possible relationship between transmitter phenotype and receptor expression because the transmitter properties of its innervation changes during development.

We have developed a protocol to examine muscarinic ligand-binding sites in gland-rich foot pad with the radiolabeled muscarinic antagonist ^{3}H-N-methyl scopolamine(^{3}H-NMS). Scatchard analysis indicated that the concentration of ^{3}H-NMS sites is 230 fmoles/mg protein. Autoradiographic studies have demonstrated that the ^{3}H-NMS sites are localized on glands. During development the distal portion of the forming glands initially expresses low levels of ^{3}H-NMS sites. As the glands mature and the innervation becomes functionally cholinergic the concentration of ^{3}H-NMS sites increases. Glands which are acutely or chronically denervated express a reduced level of ^{3}H-NMS sites which are not responsive to agonists. Our results suggest that functional cholinergic innervation is one of several factors that regulate the expression of muscarinic ligand-binding sites in rat sweat glands.
Supported by NINCDS 23678.

171.11

CHARACTERIZATION OF NEURONS DIFFERENTIATING IN CULTURES OF MOUSE NEURAL CREST CELLS. S.G. Matsumoto* (SPON: R.B. Levine) Dept. Physiology, Univ. Ariz. Coll. Med., Tucson, AZ. 85724

Neural crest cells are obtained from E 7.5 to 9.5 mouse embryos. Several thousand neurons eventually differentiate in cultures that initially contain 100-200 crest cells, indicating the presence of persistant neuroblasts. Only the morphologically differentiated neurons stain with antibodies to neurofilament. At least 2 classes of neurons differentiate in the cultures; one resembling sensory neurons and a second adrenergic class. The first class of neurons appear after 4 days to a week in vitro. Some of these show substance P immunoreactivity. These neurons do not label with antibodies to VIP, SOM, NPY, TH or display glyoxylic acid induced fluorescence (GIF). Electrophysiological recordings reveal that a subpopulation of these neurons are sensitive to capsaisin. A second, numerically larger group of neurons appears over a period of 1-3 weeks. These neurons all stain with anti-TH and show intense GIF.

171.12

A NOVEL METHOD FOR MARKING NEURAL CREST CELLS USING DiI. George Serbedzija, Scott Fraser, and Marianne Bronner-Fraser. Developmental Biology Center, University of California, Irvine, Ca. 92717.

Neural crest migratory pathways have been well-mapped in avian embryos using quail/chick chimeric techniques and the HNK-1 antibody. Far less is known about neural crest pathways in other species because the HNK-1 antibody is avian specific. Furthermore, in avians the HNK-1 antibody recognizes cell types other than neural crest cells, and quail-chick chimeras require surgical disruption of the environment through which neural crest cells migrate.

To confirm neural crest migratory pathways in chick and to define pathways in other species, we have used a nondeleterious membrane intercalating dye, diI (Honig and Hume, 1986, JCB 103:171), to label neural crest cells before they emigrate from the neural tube. Using a picospritzer, diI (0.5% in 95% ethanol/5% DMSO) was pressure injected into the lumen of the neural tube of chicks having 18-28 somites. Only neural tube and premigratory neural crest cells are labelled with the dye, because they are the only cells in contact with the lumen. As expected, migrating neural crest cells, motoraxons, and ventral root cells are the only cells types stained with diI external to the neural tube. Discreetly labeled cells were found adjacent to the neural tube and within the rostral half of each sclerotome within the first day after injection, confirming previous cell marking studies. A few cells were also found subepidermally. In embryos fixed two or more days after diI injection, dorsal root and sympathetic ganglia and some cells around the dorsal aorta were brightly labeled with diI. Recently, we have successfully used this technique for studying mouse neural crest development, indicating its broad applicability.

171.13

STABLE EXPRESSION OF A TEMPERATURE SENSITIVE V-SRC GENE IN PC12 CELLS REVERSIBLY MIMICS DIFFERENTIATION BY NGF. D.M. Rausch*, and L.E. Eiden* (SPON. D. Lewis). Unit on Molecular and Cellular Neurobiology, Laboratory of Cell Biology, NIMH, Bethesda, MD. 20892

The PC12 rat pheochromocytoma cell line has been used extensively as a model system to study neuronal differentiation. Morphologically, these cells resemble adrenal chromaffin cells, but develop into sympathetic neuron-like cells when cultured in the presence of nerve growth factor (NGF), or following infection with an avian retrovirus carrying the viral oncogene, src (Alema et al., 1985, Nature 316:557-559). In this study, a recombinant retrovirus was used to introduce a temperature sensitive src gene (provided by H. Hanafusa, Rockefeller University) along with the gene encoding for a neomycin resistance protein into PC12 cells. Following selection of these cells in G418, a stable cell line was produced that proliferates at the non-permissive temperature of 40° C with a rate similar to uninfected cells. When switched to the permissive temperature (37° C), the majority of these cells stop dividing and extend neurites resembling cells that have been cultured with NGF. Long term growth at the permissive temperature results in small colonies of cells with extensive neuritic networks.

$pp60^{c\text{-}src}$ is the normal cellular homologue of $pp60^{v\text{-}src}$. Like $pp60^{v\text{-}src}$, $pp60^{c\text{-}src}$ is a tyrosine kinase that is strongly expressed in developing neurons in vivo and may play a role in neural differentiation. This population of PC12 cells expressing v-src is now being used to study src induced neural differentiation, and to compare it with NGF induced differentiation with respect to its reversibility, effects on membrane potential (see Lewis et al., these abstracts), and expression of neuronal markers.

171.14

PC12 CELLS FORM TUMORS IN THE BRAINS OF FETAL AND EARLY NEONATAL, BUT NOT LATE NEONATAL OR ADULT RATS. J.D. Hatton, P.Y. Kelley and H.S. U. Division of Neurosurgery V-151, U C San Diego, La Jolla, CA 92093

PC12 cells represent a rat pheochromocytoma clonal line generally thought of as a neoplastic counterpart of neurons. They respond to nerve growth factor (NGF) treatment in vitro by differentiating into post-mitotic, process-bearing and action potential-competent cells. We injected PC12 cell suspensions into the brains of fetal, neonatal and adult Sprague-Dawley rats. Even small numbers of cells (10^{3}-10^{4}) produced prolific tumors within 3-4 weeks when placed into the frontal lobes of fetal (E17-E20) and early neonatal (day 1-4) rats. This tumorigenic potential declined linearly with neonatal age (day 5-10). No tumors were produced in adult hosts whether cells were placed stereotaxically or into preformed cavities. Such injections and cavities were examined in cortex, cerebellum and hippocampus. No tumors were seen in these adults even if cells were densely packed and/or in very high numbers (10^{6}-10^{7} cells pelleted before implantation). However, suviving post-mitotic cells were seen in these latter brains, suggesting that the grafts were not simply rejected. The relationship of PC12 tumor growth to the the local expression of NGF gene product(s) is being studied.

171.15

NEURONAL SPECIFIC PROPERTIES EXPRESSED BY K-RAS INFECTED PC12 CELLS. J.Bressler, H.Miyake*, C.Chiueh and M.Brightman. NIH, Bethesda, MD 20892

PC12 cells respond to ras virus infection by mitotic arrest, neuritic extension and enhanced acetylcholinesterase(AChE) activity (Noda,M.,et al., Nature 318:73, 1985). Do changes in dopamine(DA) synthesis and release also occur after ras infection? PC12 cells were seeded ($6X10^3$cells/cm^2) under standard conditions and infected with K ras virus. Control cultures were treated similarly but without virus. Four-10 days after ras infection, conditioned medium and cells were assayed for DA levels by an HPLC-Electrochemical method. Control and infected cultures were assayed for DA before and after stimulation with d-amphetamine and K^+. While amphetamine and K^+ resulted in a 7% release above basal levels in controls, there was a 25% rise in DA release by stimulated infected cells. AChE activity in these cells rose by 63%, (cf Noda et al). Tyrosine hydroxylase and choline acetyl transferase are also present in our infected cells. Protein synthesis levels were unchanged. Most PC12 cells had emitted neurites about 4-200um long. Some cells did not emit neurites; we infer from our data on DNA synthesis and from our ^{3}H-thymidine radioautography, that such cells are still mitotic. Although there was a greater release of DA from infected cells, the amount may be smaller than that from wild PC12 cells, despite an augmented neuritic outgrowth from the ras treated cells.

171.16

C-SRC EXPRESSION IN THE BRAIN OF VERTEBRATES AND INVERTEBRATES. X. Yang* and G. Walter. (Spon: J.M. Le Beau). Department of Pathology, University of California, San Diego, La Jolla, CA 92093

The proto-oncogene c-src, the cellular homolog of the transforming gene v-src of Rous sarcoma virus, encodes two closely related protein-tyrosine kinases. The larger form, pp60c-src+, differs from the smaller one, pp60c-src, by an insert of six amino acids in the amino-terminal region (Martinez R. et al., Science, 237:411, 1987; Levy J. B. et al., Mol. cell. biol., 7:4142, 1987). pp60c-src+ is neuron-specific, whereas pp60c-src is expressed in neuronal as well as non-neuronal cells. To study the evolutionary conservation of the six amino acid insert, we assayed for pp60c-src+ in the brain of different animal species using two different kinds of antibodies. One, generated against a synthetic peptide corresponding to the insert, reacted only with pp60c-src+. The other, raised against pp60v-src, reacted with pp60c-src+ and pp60c-src in different species. pp60c-src+ was detected only in the brain of higher vertebrates (mammals, birds and reptiles), but not lower vertebrates (amphibians and fish), or invertebrates (such as lobster). pp60c-src was present in the brain of all species tested. Although we cannot rule out the possibility that pp60c-src+ is expressed at low levels in brain tissues of lower vertebrates and invertebrates, our data show that significant levels of pp60c-src+ expression are correlated with increasing neuronal complexity across animal species. This finding suggests that pp60c-src+ may assume a role in events associated with higher brain function, such as plasticity.

(Supported by ACS grant CD-276 to G.W.)

171.17

Butyrate-Induced Differentiation of NG108-15 Cells is Preceded by Heterologous Desensitization of Adenylyl Cyclase and Decreases in $G_s\alpha$ and its mRNA. M.E.Charness and M.Henteleff* Department of Neurology and the Gallo Center, Univ. Cal. San Francisco, CA. 94143.

G proteins are developmentally regulated and may in turn regulate cell division, differentiation, and synaptogenesis. We studied G protein regulation during butyrate (Bu) -induced differentiation of NG108-15 cells. Addition of 1 mM Bu to NG108-15 cells cultured in defined medium arrested cell division after 24 h and induced neurite formation after 24-48 h. Cells treated with Bu for 4 days had long thick neurites, resting potentials below -50 mV and fired fast action potentials. Bu did not change intracellular cAMP after 15 min or 6 h; however, after 1 or 4 days, Bu-treated cells showed a decrease in cAMP accumulation stimulated by prostaglandin E_1 (30% and 40% of untreated cells, days 1 and 4, respectively), phenylisopropyladenosine (16% and 18%), or cholera toxin (56% and 37%) and a concomitant increase in maximal inhibition of forskolin-stimulated cAMP accumulation by δ-opioid, α_2-adrenergic, and muscarinic agonists.
Western blots of membranes from Bu-treated NG108-15 cells showed a significant decrease in $G_s\alpha$ (74% and 54% of untreated cells; $p<.05$,.005; days 1 and 4 respectively) and a small increase in $G_i\alpha$ (119% and 116% of control, $p>.05$). Bu also reduced $G_s\alpha$ mRNA after 1 and 4 days. Thus, changes in G protein-mediated signal transduction occur early during butyrate-induced differentiation of NG108-15 cells and may influence later events of differentiation.

171.18

OLFACTORY BULB INFLUENCE ON G-PROTEIN EXPRESSION IN RAT OLFACTORY EPITHELIUM. B. Mania-Farnell* and A.I. Farbman. Northwestern University, Evanston, IL, 60208.

Olfactory bulbectomy in mammals results in degeneration of olfactory epithelial neurons (ON). With time new neurons differentiate from basal cells in the epithelium, but the epithelium never reaches pre-bulbectomy conditions in cell number, thickness or olfactory marker protein expression (Costanzo & Graziadei 1983; Monti Graziadei, 1983). In vitro studies have shown that maturation of receptor cells is enhanced by the presence of the olfactory bulb (Chuah & Farbman, 1983; Chuah et al., 1985). We wanted to determine if the expression of G-proteins, believed to be involved in the transduction of stimuli in ON (Pace et al., 1985; Sklar et al., 1986), is affected by bulbectomy. Harlan rats were unilaterally bulbectomized; 17 and 30 days postoperatively frozen sections of ON were prepared for indirect immunoperoxidase staining. The primary antisera were monospecific polyclonal antibodies generated in rabbits by immunization with synthetic peptides (Mumby et al., 1986): 1) one peptide had an amino acid sequence common to the alpha subunits of Gs, Gi, Go and transducin; 2) the second peptide was specific to the 35,000 MW form of the beta subunit. With both antisera the reaction product was limited to the control side. Our results indicate that the olfactory bulb is necessary for G-protein expression in the ON.

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171.19

COMPARISON OF THE EFFECTS OF RETINOIC ACID AND NGF/db cAMP ON DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS. S.A.D. Sharma, J.T. Hansen and M.F.D. Notter. Dept. of Neurobiol. and Anat., Univ. of Rochester, Rochester, NY 14642.

P19S18O1A1 (O1A1) embryonal carcinoma (EC) cells can be induced to differentiate into a number of different cell types, including neurons. We have reported that retinoic acid (RA) or nerve growth factor/dibutyryl cyclic AMP (NGF/db cAMP) can induce neuron-like changes in O1A1 cells. Here we compare the effects of RA and NGF/db cAMP on the expression of AEC3A1-9 antigen, Band-3 protein, and on the ultrastructure of O1A1 cells.

O1A1 cells were grown for 2 days as monolayers followed by 2 days as aggregates in the presence of RA (500 nM) or NGF/db cAMP (375 ng/ml/ 500 ug/ml). The dissociated aggregates were then plated onto coverslips and maintained for 6 days in the absence (RA) or presence (NGF/db cAMP) of growth modulators.

RA had a much more striking and reproducible effect than NGF/db cAMP on inducing the neuronal phenotype in O1A1 cells. AEC3A1-9 is a surface antigen expressed on undifferentiated EC cells but not by differentiated EC cells. RA induced an almost complete loss of AEC3A1-9 expression by O1A1 cells. Treatment with NGF/db cAMP caused a major loss of AEC3A1-9 expression but there were many cells left which expressed some AEC3A1-9. Band-3 glycoprotein is found on red blood cells and, in neurons has been shown to be altered during aging. RA caused a dramatic increase in Band 3 expression compared to undifferentiated O1A1 cells. NGF/db cAMP also produced an increased Band 3 expression, though not as significantly as RA. Transmission EM of undifferentiated O1A1 cells showed cells with large nuclei and multiple nucleoli, and no evidence of neurofilaments. RA treated O1A1 cells showed microtubules, neurofilaments and nuclei with one nucleolus. NGF/db cAMP produced cells with only one nucleolus per nucleus, but with no intermediate filaments as seen by TEM. The data suggest that RA induces a more complete differentiation of O1A1 cells than NGF/db cAMP.

Funded partially by Training Grant T32AG00107 (SADS).

171.20

31P NMR STUDIES OF THE CHANGES IN INTERNAL INORGANIC PHOSPHATE, ATP, PHOSPHOCREATINE AND PHOSPHOETHANOLAMINE IN DIFFERENTIATED AND UNDIFFERENTIATED N1E-115 NEUROBLASTOMA CELLS WHICH HAVE BEEN SUBJECTED TO 80mM EXTRACELLULAR KCl. P. Glynn* and S. Ogawa*. AT&T Bell Laboratories, 600 Mountain Avenue, Murray Hill, N.J. 07974 and R.L. Chappell. Hunter College, 695 Park Avenue, New York City, N.Y. 10021.

Neuroblastoma cells were seeded on Cytodex III microcarriers, put in the life-support system, which was put into an NMR 360 WB magnet and superfused with a growth medium supplemented with 10 mM creatine to insure that the cells were charged with phosphocreatine (PCr). KCl was added to the recirculating superfusate of the life support system to a final concentration of 80 mM. The undifferentiated cells showed no difference in their phosphate profile after the addition of KCl. In the first 7.5 minute interval after the addition of KCl the differentiated cells showed a phosphocholine decrease of 12% and a phosphoethanolamine increase of 15%. Within 15 minutes these levels returned to their normal control values. Pi in the sample holder increased 33% within 30 minutes and stayed high. There were comparable decreases in the PCr and nucleotide phosphates. The PCr started to decline in concentration before the nucleotide phosphates. When the superfusate was switched to medium without KCl and the creatine was dropped to 0.2 mM, the PCr recovered in the time frame of one hour demonstrating that Cr was not lost from the cells. These experiments show that upon depolarization with 80 mM KCl, the phosphate profile of the differentiated N1E-115 cells changed. It has been shown here that chronic depolarization of these cells drains energy reserves at a rate faster than they can be restored by oxidative phosphorelation and glycolysis.

172.1

MORPHOLOGY OF NEURONS IN FETAL SPINAL CORD TRANSPLANTS J.G. Broton, R.P. Yezierski, and A. Seiger, Dept. Neurological Surgery, University of Miami School of Medicine, Miami, FL 33136.

A modified Golgi technique was used to study E14 spinal cord tissue which had developed in the anterior eye chamber of adult rats. The grafts, hemisections of single lumbar spinal cord segments, had been transplanted seven months previously and measured approximately 2.0mm L x 2.5mm W x 1.0mm H at the time the tissue was processed. Rats were overdosed with pentobarbital, perfused with aldehydes, and the graft and part of the attached iris removed from the eye. The Golgi protocol of Gobel (1978) was followed. Tissue was placed in 5% $K_2Cr_2O_7$ for 4-14 days, then 0.75% $AgNO_3$ for 24 hours. It was then dehydrated and sectioned (100um) on a Vibratome^R. Sections were transferred to xylene, put on slides, coated with DPX, and coverslipped.

Although the grafted tissue lacked projection targets and afferent input, many stained neurons examined thus far were comparable in appearance to those seen in the in situ spinal cord. Neurons often had spines on their dendrites, and axons of partly-stained neurons at the edge of the graft sent terminal arbors with varicosities near dendrites of well-stained neurons. Similar-shaped neurons were often grouped together in the graft. Large neurons (ave. cell body diam. > 30um) often resembled motorneurons and fusiform and multipolar dorsal horn neurons. Smaller neurons (ave. diam. < 12um) were also stained; extensively arborized dendrites were not seen for any of these neurons.

This study has demonstrated that many fetal spinal cord neurons which develop in the anterior eye chamber are morphologically similar to neurons seen in previous Golgi studies of the intact spinal cord.

Supported by NIH Grant NS19509 and by The Miami Project Foundation.

172.2

IMPLANTATION OF IMMATURE ASTROCYTES INTO THE NORMAL AND CONTUSED ADULT RAT SPINAL CORD. D.W. Hoovler, M.D. Castro*, G.M. Smith*, J. Silver and J.R. Wrathall. Dept. of Anat. and Cell Biol., Georgetown Univ., Washington, D.C. 20007 and Dept. of Develop. Genet., Case Western Reserve Univ., Cleveland, OH 44106.

Immature brain astrocytes reduce scar formation, support axonal growth and alleviate functional deficits following implantation into rat brains. To begin to investigate their therapeutic potential for spinal cord injury, we implanted immature astrocytes into spinal cord after standardized contusive injury (*Exp. Neurol.* **88**:108, 1985). Astrocyte cultures were prepared from embryonic rat spinal cord and labeled with ^{3}H-thymidine. Cell suspensions (10^5 viable cells in 10 µl) were implanted into adult rat spinal cord (T8) that was uninjured or had received a standardized mild contusive injury immediately prior to implantation (n = 8 each group). At 1 week, cryostat sections were examined by autoradiography, immunohistochemical staining for GFAP, and H&E staining. Samples of the cells used for implantation showed that virtually all were positive for GFAP and heavily labeled with ^{3}H-thymidine. Thymidine-labeled cells were detected in all implanted spinal cords. The labeling was less dense and the lateral and rostrocaudal distribution of labeled cells more extensive in implants into contused as compared to uninjured spinal cord. The contusion site appears to permit the survival and proliferation of implanted immature astrocytes. Investigation of the chronic effects of such implants should therefore be feasible.

(Supported by NIH-NO1-NS-7-2301)

172.3

SENSORY AXONS IN THE CHRONICALLY INJURED RAT SPINAL CORD CAN REGROW INTO AND THROUGH INTRASPINAL TRANSPLANTS OF FETAL SPINAL CORD TISSUE. J.D. Houle, Dept. of Anatomy, Univ. of Arkansas Med. Ctr., Little Rock, AR 72205

Primary afferent axons can regenerate if cut dorsal roots are immediately apposed to transplanted fetal spinal cord (FSC) tissue (Tessler *et al.*, '88). The present study utilized transplants of FSC tissue into the chronically injured spinal cord to determine whether certain classes of sensory fibers retain their capacity for regrowth long after their initial injury. A partial hemisection lesion or a complete transection of the L1 spinal cord was made in adult rats. After a delay of 3-6 weeks, encapsulating glial scar tissue was excised from the cavity and a suspension of E14 FSC tissue was placed into the lesion site. Animals were sacrificed 3-4 months later and tissue processed for calcitonin gene-related peptide (CGRP) immunoreactivity.

Ascending CGRP-like immunoreactive axons in Laminae II, III, V, and X and descending fibers in Laminae II and III of the host spinal cord crossed the graft-host inferface and spread diffusely throughout the transplant, with some extending greater than 4mm. Numerous CGRP axons also crossed from transplant tissue back into the host spinal cord rostrally and appeared to terminate directly on ventral motoneurons. These results demonstrate the ability of FSC tissue transplants 1) to promote and sustain extensive growth of ascending sensory axons from the chronically injured spinal cord and 2) to act as a bridge for the growth of these fibers across an extensive spinal cord lesion in the effort to reestablish anatomical continuity. Supported by Spinal Cord Research Foundation of the PVA.

172.4

STUDIES OF HINDLIMB REFLEXES AFFECTED BY IMPLANTS OF FETAL BRAINSTEM TISSUE IN THE SPINAL CORD OF THE RAT. S.J. Moorman*, L.R. Whalen* and H.O. Nornes (SPON: M. Anderson). Dept. of Anatomy and Neurobiology, Colorado State Univ., Ft. Collins, CO 80523 (supported in part by NS21309-02 to H.O.N.)

Cell suspensions of fetal noradrenergic (NA) tissue were implanted in the lumbar region of the spinal cord 2 weeks after catecholamine (CA) denervation by intracisternal injection of 6-hydroxydopamine (6-OHDA). Animals with implants were evaluated either 2 or 4 month post-implantation as were age matched control and lesioned animals. Force production and electrophysiologic parameters associated with the hindlimb withdrawal reflex were evaluated. Testing was performed after injection of pargyline, again after L-DOPA injection, and again after phentolamine injection. Presence or absence of the long latency EMG component of the withdrawal reflex and changes in maximal force production were used to evaluate effects of the fetal implants. CA fluorescent cells in the implants were counted and correlations with the reflex changes were determined.

Control animals have a long latency EMG component of the withdrawal reflex that can only be elicited after injection of L-DOPA. This component cannot be elicited in 6-OHDA lesioned animals and therefore the force associated with this component is significantly reduced. Lesioned animals with NA implants have a long latency EMG component of the reflex before injection of L-DOPA, and show a significant increase in maximum force production compared to lesioned animals before L-DOPA injection. This force production in implant animals is significantly increased after L-DOPA injection and blocked by injection of phentolamine. In these animals the maximum force production was correlated with the number of CA fluorescent cells (r=.78).

Conclusions: In the electrophysiologic test of the hindlimb withdrawal reflex where the long latency component has been found to be dependant on NA, implants of fetal NA tissue in lesioned animals result in a recovery of NA dependant function proportional to the number of CA fluorescent cells in the implants.

172.5

A PRELIMINARY STUDY OF COMBINED CORTICAL AND SPINAL EMBRYONIC NEURAL TRANSPLANTS IN ADULT RATS. J. B. Gelderd and J. E. Quarles,* Dept. of Anatomy, College of Medicine, Texas A&M University, College Station, Texas 77843.

Cerebral cortex was exposed 2 mm caudal to the bregma and 2 mm left of the midline in anesthetized adult Long-Evans hooded rats. A 1 mm^2 area of cortical tissue was aspirated and rat embryonic cortical tissue (E_{16-17}) from the same area was placed in the host lesion cavity. A laminectomy (T_{8-10}) was performed on the same host animal and 3-4 mm of spinal cord and meninges were removed. Embryonic spinal cord from the same embryo was placed in a rostro-caudal orientation between the stumps of host spinal cord, either on the floor of the vertebral canal or embedded in a bovine collagen matrix (Collagen Corporation, Palo Alto, CA). Animals survived for varying periods from 4 days to 8 months postimplantation. Neural tissue was processed for a variety of light microscopic stains. Eight month animals underwent spinal cord transection at the T_6 vertebral level followed by implantation of horseradish peroxidase (HRP)-impregnated Gelfoam into the lesion site. Seventy-two hours later, tissues were processed using the TMB method. Although some gliosis occurred at the host/transplant interface, all cortical transplants showed good growth and integration with host neuropil. In addition, HRP animals showed labeled neurons in the cortical transplants. Only collagen-embedded spinal cord transplants appeared to survive, with one transplant exhibiting large neurons containing two nuclei.

172.6

AXONAL PROJECTIONS BETWEEN FETAL SPINAL CORD TRANSPLANTS AND THE ADULT RAT SPINAL CORD L.B. Jakeman and P.J. Reier. Departments of Neuroscience and Neurological Surgery, Univ. of Florida, Coll. of Med. Gainesville, FL 32610.

Recent work in this laboratory has demonstrated successful transplantation of fetal spinal cord (FSC) tissue into the adult rat spinal cord (SC). In the present study, neuronal tracers and immunocytochemical markers were used to examine the neuronal interactions between host and graft tissues. Transplants of E14 FSC tissue were placed into acute hemisection lesions in adult rats. After 2 - 6 months, localized injections of HRP or Flouro-gold were made into transplants or the SC at various distances from the interface between host and graft tissues. Although 2 um plastic sections of the interface region often show extensive interdigitation of processes, these injections revealed only modest host-graft interactions. Immunocytochemical staining showed ingrowth of host fibers containing serotonin-, oxytocin-, and calcitonin gene-related peptide- like activity; however, the distance of these projections was usually limited to 1 - 2 mm. Other recipients received iontophoretic injections of PHA-L, placed into FSC transplants or the host SC in close proximity to the host-graft interface. Anterogradely labeled axons illustrated extensive intragraft projections. In addition, short distance axonal projections were observed between host and graft tissues. These results indicate that axonal growth can occur between FSC grafts and the host SC. Many of the projections across the interface may be limited to short distances, while donor neurons extend axons for long distances within the grafts. Together, intragraft connectivity and the presence of afferent and efferent axonal projections suggest that FSC grafts may provide an anatomical substratum for the formation of a relay circuit at the site of a spinal cord injury. Supported by NIH MH15737-09.

172.7

INTRASPINAL BRAINSTEM TRANSPLANTS: REINNERVATION OF SYMPATHETIC PREGANGLIONIC NEURONS. M. Gonzalez-Carvajal* and V.R. Holets (SPON: B.A. Green). Dept. of Neurological Surgery, Univ. of Miami, Miami, FL 33136

Adult rats received an intrathecal injection of either 5,7-dihydroxytryptamine (5,7-DHT) or 6-hydroxydopamine (6-OHDA) one week prior to transplantation. The medullary raphe nucleus (RN) or the locus coeruleus/subcoeruleus area (LC) from E15 rats was dissected and dissociated. The RN and LC cell suspensions were prelabeled with DiI and injected into the T10 spinal cord segment of 5,7-DHT- or 6-OHDA-treated rats, respectively.

One to 4 weeks post-transplant, DiI-labeled RN and LC cells were present in the spinal cord, and fiber outgrowth was observed. The innervation of the dorsal and ventral horns by serotonin (5-HT)-, thyrotropin-releasing hormone (TRH)- phenylethanolamine-N-methyltransferase (PNMT)-, tyrosine hydroxylase (TH)-immunoreactive fibers was sparse. However, a moderately dense innervation of the sympathetic preganglionic neurons in the intermediolateral cell column and lamina X by 5-HT-, TRH-, PNMT- and TH-postive fibers was observed, suggesting a specificity of reinnervation. Control lesion animals showed no regeneration of 5-HT-, SP- or TRH-immunoreactive fibers (5,7-DHT-treated), or of PNMT- or TH-immunoreactive fibers (6-OHDA-treated) in the thoracic and lumbar spinal cord segments. Animals with longer survival times post-transplant are being evaluated. Funded by The Miami Project.

172.8

INTRAOCULAR CO-GRAFTS OF FETAL RAT THORACIC SPINAL CORD AND BRAINSTEM AREAS: REINNERVATION OF SYMPATHETIC PREGANGLIONIC NEURONS. V.R. Holets, M. Gonzalez-Carvajal*, E. Jakobsson* and Å. Seiger. Dept. of Neurological Surgery, Univ. of Miami, Miami, FL 33136.

An E14 rat thoracic spinal cord graft was placed in the anterior eye chamber of an adult rat 1 month prior to grafting an E14 medullary raphe nucleus (RN), E15 locus coeruleus/subcoeruleus (LC) or E14 C1-2 area (C1) graft. The grafts were evaluated 6 to 12 months post-grafting for the innervation of the thoracic spinal cord graft by serotonin (5-HT)-, thyrotropin-releasing hormone (TRH)- and substance P (SP)-immunoreactive fibers from RN co-grafts; neuropeptide Y (NPY)-, tyrosine hydroxylase (TH)- and galanin (GAL)-positive fibers from LC co-grafts; and phenylethanolamine-N-methyl-transferase (PNMT)-, TH-, SP- and NPY-immunoreactive fibers from C1 co-grafts. Motoneurons and sympathetic preganglionic neurons (SPNs) were identified with an anti-acetylcholine esterase (AChE) antibody.

Clusters of AChE-immunoreactive motoneurons and SPNs were seen in the spinal cord graft. Dense innervation of the spinal cord graft by 5-HT-, SP- (RN) and TH- (LC and C1) immunoreactive fibers was observed. PNMT- (C1) positive fibers preferentially innervated SPNs, while TRH- (RN) immunoreactive fibers were seen surrounding SPNs and motoneurons. These results suggest a specificity of reinnervation of the SPNs by fibers from the brainstem co-grafts. Funded by The Miami Project.

NEUROGLIA: BIOLOGY OF ASTROCYTES I

173.1

CHARACTERIZATION OF ASTROGLIAL MEMBRANE ASSEMBLIES BY AGENTS WHICH ACTIVATE PROTEIN KINASE C. J.H. Tao-Cheng, J.P. Bressler, & M.W. Brightman. NINCDS, NIH, Bethesda, MD

The function of the astroglial membrane assemblies, the orthogonal arrays of intramembranous particles revealed by freeze-fracture electron microscopy, is unknown. Previously, we have shown that agents which raise cAMP levels increased the astroglial assembly concentrations. In order to further characterize the assemblies, we have now used agents that affect protein kinase C (PKC).

Phorbol 12-myristate 13 acetate, which activates protein kinase C, did not affect the assemblies. Choline-specific phospholipase C (PLC), which generates diacylglycerols, a PKC activator, did not change the size, shape or concentration of the assemblies. A PKC inhibitor, H-8, had no effect on assemblies either. Thus, PKC probably is not linked to the presence of assemblies in the astroglial membrane.

Background, non-assembly particles in the astroglial membrane were affected by PLC, however. After 6-7 hrs of exposure (0.1-1ug/ml), individual intramembranous particles redistributed into patches of aggregates interspersed with particle-free areas. The assemblies remained unchanged, but were flanked by other aggregated particles. By 26 hrs and without changing the media, the astroglial membrane structure recovered its pattern of evenly distributed particles and assemblies. It appears that assemblies are not affected by changes in choline containing phospholipids.

173.2

ASTROCYTIC ABNORMALITIES IN CAPRINE β-MANNOSIDOSIS: AN IMMUNOHISTOCHEMICAL ANALYSIS OF OPTIC NERVE. **P.J. Boyer and K.L. Lovell. Dept. Pathology, Michigan State Univ., E. Lansing, MI 48824.**

Central nervous system features of caprine β-mannosidosis, an autosomal recessive disease of glycoprotein catabolism, include myelin deficiency, with consistent regional variation in severity, and widespread cytoplasmic vacuolation. A previous study of one affected fetal optic nerve demonstrated, by ultrastructural analysis, a substantial increase in the number of astrocytes compared to control values. In order to further characterize astrocytic changes in affected animals, an immunohistochemical investigation of GFAP (glial fibrillary acidic protein) staining distribution was undertaken in optic nerves of animals ranging in age from 115/150 days gestation to 31 days postnatal.

At the light microscopic level, a qualitative increase in density of GFAP-positive astrocytic process profiles in affected optic nerves, compared to control optic nerves, was present at all ages studied. However, fewer GFAP-positive cell body profiles were prominent in affected goats. At 115 days gestation, optic nerve myelination is in an early stage and myelin deficiency is already evident. Thus astrocytic abnormalities are present relatively early in the progression of the dysmyelinating process. Morphometric analysis of the area occupied by GFAP reaction product showed an increase in all affected animals. This study defines quantitatively the extent of change in optic nerve GFAP-positive astrocytic processes during development in β-mannosidosis.

Supported by NS 20254 to K.L.L. and NS 16886 to M.Z. Jones.

173.3

INTERMEDIATE FILAMENT-ASSOCIATED PROTEINS AS MARKERS OF SUBTYPES OF RAT ASTROCYTES IN PRIMARY CULTURE. H-Y. Yang*, J.C. Chen*, and G.D. Pappas (SPON: E.G. Anderson). Dept. of Anatomy and Cell Biology, Univ. of IL at Chicago, Chicago, IL 60612.

Intermediate filament-associated proteins (IFAPs) have been thought to play an important role in the regulation of the organization of intermediate filaments (IFs) and the interrelationship among IFs and other cellular components. IFAP expression has been shown to be related to the functional and developmental status of the cell. Therefore, the IFAPs can be used as markers for the identification of various functional subtypes of astrocytes, that have long been speculated but little is known. Recently, we have initiated the study of the IFAPs in astrocytes. We have previously identified several IFAPs in kidney cell line (BHK-21) that contains both vimentin and desmin as the structural proteins of their IFs (Yang et al., 1985, JCB, 100:620 and 101:15a). Because vimentin and/or desmin are also found in some normal and reactive astrocytes, the immunoreactivity of these IFAPs was tested in primary cultures of astrocytes prepared from neonatal rat brain, as well as in those from stab-lesioned rat brain. The results revealed that one of the BHK IFAP (IFAP-70/280kD) is also a glial IFAP, as demonstrated by double-label immunofluorescence microscopy using antibodies to glial fibrillary acidic protein, the astrocyte specific IF structural protein, and the IFAPs. In primary cultures of neonatal astrocytes that are composed of both stellate and non-stellate astrocytes, the anti-IFAP-70/280kD staining was found only in some of the non-stellate ones, but not in the stellate ones. The immunostaining was also shown in some of the adult 'reactive' astrocytes in primary cultures that are composed only of non-stellate astrocytes. Thus, we now can divide the non-stellate ('protoplasmic'; Miller and Raff, 1984, J. Neurosci., 4:585) astrocytes into two subgroups based on the presence of a glial IFAP.

173.4

ASTROCYTE MEMBRANE DIFFERENTIATION IS INFLUENCED BY CO-CULTURE WITH ENDOTHELIAL CELLS.

L.A. Weinstein*, C.J. Skordeles*, and D.M.D. Landis, Dept Neurology, Case Western Reserve University, Cleveland, OH 44106

We have co-cultured primary astrocytes derived from neonatal rats and brain endothelial cells derived from mature rats. A larger proportion of astrocytes in these co-cultures acquire "assemblies" of intramembrane particles as compared to astrocytes cultured alone. The average density of assemblies is also increased: astrocytes cultured alone had an average density of 4.4 assemblies/μm^2, while astrocytes co-cultured with endothelial cells acquired 7.1 assemblies/μm^2. This effect is not observed when astrocytes are cultured on the basal lamina remaining after the removal of endothelial cells, though astrocytes grew very rapidly to confluence in such cultures. Potential effects of serum supplementation and of culture medium composition seemed not to play a role in the influence of endothelial cells on astrocyte differentiation; similar results were obtained when cells were cultured in HAM F12 media supplemented with calf serum and when cells were cultured in L15CO_2 media supplemented with fetal calf serum. No consistent differences in intramembrane structure were evident as endothelial cells in co-culture with astrocytes were compared to endothelial cells cultured alone.

The increased concentration of assemblies in the co-cultured astrocytes may reflect in vivo interactions. In the adult mammalian central nervous system, astrocytic membranes adjacent to vascular structures are characterized by a high concentration of assemblies. Co-culture thus replicates certain aspects of the normal blood-brain interface, and suggests that trophic interactions between the two cell types may occur during development.

173.5

DEXAMETHASONE AND FORSKOLIN EXERT OPPOSITE INFLUENCES ON MEMBRANE DIFFERENTIATION IN CULTURED ASTROCYTES.

C.J. Skordeles,* L.A. Weinstein,* and D.M.D. Landis (Spon: R.B. Daroff). Dept. Neurology, Case Western Reserve University., Cleveland, OH 44106

Astrocytes in confluent secondary cultures exposed to 5 μM dexamethasone acquire very high densities of "assemblies," aggregates of intramembrane particles packed in orthogonal array: after 7 days of culture in dexamethasone, average density was 13.65 assemblies/μm^2, as compared to control values of 3.98 assemblies/μm^2. Not all cells in the culture are affected. While the proportion of cells expressing assemblies is somewhat increased, the more striking effect is that some cells acquire densities of assemblies greater than that observed under any other conditions in vitro. We hope to learn whether this glucocorticoid effect in vitro is related to the therapeutic benefits of glucocorticoids used to treat cerebral edema.

When 50 μM forskolin is added to confluent secondary cultures of astrocytes for 72 hours, there is a significant but transient increase in c-AMP levels (50-131 fold increase at 24 hours, 4.5-4.7 fold increase at 48 hours, and 1.5-5.0 fold increase at 72 hours). As previously observed in cultures of astrocytes exposed to elevated c-AMP or analogues, the flat, epitheliod cells undergo a remarkable shape transformation to become process bearing. Many cells detach from the substrate, and cells remaining adherent have a lower concentration of assemblies. In one plating, densities at 24 hours were 2.34 assemblies/μm^2 (control 3.39 assemblies/μm^2), at 48 hours were 0.66 assemblies/μm^2 (control 4.14 assemblies/μm^2), and no cells bearing assemblies could be found at 72 hours. Astrocytes in such cultures thus resemble astrocytes in white matter.

173.6

DIFFERENCES IN ASTROCYTE DEVELOPMENT IN STRAINS OF MICE INBRED FOR DIFFERENT ETHANOL SENSITIVITY. N. Sakellaridis, D. Mangoura, J. M. Masserano, V. Detsis,* C.J. Leoni,* J.D. Jones,* R. Deitrich,* and A. Vernadakis.* Depts. of Pharmacology and Psychiatry, Univ. of Colorado School of Medicine, Denver, CO 80262

Differences in astrocyte development were investigated in mice selectively bred for ethanol sensitivity: a) Short Sleep (SS) or Long Sleep (LS) and b) Severe Ethanol Withdrawal (SEW) or Mild Ethanol Withdrawal (MEW). Astrocyte development was assessed using glutamine synthetase (GS) activity as a marker. GS was assayed in the forebrain, cerebellum and remaining brain in LS and SS mice at 8, 10, 12, 15, 30, 45, and 60 days of age. GS activity rose linearly up to 30 days of age and leveled off thereafter in all brain areas. Activity was lower early in development in SS mice in all three brain areas until day 15 when the activities were identical except in 60 day old forebrain when again GS was lower for SS mice. The same ages / brain areas were assayed for MEW, SEW and were compared to CEW their controls. GS activity was lower in the cerebellum and forebrain of both SEW and MEW mice as compared to the control line before 15 days of age. Comparing the developmental profiles of GS activity with the developmental emergence of ethanol sensitivity we propose that astrocytes play a role in the ethanol related line differences. (Supported by AA-03527)

173.7

EVIDENCE FOR THE EXISTENCE OF SUBTYPES OF TYPE-1 RAT ASTROCYTES IN CULTURE. Y.J. Oh*, G.J. Markelonis and T.H. Oh, Dept. of Anat., Univ. of MD Sch. of Med., Balto., MD 21201

Studies of certain cell surface receptors or intermediate filament markers suggest that cultured CNS astrocytes exhibit different subtypes. Experiments in our laboratory have focused upon other appropriate metabolic cell markers which can be used to clearly differentiate astrocytic subtypes. Antibodies against mitochondrial malate dehydrogenase (MDH) appear to be excellent markers for this purpose. We have examined MDH immunoreactivity in primary cultures of type-1 astrocytes (non-stellate) or type-2 astrocytes prepared from neonatal rat brain by standard methods. Double immunofluorescent staining with anti-MDH serum (rabbit) and anti-GFAP antibody (mouse monoclonal) suggested that there are at least two different subtypes among type-1 astrocytes. The predominant pattern consisted of numerous punctate fluorescent vesicles; the remaining astrocytes showed far fewer of these discrete flourescent profiles. Some type-1 astrocytes did react in an intermediate pattern to anti-MDH. These MDH results were consistent for cells cultured for 3-21 days. Experiments with rhodamine 123, a mitochondrial-specific fluorochrome, showed that all astrocytes contained mitochondria. In contrast, type-2 astrocytes uniformly showed strong immunoreactivity to anti-MDH with no apparent subtypes being evident. We conclude that different metabolic subtypes of type-1 astrocytes can be distinguished by anti-MDH. (Supported by NIH grants NS20490 and NS 15013)

173.8

IMMUNOHISTOCHEMICALLY LABELED ASTROCYTES IN RAT OLFACTORY BULB WITH MONOCLONAL ANTIBODIES. M.S. Bailey*, M.T. Shipley, R.A. Akeson. (SPON: R.V.W. Dimlich) Dept. of Anatomy & Cell Biology and Division of Basic Research, Children's Hospital Research Foundation, Univ. Of Cincinnati Med. Ctr., Cincinnati, Ohio 45267.

Three monoclonal antibodies (Mabs) 1F10, 2C8 and 2F10 yeild excellent immunohistochemical staining of astrocytes in the rat CNS. These Mabs were generated by immunizing against neonatal rat olfactory bulb after tolerizing the mice to cerebellum with cytoxan treatment. Western anaylsis suggests that the Mabs bind protein(s) that co-migrates with GFAP on SDS-PAGE. Mabs 1F10, 2C8, 2F5, as well as a polyclonal anti-GFAP antibody, demonstrate differential distribution of astrocytes in the rat olfactory bulb. The heaviest label is in the synaptically active glomerular and external plexiform layers. The internal plexiform and granule cell layers have much lower density of astrocytes, with higher density in the subependymal zone. 2C8 and 2F5, but not 1F10 or anti-GFAP, also label the nerve layer. In addition, 2C8 strongly labels mitral and tufted cells in the postnatal day 9 rat olfactory bulb and only faintly labels mitral cells in the adult. The Mabs will be used for further study of organization and development in the rat olfactory bulb. (Supported by : NIH 23348, 20643, 22053, U.S. Army DAMD 17-86-C-6005)

173.9

INCREASED GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) WITHIN THE HYPOGLOSSAL NUCLEUS FOLLOWING HYPOGLOSSAL NERVE TRANSECTION OR ELECTRICAL STIMULATION. L.L. Hall[1] and R.C. Borke[2]. Depts. of Physiology[1] and Anatomy[2], Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.

Astrocytic processes, containing intermediate filaments, hypertrophy around axotomized neurons. The stimulus and function for this response remain unknown. In this study, GFAP expression within the hypoglossal nucleus was investigated following axotomy. The influence of hypoglossal nerve stimulation on the astrocytic expression of GFAP within the hypoglossal nucleus was also examined. At various intervals following either unilateral hypoglossal nerve transection or electrical stimulation (10 mins. of stimulation, 0.5-2.0 V, 1 Hz pulses of 0.05 ms duration), the hypoglossal nuclei from Sprague-Dawley rats were analyzed for GFAP immunoreactivity. Hypoglossal nerve transection induced a marked, unilateral increase in GFAP expression within the hypoglossal nucleus 4 to 20 days following axotomy. A dramatic enhancement of GFAP immunoreactivity within the hypoglossal nucleus was also observed 5 to 20 days following nerve stimulation. Thus, either hypoglossal nerve lesion or electrical stimulation provoked an increase in GFAP-positive staining astrocytes within the hypoglossal nucleus.

173.10

3-DIMENSIONAL RECONSTRUCTION OF VOLUME REGULATION IN SWOLLEN ASTROCYTES USING HIGH VOLTAGE ELECTRON MICROSCOPY (HVEM). H.K. Kimelberg[1], S. Goderie[1]*, R. Cole[2]* & D.F. Parsons[2]*, Albany Medical College[1] & Wadsworth Center for Laboratories & Research, N.Y.S. Dept of Health[2], Albany, NY

During exposure to hypotonic media astrocytes in primary monolayer culture swell and then regulate their volume back to control levels, a property termed regulatory volume decrease (RVD). Using thick (0.5µm) sections viewed by a 1MV HVEM and using a 3-dimensional graphic reconstruction computer program, we confirmed these results previously obtained using radiolabelled markers of the intracellular space. We found that upon swelling the shape of the cell changes from being relatively flat to cone-shaped, and then regains its initial volume and shape during RVD. Transmission electron microscopy (TEM) showed that the cytoplasm became much less electron-dense during swelling, the bundles of intracellular filaments appeared to dissociate into single filaments, the mitochondria showed marked swelling and the numerous surface infoldings became smoothed-out and deconvoluted. All these effects were reversible. We have found that in swollen cells the "normal" stress fibers, composed of longitudinal actin filaments are reconfigured into a radial pattern, which rapidly reverts to the "normal" pattern with RVD. There is a loss of poly-peptides of both low (< 40 kDa) and high (> 66 kDa) MW from a detergent extract of swollen cells, and partial recovery of these poly-peptides after 30 mins exposure to hypotonic medium. (Supported by NIH grants NS23750(HKK) & PHS RR01219 (DFP)).

173.11

ASTROCYTE VOLUME REGULATION FOLLOWING EXPOSURE TO SERA FROM REYE'S SYNDROME (RS) PATIENTS. J.E. Olson, J.F.S. Crocker*, and M.G. Murphy, Dept. Emerg. Med., Wright State Univ., Dayton, OH and Depts. Pharmacol. and Pediatr., Dalhousie Univ., Halifax, NS, Canada.

Elevated intracranial pressure, one of the neurological hallmarks of RS, is thought to be caused by astrocytic swelling. This cytotoxic brain edema may be caused by toxic factors in the circulation. To test this hypothesis directly, we determined the morphology and volume regulation of cultured astrocytes following exposure to sera from RS patients.

Astrocyte cultures (more than 85% GFAP positive) were exposed to media containing 5% serum from RS patients in the acute (ARS) or recovery (RRS) phases of the disease or normal human serum (NHS) for 30 min or 2-5 days. Volume regulation was analyzed by measuring cell volumes for 30 min during suspension in hypoosmotic medium (165 mOsm). Astrocytes exposed to ARS or RRS sera for 30 min showed volume regulation similar to cells exposed to NHS. Cells cultured in ARS sera for 2-5 days had normal cell volumes in isoosmotic medium (257 mOsm), but in hypoosmotic medium these cells were initially 10% smaller than cells grown in RRS sera from the same patient. Volume regulation following hypoosmotic swelling was reduced or absent for cells grown in ARS serum and, in general, correlated with abnormal cell morphology. These observations support the hypothesis that during the acute phase of RS, serum contains a factor(s) that contributes to the pathogenesis of cytotoxic edema.

Supported by NIH, the Robert Katz Medical Research Foundation (JEO) and the Canadian MRC (JFSC and MGM).

173.12

REACTIVE ASTROCYTES EXPRESS HIGH LEVELS OF MICROTUBULAR ASSOCIATED PROTEIN 2 (MAP2): A POSSIBLE MARKER FOR REACTIVE GLIOSIS. E.E. Geisert and L.I. Binder.* Dept. Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

Reactive astrocytes are a major component of the scar that forms following injury to the CNS. To study the process of scar formation, 26 Sprague-Dawley rats received lesions in different regions of the CNS: 16 lesions of the internal capsule; 6 lesions of the corpus callosum; and 4 lesions of the spinal cord. The animals were allowed to survive from 0 to 50 days. Frozen sections were stained with three different monoclonal antibodies against MAP2. Two different monoclonal antibodies and one rabbit polyclonal antiserum were used to stain for GFAP. From an analysis of these sections, reactive astrocytes appeared to express high levels of MAP2. In sections double stained for both MAP2 and GFAP, all of the GFAP-positive astrocytes near the edge of the lesion were also positive for MAP2, while astrocytes in normal tissue were not. The high levels of the cytoskeletal protein MAP2 in reactive astrocytes may represent a terminal differentiation of the astrocyte as it becomes reactive and may serve as a marker for reactive gliosis.

173.13

PROTEIN KINASE C INHIBITORS ALTER ASTROCYTE MORPHOLOGY. C. A. Bedoy* and P. L. Mobley, St. Mary's University and The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Shape changes appear to play an important role in astrocyte function. In astrocyte cultures activators of either protein kinase C (PK-C) or cyclic AMP activated protein kinase (PK-A) can alter cell shape. While conducting studies to determine if kinase inhibitors could block morphological changes induced by the PK-C activator phorbol 12-myristate 13-actetate (PMA), we found that PK-C inhibitors also induced morphological changes similar to those observed with PMA. In confluent cultures treated with 50 μM H-7 cells rapidly converted from their flat, polygonal form to stellar shaped cells with processes. Changes in morphology were also observed with 500 nM staurosporine, another inhibitor of PK-C. No effects were observed with 100 μM or 200 μM HA-1004, a PK-A inhibitor. In cells pretreated 24 hrs with PMA, no measurable PK-C activity is found using a histone substrate, and subsequent exposure to PMA does not affect morphology or alter protein phosphorylation; however morphological changes are still observed with H-7. These studies indicate the both activators and inhibitors of PK-C can alter astrocyte morphology and suggest that this kinase plays an important role in astrocyte function.
(Supported by NIH grants NS25766 and RR08170)

173.14

PROTEIN KINASE C SUBSTRATES AND OTHER MEMBRANE PHOSPHOPROTEINS IN RAT ASTROCYTES. L. Vitković, V. J. Aloyo, H. W. Steisslinger*, D. Benzil*, and J. P. Bressler. Lab. Mol. Biol. & Surgical Neurol. Branch, NINCDS, NIH, Bethesda, MD 20892 and Dept. Pharmacol., Med. Col. Penn., Phyladelphia, PA 19129.

Membrane phosphoproteins are likely mediators of signal transduction and their characterization in astrocytes contributes to defining these cells' ability to interact with their environment. Among polypeptides with relative molecular weights (Mr) between 20 kDa and 120 kDa and isoelectric points (pI) between 4.2 and 8.5, 10 were phosphorylated when crude membranes from cultured rat astrocytes were incubated under conditions optimal for activities of Ca^{2+}-dependent kinases. The same acidic plasma membrane proteins from astrocytes and 1-day old cortex were phosphorylated, but to an apparently different degree, by partially purified rat brain protein kinase C (pkC). The two most prominent among these were characterized by four criteria as major substrates of pkC in brain: growth-associated protein 43 (GAP-43) and the Mr 87 kDa protein. Addition of phorbol 12-myristate 13 acetate to cultured astrocytes led to an increase in the degree of phosphorylation of these two proteins. These results suggest that the two prominent pkC substrates in brain, GAP-43 and the 87 kDa protein, are endogenous substrates of pkC in astrocytes.

173.15

EXTRACELLULAR POTASSIUM AND OSMOLARITY INFLUENCE DNA SYNTHESIS AND GLIAL FIBRILLARY ACIDIC PROTEIN EXPRESSION IN CULTURED GLIAL CELLS. K. S. Canady*, F. Ali-Osman* and E. W Rubel (SPON: A. Towe), Depts. of Physiol. & Biophys., Otolaryngol. & Neurol. Surg., Univ. of Washington, Seattle, WA 98195.

Extracellular potassium (K^+_o), which varies with neuronal activity, is thought to play an important role in neuron-glia interactions. We have previously shown that changes in glial fibrillary acidic protein (GFAP) immunopositive astrocytic processes can result from changes in neuronal activity levels. Here we tested the hypothesis that changes in $[K^+]_o$ stimulate these glial reactions by varying the $[K^+]$ of the media in which glial cells were cultured. We varied $[K^+]_o$ in two ways: (1) by adding KCl to Dulbecco's modified Eagle's medium (DME) to achieve final $[K^+]$'s of 5-50 mM; or (2) by adding both KCl and NaCl to K^+,NaCl-free DME. The latter was done to hold osmolarity constant and to achieve final $[K^+]$'s of 1-50 mM. To vary osmolarity without altering K^+_o, we added NaCl to DME to achieve final [NaCl]'s of 110-155 mM. In these cultures, we measured GFAP expression by a dot-immunobinding assay, and we measured the incorporation of ^{3}H-thymidine and ^{3}H-leucine into cellular DNA and protein, respectively. Glial cells cultured in hyperosmolar media (50 mM K^+ or 155 mM NaCl) showed decreased DNA synthesis and increased GFAP expression. When osmolarity was held constant, however, GFAP expression was inversely related to K^+_o over the 1-50 mM range. The syntheses of DNA and protein did vary with physiologically significant $[K^+]_o$'s (1-10 mM), regardless of the small changes in osmolarity. We conclude that the DNA synthesis and GFAP expression of cultured glial cells are sensitive to both small changes in $[K^+]_o$ and relatively large changes in osmolarity. These data suggest that changes in neuronal activity, by altering K^+_o, may play a role in glial reactions observed *in vivo*. Supported by PHS grants NS24518 and GM07108.

173.16

DIFFERENTIATION OF CULTURED RAT ASTROCYTES: LIMITATION IN MINIMAL MEDIUM. E. T. Browning, E. J. Hoff* and S. E. Farinelli*, Dept. of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Past studies indicated that glial fibrillary acidic protein (GFA) containing rat astrocytic cultures express monoamine oxidase-B (MAO-B), and glutamine synthetase (GS) only if culture conditions are properly selected. Expression of significant levels of MAO-B activity occurred only if culture pH was kept near 7-7.4 and if culture continued for 40 days or longer. GS expression required control of pH but was also greatly increased in cultures grown in DMEM compared to those grown in BME. Present work indicates that cultures (25 cm^2 growth surface/3ml medium) deplete their growth medium of three essential amino acids, leucine, isoleucine and valine, within approximately 24 hr indicating that these cultures are starved for these amino acids for the remaining 24-48 hr of the usual period between feedings. Cultures grown in DMEM contain 4-times the initial concentration of amino acids present in BME and are not depleted during this usual feeding cycle. Isotope studies indicate that 2/3 of the C^{14}-labeled leucine, isoleucine or valine which is taken up by the cultures is incorporated into protein and 1/3 is oxidized to CO_2. In addition the cultures grown in either medium consume the 5.5 mM glucose of their medium within 24 hr, converting it largely to lactic acid. Experiments are in progress to determine if addition of leucine, isoleucine and valine to BME will increase GS expression.
(Supported by NSF Grant NSF-BNS-8708539)

173.17

COMPACT ASTROGLIAL SCAR FORMATION IN VIVO AND IN VITRO IS ALTERED WHEN MYELIN IS PRESENT. P.A. Trimmer. Dept. of Neurosci., Univ. of Virginia, Charlottesville, VA 22908.

Little is known about what factor(s) influence compact glial scar formation by reactive astrocytes. To study this question, the retina was unilaterally removed surgically to induce scar formation in the optic nerve. Light and electron microscopic examination of scars formed in newborn, juvenile and adult rat optic nerves suggested that the presence of myelin affects the time course of compact glial scar formation. Before myelination in vivo, scar formation took less than one week. In older rats, compact scar formation took from one to five months. Astroglial scar formation was also studied in explant cultures prepared from newborn, juvenile, and adult rat optic nerves as previously described (Trimmer, P.A., Soc. Neurosci.,11:396,1985). Compared with in vivo, the time course of myelin removal in vitro seemed to be delayed. Even after several weeks in vitro, explants from older rats consisted of degenerating myelin. The number of reactive astrocytes in explants appeared to be greatly reduced. Glial scar formation in vitro was more successful in optic nerve explants which did not contain myelin. This suggests that the formation of a compact glial scar by reactive astrocytes in vivo and in vitro is altered by conditions which favor the persistance of myelin debris. Supported by NIH grant NS25055.

173.18

MIGRATION AND PHENOTYPE OF TRANSPLANTED ASTROCYTES C.J.Emmett, J.M.Lawrence, P.J.Seeley and G Raisman (SPON: R.E.Rydel), Norman & Sadie Lee Res.Ctr., Lab of Neurobiology, N.I.M.R., The Ridgeway, London NW7 1AA, U.K.

Type 1 astrocytes were derived from post-natal (P5-8) corpus callosum rat. They were characterized by histology and immunocytochemically. After rotational purification and a complement-mediated cytolosis with antibodies to G_{GIC} (A2B5) and galactocerebroside (GalC), the final population of cells contained 99% GFAP-positive astrocytes. The astrocytes were labelled by mitotic uptake of tritated thymidine prior to grafting. Over a series of transplant survival times astrocytes were visualized autoradiographically.

They divided *in vivo* and migrated extensively into host brain within one week of implantation. As routes for migration, they commonly appeared to use blood vessels and neuronal dendrites. Labelled cells were found in the optic track and thalamus. Ultrastructural autoradiography of transplanted astrocytes demonstrated a bimodal distribution of cell morphology, although the original population was homogeneous. One cell phenotype was small, darkly-stained, relatively heavily radiolabelled and found at quite large distances from the original grafting site (class S cells). The other was large, lightly-stained, with relatively low levels of radioactivity (class L cells). They were found close to the host transplant interface. Material from longer survival times revealed an apparent progressive conversion of class S cells to the L form.

173.19

COMPARISON OF MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) ANTIGEN EXPRESSION ON HUMAN ADULT ASTROCYTES IN SITU AND IN VITRO. Y. Grenier*, C. Szwarc*, J.P. Antel (SPON: I. Hunter). Montreal Neurological Institute, Montreal, Quebec H3A 2B4.

We compared in situ and in vitro (tissue culture) expression of MHC antigens by astrocytes of young adult humans (i.e. age most at risk for autoimmune CNS disease) by using surgically resected temporal lobe tissue and immunohistochemical labelling of cells, both in cryostat sections and in tissue culture. Histopathologic examination indicated variable degrees of gliosis in the tissue specimens. Monoclonal antibodies (mcAbs) W6/32 and SG465 directed to framework epitopes of the class I and class II molecules respectively, as well as B7-21, TV22A, D1-12, which recognize the class II DP, DQ, DR gene products respectively, were used. Astrocytes were identified by double immunostaining the cultured cells or tissue sections with anti-GFAP antibody. In tissue culture, a mean 75% of GFAP+ cells express MHC class I. A mean of 25% of astrocytes grown in DMEM + 10% fetal calf serum express MHC class II compared to <10% of such cells grown in DMEM 10% human serum. MHC reactivity on GFAP+ cells was absent in tissue sections, including examples with severe gliosis. MHC expression was detected using all 5 mcAbs on non-GFAP+ cells in sections of a case of encephalitis. Our results parallel studies of fetal murine tissue, in which MHC antigen expression on astrocytes is immunohistochemically detected in vitro but not in situ.

173.20

PRIMARY ADULT HUMAN ASTROCYTES CONTAIN FACTORS SUFFICIENT FOR JC VIRUS REPLICATION. A. J. Aksamit* and J. Proper*. (SPON: Julie Cunningham) Dept. of Neurology, Mayo Clinic, Rochester, MN 55905.

Factors specific for glial cells have been shown to be important in determining the selective transcription and replication of JC virus, the etiologic agent of progressive multifocal leukoencephalopathy, a demyelinating disease of the human central nervous system. These factors determine tropism. Cultured, purified adult human astrocytes taken from temporal lobectomy specimens and identified by staining with anti-glial fibrillary acidic protein antibody were examined for the ability to support JC virus production. Infected cells underwent nuclear enlargement but showed no signs of transformed growth characteristics. Early viral gene translation was demonstrable by immunohistochemistry. JC virus DNA replication quickly followed, as demonstrated by biotinylated DNA:DNA in situ hybridization. Late gene structural protein synthesis followed and was accompanied by infectious virion assembly, as demonstrated by immunocytochemistry and hemoglutination inhibition assay, respectively. This is the first demonstration that adult human astrocytes are capable of productive JC virus infection, whereas human oligodendrocytes or human fetal spongioblasts were thought to be the only primary cells containing specific glial factors capable of replicating this virus. We conclude that glial factors required for JC virus expression are sufficiently present in primary adult human astrocytes.

HORMONAL CONTROL OF BEHAVIOR II

174.1

NEURAL PROGESTIN RECEPTORS FOLLOWING DIFFERENT ESTRADIOL TREATMENTS IN FEMALE GUINEA PIGS: BIOCHEMISTRY AND IMMUNOCYTOCHEMISTRY. D.H. Olster and J.D. Blaustein. Neuroscience and Behavior Program and Psychology Department, Univ. of Mass., Amherst, MA 01003

Estradiol (E_2), administered as two pulses or as a single injection of estradiol benzoate (EB), is effective in priming ovariectomized (OVX) guinea pigs for progesterone facilitation of lordosis. Because E_2-induced progestin receptors (PRs) appear to mediate progesterone-facilitated lordosis, neural PRs were evaluated biochemically (by in vitro binding) and immunocytochemically following EB and E_2 pulse treatment. The concentration of combined hypothalamic-preoptic area PRs measured biochemically was higher in EB (10 µg) as compared to E_2 pulse (two 2-µg injections) treated OVX guinea pigs. Immunocytochemistry revealed this to be an effect localized primarily to the preoptic area, where PR-immunoreactivity (IR) was markedly reduced in the E_2 pulse- as compared to EB-treated animals. In contrast, PR-IR in hypothalamic areas appeared similar following EB and E_2 pulse treatment. These data suggest that hypothalamic cells are exceptionally responsive to the inductive effects of E_2 pulses on PRs, and further support the hypothesis that hypothalamic PRs may mediate the facilitation of lordosis by progesterone in E_2-primed guinea pigs.
(Supported by NS 19327 and RCDA NS 00970 from the NIH)

174.2

POSSIBLE INTERACTION OF VASOTOCIN AND CENTRAL VISUAL PATHWAYS IN THE FROG, RANA PIPIENS. G.J. De Vries, K. Fite, J. Brewer*, and S. Numan*. Neuroscience and Behavior Program and Department of Psychology, University of Massachusetts, Amherst, MA 01003.

Visually guided behaviors involved in prey-catching vary seasonally in anuran amphibians. So do certain responses of visual neurons. These seasonal variations may have a hormonal basis. Specific hormonal effects on visual systems, however, have not been described. Here, we are identifying neurotransmitter systems that may be involved in seasonal modulation of visual functions. We have started to stain brains of the leopard frog, Rana pipiens, immunocytochemically for vasotocin. This peptide shows seasonal variations in certain anura, just like its homologous peptide vasopressin in mammals, where it is under strict steroid hormone control. Vasotocin fibers were found in several thalamic and mesencephalic regions that receive direct retinal afferents: specifically, in the dendritic zone between visual terminals in the Nucleus of Bellonci and the postsynaptic neurons in the anterior thalamus, the ventral thalamus including the rostral visual nucleus, the pretectal nucleus lentiformis mesencephali, and the deep layers of the tectum. As all these areas appear to be involved in visually guided behaviors associated with prey-catching, we suggest that vasotocin may play a role in their seasonal variations.

174.3

SOCIAL STIMULI INCREASE ESTROGEN RECEPTOR BINDING IN CELL NUCLEI OF THE FEMALE BRAIN. M. Cohen-Parsons and E.J. Roy. Department of Psychology, University of Illinois, Champaign, IL 61820.

In female prairie voles, the onset of puberty is induced by exposure to an unfamiliar male. Circulating estrogen levels rise after a female is exposed to a male and consequently, the binding of estrogen within cell nuclei of the brain also rises. The present study was conducted to determine whether social stimuli can also increase the level of estrogen receptor binding in brain cell nuclei (ERN) independent of corresponding alterations in circulating estradiol. When ovariectomized females were given a fixed amount of estradiol and exposed to males, the females were found to have 31 to 101% higher levels of ERN in the preoptic area than did females given estrogen and not exposed to males. This treatment difference was statistically significant ($p<.01$). Differences were not observed within the medialbasal hypothalamus although the area is also rich in estrogen receptors. These results suggest that social stimuli can influence estrogen concentrating cells within the brain by modifying estrogen receptor activity within select areas.

174.4

MODULATORY EFFECTS OF NOREPINEPHRINE (NE) ON ESTROGEN RECEPTORS IN THE HYPOTHALAMUS OF THE FEMALE RAT. M.E. Montemayor and E.J. Roy. Neural and Behavioral Biology Program, Univ. of Illinois, Champaign, IL 61820.

It has been demonstrated that the NE neurotransmitter system modulates estrogen receptors and accompanying synthesis of progestin receptors in the medialbasal hypothalamus (MBH) of female rats and guinea pigs (Blaustein, 1987; Clark et al.,1985; Nock et al., 1981, 1984). After administration of alpha-1 antagonists, in the presence of estradiol, decreases in nuclear estrogen receptors and cytosol progestin receptors appear to be associated with a decrease in estrogen dependent lordosis behavior. We have replicated the finding that prazosin decreases nuclear estrogen receptors after estradiol administration in rats (Blaustein, 1987). In addition, NE input to the hypothalamus has been manipulated by direct infusion of 6-OHDA onto NE cells in the medullary brainstem. After significant depletion of NE input, as verified by HPLC measurement of NE from the same tissue, differences in the levels of estrogen receptors measured by exchange assay were not detected in the MBH or preoptic area (POA). Are the pharmacological effects of prazosin generated by blocking NE receptors other than those on estrogen concentrating cells, i.e. effects on cerebral vasculature? At present, we are investigating whether MBH and POA tissue levels of estrogen measured by RIA are altered as a result of prazosin treatment.

174.5

EFFECTS OF HYPOPHYSECTOMY ON SALT HUNGER AND ON BRAIN AND LIVER ANGIOTENSINOGEN mRNA LEVELS. Jay Schulkin, Bruce S. McEwen, Jesus Angulo and Randall Sakai, Dept. of Anatomy Univ. of Pennsylvania and Lab of Neuroendocrinology Rockefeller University. (SPON: S. Williamson)

Hypophysectomized (HYPOX) rats ingest less salt following body sodium depletion than pituitary intact rats. The hormones angiotensin and aldosterone that are active during body sodium depletion generate the behavior of salt ingestion in the rat. Mineralocorticoid-induced salt hunger is normal in the HYPOX rat, while salt hunger generated by increasing the angiotensin levels is reduced in the HYPOX rat. We next examined whether the message for angiotensinogen, the precursor for the production of angiotensin, is reduced in the sodium replete HYPOX rat. We found that HYPOX rats mRNA for angiotensinogen is reduced in preoptic, medial amygdala and the ventral and dorsal medial hypothalamus when compared to control rats. The mRNA in liver was also reduced in the HYPOX rat. Therefore, the diminished ingestion of salt that results from body sodium depletion in the HYPOX rat may be due to a reduced production of angiotensin in both brain and liver.

Supported by NIMH 00678

174.6

DIFFERENTIAL EFFECTS OF ANDROGENS AND ESTROGENS ON CATECHOLAMINE LEVELS AND TURNOVER IN HYPOTHALAMIC AND VOCAL CONTROL NUCLEI IN THE MALE ZEBRA FINCH. S.R.Barclay and C.F.Harding. Biopsychology Program, Hunter College, NYC 10021

Behavioral and biochemical studies indicate that most male courtship behaviors, including singing, are induced by the combined actions of estrogens and androgens. The precise mechanism by which these steroids exert their effects is unknown. We recently presented evidence that castration alters catecholamine (CA) levels and turnover in steroid-sensitive brain areas which modulate sexual behavior and vocalizations in the male zebra finch and that these changes in CA levels and turnover can be reversed by treatment with an aromatizable androgen.

This study separated the effects of estrogenic and androgenic metabolites on CA function in the hypothalamus and vocal control system. Castrated male zebra finches were implanted with hormone-filled Silastic capsules (containing estradiol, dihydrotestosterone or both) and housed with a female for one week. To determine turnover as well as initial levels of CA, the males were divided into two subgroups: one group received αMPT, the other saline. The appropriate dosage and time course of αMPT treatment was empirically determined in a preliminary study. Brain nuclei were microdissected from 180μ frozen sections and expelled into sodium acetate buffer containing an internal standard. The concentrations of NE, DA and 5HT were determined by HPLC with electrochemical detection.

Differential control of steroid induced changes in CA levels and turnover in various vocal control and hypothalamic nuclei was found. Changes in NE levels and turnover were induced by estrogen alone. However, in most nuclei, changes in DA levels and turnover required both estrogen and androgen, or in a few areas, only androgenic stimulation. *(Supported by HD15191 and MH00591 to CFH, and MH09425 to SRB)*

174.7

IMMUNOCYTOCHEMICAL COLOCALIZATION OF SUBSTANCE P AND PROGESTIN RECEPTORS IN NEURONS OF THE FEMALE GUINEA PIG HYPOTHALAMUS. K.H. Nielsen*, J. Farkas* and J.D. Blaustein. (SPON: E.W. Westhead). Neuroscience and Behavior Program, and Psychology Department, University of Massachusetts, Amherst, MA 01003.

Recent studies have suggested that substance P (SP) is involved in the steroid-induced sexual behavior of female rats (W.A. Dornan, et al., Neuroendocrinolgy, 45:498, 1987). Adult female guinea pigs were ovariectomized and subsequently estradiol-primed. Two days before perfusion, 25 μg of colchicine were injected intraventricularly. SP and progestin receptors were labelled by a double immunocytochemical sequence. Distributions of SP-containing cells and progestin receptor-containing cells overlapped in regions such as the periventricular nucleus, the ventrolateral area of the hypothalamus (VLA), and the caudal arcuate nucleus. Colocalization of SP and progestin receptor immunoreactivity within the same cells was observed only in the VLA, where most of the progestin receptor-containing cells were also SP-immunoreactive. This provides neuroanatomical evidence that the mode of action of estradiol and progesterone may involve SP. (Supported by NS 19327 and RCDA NS 00970 from the NIH).

174.8

NEURAL SITE OF ACTION OF ESTRADIOL IN THE INDUCTION OF SEXUAL RECEPTIVITY IN FEMALE GUINEA PIGS. Y. Delville* and J.D. Blaustein (SPON: N.R. Carlson). Neuroscience and Behavior Program and Psychology Department, Univ. of Mass., Amherst, MA 01003.

Recent immunocytochemical studies performed in our laboratory have demonstrated that estradiol treatments that result in induction of progesterone-facilitated sexual behavior in guinea pigs also induce progestin receptor-immunoreactivity (PR-IR) in the preoptic area and the hypothalamus. To localize discrete areas sufficient for estradiol-induced sexual receptivity, behavioral response and PR-IR were evaluated after bilateral implantation of estradiol-containing cannulae in the the mediobasal hypothalamus. The animals were exposed to the estradiol released by the cannulae for two days. They were then injected sub cutaneously with progesterone. A dilution of 1% crystalline estradiol in cholesterol was sufficient to induce progesterone-facilitated sexual receptivity only when the implants were located in the rostral part of the ventrolateral area of the hypothalamus. These implants resulted in localized induction of PR-IR. This suggests that estradiol stimulation of a site in the rostral ventrolateral area of the hypothalamus is sufficient to induce behavioral response to progesterone.
(Supported by NS 19327 and RCDA NS 00970 from the NIH)

174.9

AUTORADIOGRAPHIC LOCALIZATION OF PROGESTIN-CONCENTRATING CELLS IN THE BRAIN OF THE ZEBRA FINCH (POEPHILA GUTTATA). J.L. Lubischer and A.P. Arnold. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

In the male zebra finch, the steroid hormones testosterone and estradiol are known to control the development and production of song. Although the role of progesterone in male behavior is not well understood, plasma levels of progesterone in young male zebra finches are higher than those observed for testosterone or estradiol (Prove, 1983). In the current study, the autoradiographic method was used to investigate the distribution of progestin-accumulating cells in the male zebra finch brain.

Three adult male zebra finches were castrated 24 hr before intramuscular injection of the synthetic progestin [17alpha-methyl-^{3}H]-promegestone (5.7 μCi/g), then decapitated 90 min after injection. The brain was immediately removed and processed for autoradiographic analysis. Cells were defined as being progestin-labeled if the density of silver grains over their somata exceeded by a magnitude of three the density over an adjacent area of neuropil. The loci of such cells were noted on section sketches drawn with the use of a microprojector.

Groups of progestin-accumulating cells were found in the dorsolateral region of the telencephalic hyperstriatum accessorium, the medial lobus parolfactorius, the preoptic area, and through much of the medial hypothalamus, including nucleus periventricularis magnocellularis, nucleus medialis hypothalami posterialis, and area infundibularis. Known song regions were not found to be sites of progestin accumulation. The general pattern of progestin-accumulating cells reported here is consistent with the pattern of steroid hormone accumulation observed in vertebrates studied to date, and suggests possible sites of progesterone action.
Supported by NIH grant NS 19645 and an NSF Graduate Fellowship.

174.10

THE CENTRAL FACILITATION OF RESPIRATION BY PROGESTERONE IS ESTROGEN-DEPENDENT. D.A. Bayliss, J.B. Dean, J.A. Cidlowski*, and D.E. Millhorn. Dept. of Physiology, University of N. Carolina, Chapel Hill, NC 27599.

We examined the possibility that the central, receptor-mediated facilitation of respiration by progesterone (PNAS,84:7788,1987) is estrogen-dependent. Ovariectomized cats were anesthetized, paralyzed and ventilated. Carotid sinus and vagus nerves were cut and end-tidal PCO_2 and temperature were maintained constant. Phrenic nerve activity was used as an index of central respiratory output. Repeated i.v. doses of progesterone (0.1 to 2.0 μg/kg, cumulative) caused a long-lasting (>45 min) facilitation of respiration in animals pretreated with 17β-estradiol (20 μg/kg/day) the 3 days prior to study; progesterone was without effect in animals not pretreated with 17β-estradiol. Thus, prior estrogen exposure is necessary for the respiratory response to progesterone in ovariectomized cats. The estrogen-dependent respiratory response to progesterone was attenuated by the progesterone receptor antagonist, RU486 (500 μg/kg). An estrogen receptor antagonist, CI628 (10 mg/kg/day), administered jointly with 17β-estradiol, caused a marked diminution of the respiratory response to progesterone. These results indicate that interaction of both progesterone and estrogen with their receptors is required in the mediation of this response.
Supported by HL-33831, AM-32459, AM-32460, & AM-32078.

174.11

STRESS, ESTROGEN RECEPTORS AND SEXUAL BEHAVIOR IN MALE ZEBRA FINCHES. M. Walters, A. Gonzalez* and C. F. Harding. Biopsychology Program, Hunter College, CUNY, New York, NY 10021.

Although male zebra finches exhibit a variety of estrogen-dependent sexual behaviors, binding studies have revealed relatively low levels of neural ^{3}H-estrogen binding in castrated males. To determine if these low levels of binding could have resulted from competition from endogenous estrogen, we conducted a series of *in vitro* exchange assays to measure occupied nuclear estrogen receptors (nER) in the basal hypothalamus-preoptic area (BH-POA) of castrated males. BH-POA cell nuclei were isolated and purified by resuspension and centrifugation in a series of sucrose solutions, and hormone-receptor complexes were extracted by dispersing the nuclei in a 0.4 M KCl solution. Extracts were then incubated with ^{3}H-estradiol for 5 hr at 25°C. Parallel incubations contained a 500-fold molar excess of unlabelled estrogen to correct for nonspecific binding. The assays revealed substantial levels of specific nER's in the BH-POA of castrated males (Kd = 0.39 nM; Bmax = 50 fM/mg DNA). However, substantial nER levels were also detected in the BH-POA of castrates treated with ATD, an inhibitor of estrogen synthesis. The nER levels in these birds appeared to be related to the stress of being transported from the housing facilities to the laboratory: nER levels were significantly reduced when the animals were not subjected to the stress of the transport procedure. These results suggest that neural estrogen receptors in male zebra finches bind a hormone which is i) stress-related and ii) not an estrogen. In behavioral experiments, intact males subjected to a simulated transport procedure exhibited significantly less estrogen-dependent behavior than unstressed controls. These data suggest that the unknown hormone may play a role in suppressing normal breeding behavior during periods of acute stress.
(Supported by grants MBRS RR08176, HD15191 and MH00591 to CFH)

174.12

MACRONUTRIENT INTAKE OVER THE ESTROUS CYCLE S. Montanez*, J. Williams*, N. DiMarco*, K.Eckols*, and L. Uphouse, Texas Woman's University, Denton, Texas, 76204

Carbohydrate and protein intake were examined during the female rat estrous cycle and in response to gonadal hormones. There was a significant effect of the estrous cycle on macronutrient intake (ANOVA, P< .001). The lowest levels of carbohydrate intake occurred during the stages preceding ovulation. In a second study, females were ovariectomized and injected for 5 consecutive days with 10 μg estradiol, 500 μg progesterone, or 1 day each of 10 μg estradiol, vehicle, 500 μg progesterone. Estrogen treatment produced a rapid (within 24 hours) reduction (20-40%) in carbohydrate intake which was partially reversed by the progesterone treatment. Progesterone alone had no effect on macronutrient intake. The present results suggest that it is the macronutrient intake, rather than total caloric intake, which is regulated during the estrous cycle. Since standard laboratory rat chow is high in carbohydrates, observations of a reduced caloric intake may result from the females' attempt to modulate carbohydrate intake. The pattern of estrous cycle changes and the effects of estradiol in ovariectomized females support the hypothesis that estrous cycle modulation of carbohydrate intake results from cyclic variations in gonadal steroids.
(Supported by NIH ESO-3351)

174.13

ANDROGEN, NOT ESTROGEN, AFFECTS ELECTRIC ORGAN DISCHARGE AND EXTERNAL MORPHOLOGY IN *GNATHONEMUS PETERSII*. R.E. Landsman*, C.F. Harding and P. Moller. Psychology Dept., Hunter College, CUNY, New York, NY 10021.

Electric fish use electric organ discharges (EODs) to communicate. Our previous work showed adult male *G. petersii* have shorter wave-form durations than females, but surprisingly, methyltestosterone treatment increased duration and decreased peak power spectrum frequency (PPSF) of Fourier-transformed EODs. To investigate the hormonal control of EODs and sex-related anal fin indentations of *G. petersii*, immature fish were gonadectomized and implanted with low-dose (1mm/2.5g b.w.) or high-dose (1mm/1g b.w.) silastic capsules of testosterone (T), DHT, estradiol (E) or cholesterol (C). At low doses, T increased EOD durations and decreased PPSFs compared to nonhandled controls, while the other implants had no effects. High-dose implants of T and DHT, but not E or C, increased EOD durations. High doses of T caused faster, more profound duration changes. Both doses of T and DHT induced typical male anal fin indentations.

Thus, EOD waveforms are affected by androgens, not estrogens. Similarly, the anal fin indentation characteristic of adult males appears to be under androgenic control. However, the effect of exogenous androgens on EODs is in the opposite direction from the reported sex difference in untreated fish.
Supported by MH09664 and a grant from Sigma Xi to REL.

174.14

EFFECTS OF SUSTAINED STRESS ON ADENYLATE CYCLASE ACTIVITY IN PITUITARY AND RAT BRAIN REGIONS. G.S. Dhillon* & G.J. Kant (Sponsor: M.L. Koenig) Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307-5100.

Acute stress activates brain adrenergic and CRF neuronal pathways. Chronic stress has been shown to decrease the number of brain beta-adrenergic receptors and to lower NE-stimulated cAMP responses in cortex and hypothalamus. Recently, we found that following chronic intermittent footshock, CRF receptor binding is decreased in pituitary, cortex and hypothalamus. The present study investigated NE and CRF-stimulated adenylate cyclase activity in pituitary, frontal cortex and hypothalamus following intermittent footshock.

Rats were sacrificed following 1 hour or 1, 3, 7 or 14 days of around-the-clock intermittent footshock (1 trial/5 minutes, avoidable on 90% of trials). Basal, NE and CRF-stimulated adenylate cyclase activity was measured in the P_2 fractions. Following one hour of footshock, there was an increase in basal adenylate cyclase activity in the hypothalamus. One day of footshock resulted in decreased basal adenylate cyclase activity in the anterior pituitary. In fractions prepared from three day stressed rats, basal adenylate cyclase activity increased in the hypothalamus and decreased in the frontal cortex. At all other time intervals, we did not observe any significant changes in basal or stimulated adenylate cyclase activity.

174.15

SEXUAL BEHAVIOR, EPISODIC GONADOTROPIN SECRETION, AND BRAIN NEUROPEPTIDE AND MONOAMINE LEVELS IN AGING MALE RATS. A.M. Clancy, F. Macrides, F.H. Bronson*, M.S.A. Kumar* and R.M.Kream*. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, University of Texas, Austin, TX 78712, Tufts University Schools of Medicine, Boston, MA 02111.

The relationships among sexual interest in estrous females, episodic secretion of luteinizing hormone (LH), and brain levels of luteinizing hormone-releasing hormone (LHRH), methionine-enkephalin (MET), beta-endorphin (END), dynorphin$_{1-17}$ (DYN), substance P (SP), cholecytokinin$_{1-8}$ (CCK), vasoactive intestinal polypeptide (VIP), norepinephrine (NE), dopamine (DA) and serotonin (5HT) were studied in male naive or retired breeder Fischer-344 rats across the age range of 8-29 mos. Each subject was paired with females on 3 occasions, then prepared for serial blood sampling via a femoral arterial bypass catheter. Following 3 hrs of blood collection, a 4th female pairing was initiated with sampling continued for another hour. Each male was then sacrificed for pathological workup and its brain dissected for measurements of neuropeptides by RIA and monoamines and metabolites by HPLC combined with electrochemical detection. The ratio of 5HT to 5HIAA was used as a measure of 5HT turnover. Time and amplitude series analyses of circulating LH levels demonstrated an aging-related decline in the pulsatility of spontaneous LH secretion, and both age and experience dependent changes in the ability of female-related olfactory and/or behavioral stimuli to elicit secretion episodes. The differences in LH secretion patterns were correlated with age and experience dependent differences in copulatory behavior. There were no significant neurochemical changes in the olfactory bulbs or rhinitic pathology that could account for these findings on the basis of a sensory impairment. LHRH, SP, CCK and VIP levels in the hypothalamus showed no significant changes with age or correlations with sexual behavior. In contrast, the hypothalamic levels of opioid peptides and monoamines, and 5HT turnover in the hypothalamus, did covary in relation to age, behavioral measures of sexual interest, and differences in LH secretion patterns. The changes in MET and NE levels showed the closest correlations with the endocrine and behavioral changes, suggesting that these transmitters or neuromodulators are integrally related to the regulation of episodic gonadotropin secretion and sexual arousal.

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174.16

AUGMENTATION OF ANDROGEN-DEPENDENT BRAIN AROMATASE ACTIVITY BY ESTROGEN IS NOT ANDROGEN RECEPTOR MEDIATED C.E. Roselli and T.A. Fasasi*. Physiology Dept., Oregon Health Sciences University, Portland, OR 97201

Estradiol (E) appears to increase cytosolic androgen receptor levels in the preoptic area (POA) of male rats. Thus, we were interested to determine whether a paradigm of combined treatment with E and dihydrotestosterone (DHT) would: 1) augment the stimulatory effect of DHT on aromatase activity (AA) and 2) increase the concentration of nuclear androgen receptor (ARn). To this end we measured simultaneously the concentrations of ARn and microsomal AA in POA and basal hypothalamus (BH) of adult male rats that were castrated (CX) and treated for 1 week with either vehicle, E (2 ug/day, sc), DHT (3 cm Silastic implant), E + DHT, or testosterone (T, 3cm Silastic implant). DHT stimulated AA ($p<.05$ vs. CX) in both POA and BH, but not as effectively as T. E alone did not change AA in POA, but potentiated the stimulation of AA by DHT ($p<.05$, DHT vs. E+DHT) to levels comparable to the T group. By comparison E did not enhance the induction of AA by DHT in the BH. Despite the ability of E to augment the induction of AA in POA, combined treatment with E and DHT had no effect on the concentrations of ARn in either the POA or BH. Futhermore, T appeared to be the most effective treatment for increasing the levels of ARn in both tissues. These results suggest that E augments androgenic responses in the POA by a mechanism that is independent of its effects on ARn levels. Supported by HD 23293.

174.17

ESTRADIOL (E) REGULATION OF RIBOSOMAL RNA (rRNA) PROCESSING IN RAT VENTROMEDIAL HYPOTHALAMIC (VMN) NEURONS : EARLY DETECTION BY QUANTITATIVE *IN SITU* HYBRIDIZATION. K. J. Jones[1,2], C. A. Harrington[3], D.M. Chikaraishi[3], and D.W. Pfaff[2]. [1]The Chicago Medical School, North Chicago, IL 60064, [2] The Rockefeller University, New York, NY 10021, and [3]Tufts University, Boston, MA 02111.

We have previously demonstrated that E acts very early, at structural, RNA and protein levels, in the rat VMN, a brain region critical in the control of reproductive behavior. Cellular organelles involved in ribosome production and function, including the nucleolus and RER, are morphologically altered, and nucleoplasmic changes consistent with increases in overall gene activity occur, in VMN neurons after 2 h of E. Six h of continuous E administered to ovariectomized (OVX) rats is sufficient to increase product rRNA levels in the VMN. In this study, we used quantitative *in situ* hybridization to determine the early effects of E on rRNA processing. Three OVX rats per group received E implants for 15 min, 30 min, or 2 h; OVX rats, sham-implanted under the same time courses, served as controls. Fresh, frozen brain sections containing the VMN were prepared. For detection of precursor and product levels of rRNA, 2 ^{3}H-labeled DNA probes, complementary to an external transcribed spacer region near the beginning of the rRNA gene and 28S product rRNA, respectively, were used. We additionally used ^{3}H-labeled poly U fragments to detect poly A$^+$ sequences in VMN neurons. Following prehybridization to eliminate nonspecific binding, hybridization was done overnight at Rm T. The sections were washed, dried, and processed for autoradiography. Quantitative analysis was accomplished using a computerized image analysis system to measure number of grains per cell, somal size, and grain density. Precursor rRNA levels rose to a statistically significant 30% increase after 30 min and a 110% increase after 2 h of E, relative to control values. Product rRNA levels were unaffected at any of the time points examined. Poly A$^+$ levels increased to 50% above control after 2 h of E. The rRNA data are consistent with the hypothesis that the increase in ribosomes after 6 h of E is preceded by rapid alterations in processing of newly transcribed pre-rRNA. We are currently exploring whether the preliminary data obtained with the poly U probes are reproducible and represent actual changes in poly A$^+$ sequence levels. Supported by BRSGSO7RR0526-27 and HD05751.

174.18

THE AROMATIZATION INHIBITOR, ATD, INHIBITS ANDROGEN RECEPTOR BINDING IN MALE RATS. M.Y.McGinnis and M.E.Kaplan*(SPON:J.E.Shriver). Dept. Anatomy, Mt. Sinai Sch. Med., NY, NY 10029.

The aromatization inhibitor, ATD, has been shown to inhibit sexual behavior, suggesting that aromatization to estradiol is required for the activation of male sexual behavior. In the present study, we examined 1) the effects of both systemic and intracranial ATD on male sexual behavior, and 2) the effects of systemic ATD on androgen receptor (AR) and estrogen receptor (E_2R) binding. Daily injections of 15 mg ATD suppressed sexual behavior in T-treated males. Bilateral MPOA implants of ATD inhibited sexual behavior, but were less effective than systemic injections. To determine whether ATD affects cell nuclear AR or E_2R binding, T-treated or castrated males were given ATD (15 mg/day for 7 days). Binding of T to AR's was significantly decreased in the presence of ATD. There was no effect of ATD on E_2R's. In vitro competition studies indicated that ATD markedly reduced cytosol AR binding, though it did not compete for nuclear AR's. Thus ATD appears to act both as an antiandrogen and as an aromatization inhibitor, and may inhibit male sexual behavior by blocking AR's rather than by inhibiting aromatization.

SECOND MESSENGERS VI

175.1

IDENTIFICATION OF THE AUTOPHOSPHORYLATION SITE AND REGULATORY DOMAINS IN BRAIN Ca^{++}/CALMODULIN-DEPENDENT PROTEIN KINASE II. T.R. Soderling, R.J. Colbran, and C.M. Schworer*. Howard Hughes Medical Institute, Vanderbilt University, Nashville, TN 37232

Ca^{++}/calmodulin-dependent protein kinase II (CaM-kinase II) is a multifunctional kinase, highly concentrated in neural tissues, which can be converted to a Ca^{++}-independent (indep.) form by intramolecular autophosphorylation. Through analysis of CNBr ^{32}P-peptides, we have identified thr-286 as the site of this autophosphorylation. A synthetic peptide containing residues 281-309 of the 50 kDa subunit sequence contains this phosphorylation site and also the CaM-binding domain and an inhibitory domain. Peptide 281-309 is not phosphorylated by the Ca^{++}-indep. form of CaM-kinase II, and it inhibits the kinase activity against other substrates. However, when Ca^{++}/CaM binds to peptide 281-309, the peptide is no longer inhibitory and it is phosphorylated. Additionally, the phosphorylated peptide in the absence of Ca^{++}/CaM is also less inhibitory. These results suggest the following model: 1) the inhibitory domain in residues 281-309 of CaM-kinase II is responsible for basal inhibition of the kinase, 2) binding of Ca^{++}/CaM to the adjacent CaM-binding domain relieves this inhibition, allowing phosphorylation of thr-286 and substrate proteins, 3) the phosphorylation of thr-286 diminishes the effect of the adjacent inhibitory domain, allowing for the Ca^{++}-indep. activity of the kinase.

175.2

CALCIUM/CALMODULIN STIMULATED Mg-ATPASE ACTIVITY ASSOCIATED WITH A NOVEL CALMODULIN-BINDING PROTEIN. R.E.Larson*, F.S.Espindola*, E.M. Espreafico*, M.V.Coelho* and D.E.Pitta*(SPON:J.A. Ricardo). Dept♀Bioquímica, Fac.Med.Rib.Preto Univ.Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil

Ca^{2+}/calmodulin(Ca/CaM) is the essencial link of a major signal transduction system in brain. Cytoskeletal proteins are important targets for the Ca/CaM complex. We reported the presence in mammalian brain of a novel calmodulin-binding protein(CaM-BP$_{190}$) which is tightly associated with brain actomyosin(Braz.J.Med.Biol.Res.21:213 1988).CaM-BP$_{190}$ precipitates with actomyosin from 1% Triton X-100 and 10mM EGTA, and accompanies actomyosin thru a solubilization-precipitation cycle by 1-0.1M KCl.Here we report the separation of CaM-BP$_{190}$ and brain myosin by differential centrifugation, gel filtration and ion exchange chromatography, and show that distinct ATPase activities are associated with the two proteins. Purified brain myosin has characteristic K-EDTA ATPase activity,whereas CaM-BP$_{190}$ has Mg-ATPase activity stimulated 5 fold by Ca/CaM and no detected K-EDTA activity. Polyclonal antibodies of CaM-BP$_{190}$and brain myosin show specific reactions for their respective antigens with no immunological cross-reaction detected by immunoblot technique.Grants:FAPESP 2056-7;CNPq 40.3824.

175.3

Involvement of Calmodulin in the potentiation of synaptic transmission by 4AP and the modulation of the AHP in the rat hippocampus. P. Perreault and M. Avoli MNI, McGill University, Quebec, Canada, H3A-2B4.

It has been suggested that 4-Aminopyridine (4AP) potentiates neurotransmission by directly promoting Ca^{++} entry in nerve terminals or indirectly, by blocking K^+ conductances. This enhanced Ca^{++} entry might in turn activate Ca^{++}-dependent second messengers. Here, we recorded intracellularly CA1 pyramidal neurons of rat hippocampal slices to asses whether the Ca^{++}-binding protein Calmodulin was related to 4AP effects. Bath application of the Calmodulin antagonist Trifluoroperazin (TFP,10-50uM) resulted in a marked decrease of the amplitude of orthodromic EPSPs and IPSPs. 4AP (50uM) applied on TFP-treated slices failed to potentiate synaptic potentials although its ability to increase neuronal intrinsic excitability was not impaired (n=5). After TFP washout, the amplitude of EPSPs and IPSPs increased markedly (above control levels) and spontaneous synaptic potentials became prominent. TFP also reduced the firing adaptation and decreased by up to 60% the amplitude of the slow AHP evoked by depolarizing current pulses. This effect was not due to a decrease in Ca^{++} entry since Ca^{++} spikes observed in the presence of TTX (1uM) and TEA (5mM) were not reduced. These results suggests that Calmodulin might be involved in the regulation of neurotransmitter release and modulate Ca^{++}-dependent K^+ conductances.

175.4

STIMULATION OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II BY BRAIN GANGLIOSIDES. K. Fukunaga and T.R. Soderling (SPON: D. Buxbaum), Howard Hughes Med. Inst., Vanderbilt Univ., Nashville, TN 37232

Gangliosides are ubiquitous, sialic acid-containing glycosphingolipids which are thought to play a role in the regulation of growth and proliferation. The actions of gangliosides may be partly related to modulating protein phosphorylation. The purified rat brain Ca^{2+}/calmodulin (CaM)-dependent protein kinase II (CaM kinase II) is stimulated by brain gangliosides. Among the various gangliosides tested, GT1b was found to be the most potent activator, whereas GD1a and GM1 were slightly less effective and asialo-GM1 had no effect. The activation process was rapid and did not require the presence of Ca^{2+}. Half-maximal activation by GT1b was observed at 25 μM and was nearly full at 125 μM. Using syntide 2 or synapsin 1 as substrate, the phosphorylation rate by CaM kinase II in the presence of GT1b was approximately 50% of the rate in the presence of Ca^{2+} and CaM. Gangliosides also stimulated the autophosphorylation of CaM kinase II, mainly on serine residues, but resulted in little formation of the Ca^{2+}-independent species. This ganglioside-stimulated autophosphorylation was also found in the synaptic membrane fractions from rat brain. These observations suggest that the activation of CaM kinase II by gangliosides may be different from activation by Ca^{2+} and CaM and relate to certain functions of gangliosides in the nervous system.

175.5

EFFECTS OF ADRENALECTOMY AND STRESS ON cAMP ACCUMULATION AND CALMODULIN SENSITIVE ADENYLATE CYCLASE IN RAT BRAIN REGIONS M.N. Gannon, R.E. Brinton, P. Choo* and B.S. McEwen, Laboratory of Neuroendocrinology, Rockefeller University, New York, N.Y. 10021

Glucocorticoids (GC's) have widespread effects on cAMP generating systems. Since GC's induce Ca2+ binding proteins in peripheral tissues, we studied whether GC`s modify calmodulin-sensitive (CaM) adenylate cyclase (AC) activity in rat brain membranes and measured in parallel their effects on cAMP accumulation in slices. Adrenalectomy (ADX, 6d) reduced CaM AC activity by 25% in hippocampus, dentate gyrus and Ammon's horn, with a similar trend in cerebral cortex. No effect of ADX on basal or forskolin AC activities was found. Noradrenaline-stimulated accumulation of cAMP in hippocampal slices in the presence of a phosphodiesterase inhibitor was also reduced by ADX. In contrast, 21d repeated, daily stress decreased maximal CaM AC activity in cortex membranes, which is consistent with work of Stone et.al. JPET 237:702,1986 who subsequently showed that adrenal steroids mediate this effect. Hippocampus is less affected. Thus adrenal secretions may have biphasic effects on the adenylate cyclase system of brain, although the effects of repeated stress are more pronounced than those of ADX. (Supported by MH41256.)

175.6

REGULATION OF PROTEIN TYROSINE KINASE BY CORTICOSTERONE IN DISCRETE REGIONS OF RAT BRAIN. E.J. Nestler, R.Z. Terwilliger*, and E. Halm*. Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Several lines of evidence suggest that protein tyrosine kinases play an important role in neuronal function, even though it has been difficult to demonstrate regulation of the enzyme in nervous tissue by first or second messengers. We now report such regulation for the first time: chronic corticosterone (CORT) treatment increases protein tyrosine kinase activity in discrete regions of rat brain.

Rats were treated with CORT for 1 week, and then isolated brain regions were analyzed for protein tyrosine kinase activity. It was found that such treatment increases protein tyrosine kinase activity by 20-25% in the locus coeruleus (LC), dorsal raphe, and ventral tegmentum, brain regions known to contain high levels of corticosterone receptors, but not in several other brain regions studied.

This phenomenon was characterized further in the LC. CORT was found to produce a time-dependent increase in protein tyrosine kinase activity, which plateaued between 4 and 7 d of treatment. This increase was found to be localized to the microsomal fraction of the LC, and to be associated with a 60% increase in the amount of phospho-tyrosine present in the LC, as determined by immunoblot analysis. Bilateral adrenalectomy led to a 25% decrease in enzyme activity, an effect reversed by CORT replacement.

These results demonstrate that protein tyrosine kinase is regulated by corticosterone in specific regions of the nervous system, and raise the possibility that some of the effects of glucocorticoids on neuronal function are achieved through alterations in the phosphorylation of target proteins on tyrosine residues.

175.7

GLYCOPROTEIN-ASSOCIATED SERINE PROTEIN KINASE FROM RAT BRAIN SYNAPTOSOMES. E. Suwita* and N. Sahyoun. (SPON: J. Reinhard). The Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709.

A glycoprotein preparation was obtained from Triton X-100 extracts of purified rat brain synaptosomes by wheat germ agglutinin (WGA) affinity chromatography. The glycoprotein eluate was examined for protein kinase activity in the presence of several potential substrates. A broad spectrum of substrates was phosphorylated by a putative glycoprotein-associated protein kinase including myelin basic protein, protamine, tubulin, casein and a mixed histone preparation. Myelin basic protein was phosphorylated solely on a serine residue with a substrate K_m of 0.33 µM. The K_m for ATP was 92 µM and GTP could not replace ATP. The protein kinase had a K_m of 0.38 mM for $MgCl_2$ and was inhibited by $MnCl_2$. Several protein kinase regulators such as cyclic AMP, Ca^{2+}, calmodulin and phosphatidylserine did not alter enzyme activity. The presence of a glycoprotein-associated serine protein kinase suggests an additional mechanism for synaptosomal transmembrane signalling.

175.8

A RAT BRAIN NA^+/K^+-ACTIVATED PROTEIN KINASE. C.H. Reynolds* and N. Sahyoun. (SPON: R. Ferris) The Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709.

An endogenous brain polypeptide (Mr 42,000) was avidly phosphorylated by a soluble brain protein kinase in a reaction which required the presence of K^+ or Na^+. NH_4Cl but not LiCl or Tris-HCl could substitute for KCl or NaCl. The activation process was highly cooperative with respect to monovalent cation concentration with an n value of 3.4 for KCl such that maximum change in activity occurred between 100 mM and 150 mM salt. NaCl alone was less potent than KCl; however, when the enzyme was 'primed' with 100 mM KCl, further addition of NaCl was as effective as KCl. The apparent K_m values for ATP and protein substrate at 150 mM KCl were about 10 µM and 50 nM, respectively. Enzyme activation largely involved an increase in the V_{max} values. GTP replaced ATP as a phosphate donor and the enzyme activity was inhibited by 2-10 µM heparin. The protein kinase was purified on octyl-Sepharose and heparin-Sepharose affinity columns, and appeared to have a narrow spectrum of substrates although it did phosphorylate casein poorly and phosvitin more effectively. The detection, characterization and purification of a brain protein kinase which has an obligatory requirement for K^+ or $Na^+ + K^+$, at physiological intracellular concentrations, suggests a significant regulatory role for these ions whose levels can be altered by neuronal activity.

175.9

ISOLATION AND CHARACTERIZATION OF PKCAM-GR.
C.A. Ohmstede*, K.F. Jensen and N. Sahyoun. The Wellcome Research Laboratories, 3030 Cornwallis Road, Research Triangle Park, NC 27709.

Previously, we described the isolation of cDNA clones from a rat brain expression library which encoded a novel brain-specific, calmodulin-dependent protein kinase (Soc. for Neurosci. 13:1136 1987). Comparison of the cytosolic fractions from forebrain and cerebellum by Western blotting with a polyclonal antibody against the recombinant protein showed a greater concentration of the enzyme in cerebellum than in forebrain with the presence of a common Mr 65,000 polypeptide and a unique Mr 67,000 polypeptide only in cerebellum. We purified the enzyme to apparent homogeneity from rat cerebellum and obtained a fraction with two subunits (the Mr 65,000 and 67,000 polypeptides) both of which bound the specific antibody by Western blot analysis. In the presence of calmodulin, the purified enzyme phosphorylated homogeneous synapsin I as well as a Mr 100,000 polypeptide from 55°C-treated cerebellar cytosol. This Mr 100,000 polypeptide was also phosphorylated in non-heated extracts in a calmodulin-dependent fashion but was not phosphorylated by the addition of purified calmodulin-dependent kinase type II. The immunohistochemical localization of the kinase showed the protein to be highly enriched in the perikaryon of cerebellar granule cells, therefore we have named this enzyme PKCAM-Gr.

175.10

CASEIN KINASE II AND ITS SUBSTRATES IN BRAIN.
J.-A. Girault*, H.C. Hemmings Jr., S.H. Zorn, and P. Greengard, Lab. of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021

We have studied the characteristics and distribution in brain of casein kinase II (CK II). CK II purified from bovine caudate nucleus or cerebral cortex exhibited a subunit composition and many properties similar to those reported for CK II from other tissues, including inhibition by heparin and stimulation by polylysine. CK II activity was higher in rat brain than in liver and, within brain, was highest in hippocampus and caudate-putamen. CK II activity decreased by 50% following injection of the neurotoxin quinolinic acid into the caudate-putamen showing relative enrichment of CK II in neurons. In addition to known substrates for CK II, several major substrates for this enzyme could be identified in all brain regions studied that were not found in peripheral tissues. Moreover, three substrates had a limited regional distribution. These included DARPP-32, enriched in caudate-putamen, and proteins of Mr 77,000 and 240,000 enriched in spinal cord and cerebellum, respectively. In conclusion, the high activity of CK II in brain as well as the number of its substrates suggest that this enzyme may play an important role in signal transduction in the CNS.
Supported by USPHS MH40899.

HYPOTHALAMIC - PITUITARY - GONADAL REGULATION I

176.1

EVIDENCE OF LOCALIZED DA-LHRH INTERACTION IN EWE HYPOTHALAMUS. JP Advis*, CD Conover*, AM Contijoch* and RO Kuljis. (SPON: L. Grandison) Animal Sci, Rutgers, New Brunswick, NJ 08903 & Sec Neuroanat, Yale Sch Med, New Haven, CT 06510.

Pharmacological evidence has implicated hypothalamic (hyp) catecholamines in the neuroendocrine control of reproduction. We assessed: **a)** localization of Lutainizing Hormone (LH)-Releasing Hormone (LHRH)-, tyrosine hydroxilase (TH)-, and dopamine (DA)-β-hydroxylase (DBH)-like immunoreactivities (IR, PAP), and **b)** contents of LHRH, DA and its metabolites (HVA, DOPAC) in samples of discrete hyp regions from ewes killed in anestrus and at each estrous cycle stage (1-2 PM, RIA & LCED, n=26). Reproductive stages were assessed by estrous behavior and serum progesterone and serum pulsatile LH. Ewes were killed in early luteal (EL), mid-luteal (ML), late luteal (LL), estrus (E), and anestrus (A). Perikarya containing TH-IR but not DBH-IR were scattered throughout the hyp. Particularly dense collections of them were situated in the region of the arcuate (arn) and paraventricular nuclei and the medial preoptic area (mpoa). Most LHRH-IR cell bodies were observed in the mpoa. Many of their axons descend into the infundibulum (inf) through the arn. In EL ewes, inf-content of DA (81± 3 pg/µgP, mean ±sem) and LHRH (24±2 pg/µgP) was 4 times higher than in the arn ($p<.01$) and a medio-lateral gradient of decreasing content exist through inf, arn, peri-arn, lateral (l) peri-arn, and l-hyp. In inf DA, DOPAC, and HVA-content increases continuosly from EL through LL (100%) to suddenly decrease in E (to EL values). In contrast, LHRH content kept increasing ($p<.01$) even further in E ewes (400%). DA and LHRH inf-content in A was 10-20% of EL ($p<.01$). LHRH mpoa-content was highest in EL (5±1 pg/µgP) and gradually decreased ($p<.01$) in lpoa, lhyp, diagonal band of Broca, as well as in ML, LL, E, and A ewes. DA, DOPAC, and HVA contents were similar in mpoa, lpoa and lhyp at all times studied, but gradually decreased ($p<.01$) in the diagonal band of Broca from their highest values in ML to their lowest values in EL, which were similar to those shown in A. These findings support the possibility that regulatory DA-LHRH interactions might occur at the level of the median eminence in adult cycling ewe. (Supported by Wetterberg Foundation, Hatch 08616 and USDA 87CRCR-1-2558).

176.2

ACCESS OF LHRH NEURONS TO THE VASCULATURE IN RATS.
J. Witkin, A. Giordano and A.J. Silverman. Anat.& Cell Biol., Col. U. Coll. P&S, New York, NY 10032.

LHRH neurons form neurosecretory terminals on fenestrated blood vessels and make synapses on neurons. The distribution of neurons making these contacts is not completely understood. Wheat germ agglutinin, when applied directly to the median eminence (ME), retrogradely labeled about 50% of the LHRH neurons in all brain regions in male rats (J. Neurosci. 7: 2312,'87). Intravenous infusion of horseradish peroxidase labeled a similar subpopulation of LHRH neurons in the female mouse (Jennes and Stumpf, Neurosci. 18:403,'86). We used intraperitoneal injections (2 doses: 20mg/kg, 8 and 4 d. prior to sacrifice) of fluorogold (FG). LHRH was demonstrated (Texas red) in 40um brain sections from septum to medial basal hypothalamus. More than 90% of all LHRH neurons contained tracer in intact males (2mo and 24 mo), in cycling females (when FG injections were timed around the LH surge), and in ovariectomized (30d) rats treated with estradiol benzoate and progesterone. Since uptake of the tracer accompanies exocytosis, virtually all LHRH neurons in these animals apparently have access to the vasculature. Which of these neurons projects to the ME must be dissected by additional studies. USPHS AG05366 (JW) and HD10665 (AJS).

176.3

DYNAMIC CHANGES IN LHRH CONCENTRATION IN HYPOTHALAMIC SITES FOLLOWING PREMATURE INDUCTION OF THE PROESTRUS LH SURGE WITH AN OPIOID ANTAGONIST. W. Jacobson*, A. Sahu*, and S.P. Kalra, (SPON: P.S. Kalra). Dept of Ob Gyn, University of Florida, Gainesville, FL, 32610.

An infusion of the opioid receptor antagonist naloxone (NAL) can advance the onset of the LH surge during proestrus (PE) in the rat. However, the effects of this decrease in opioid tone on dynamic changes in hypothalamic LHRH have not been examined. Because the cell bodies and neuronal processes of the LHRH neurons are widely distributed within the preoptic area (POA) and the mediobasal hypothalamus (MBH), we examined naloxone-induced changes in the LHRH concentration of several hypothalamic nuclei previously implicated in the generation of the proestrus LH surge. Female rats which had demonstrated at least 2 consecutive 4 day estrous cycles were implanted with an indwelling atrial cannula at 0800-0900 hours on the morning of PE. Naloxone was infused (2 mg/hr; 600 µL/min) starting at 1100 hours, and animals were sacrificed at 0, 90, and 180 minutes later. Trunk blood was collected for LH determination and brains were removed, frozen on dry ice, and sectioned at 300µ. Median eminence (ME), medial preoptic area , arcuate nucleus , and ventromedial hypothalamic nuclei were punched out by the method of Palkovits, and the concentration of LHRH was determined. Serum LH was significantly increased over control levels in animals infused with both SAL and NAL by 180 minutes ($p<.025$; $p<.005$ respectively), however, the NAL group had significantly higher levels than the SAL group at 180 minutes ($p<.025$). LHRH concentrations (pg/µg protein) were unchanged in all hypothalamic nuclei except the ME at 180 minutes after the onset of NAL infusion, where it was significantly elevated ($p<.01$). We conclude from these studies that NAL can activate hypothalamic LHRH neuronal systems in association with the LH surge, but such action is restricted to the ME. Consequently, opioid interactions with LHRH neurons for the induction of the LH surge may occur in the median eminence. (NIH HD08634.)

176.4

LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) NEUROSECRETION FOLLOWING NEUROTRANSMITTER EXPOSURE IN PERIFUSION TISSUE CULTURE: DIFFERENTIAL REBOUND RESPONSES TO OPIOID AND ADRENERGIC AGENTS. Richard W. Clough. Department of Anatomy, Southern Illinois University School of Medicine-Carbondale, Carbondale, Illinois 62901

Previous studies in this laboratory have demonstrated the utility of preoptic area-mediobasal hypothalamic-median eminence (POA-MBH) explants for the study of LHRH neurosecretory control. In this regard, we and others have described a stimulatory role for adrenergic agents (Clough, et al., Br. Res. 446, 1988) and an apparent inhibitory effect of endogenous opiates (Clough, et al, Brain Opioids in Repro. Cambridge, UK, 1987) on LHRH neurosecretion in vitro. Modulation of luteinizing hormone and follicle stimulating hormone secretion by adrenergic and opiate systems in vivo have been described which parallel our findings on LHRH release in vitro. The present abstract reports that a consistent observation in the perifusion of POA-MBH explants is a rebound reduction in LHRH release to sub-baseline levels following a stimulatory episode such as provided by phenylephrine or norepinephrine. A similar rebound reduction is found following exposure of POA-MBH explants to the opiate antagonist naloxone, which also provides a stimulatory signal to LHRH neurosecretion. Concomitant naloxone and phenylephrine which have additive stimulatory effects also induces a rebound reduction in LHRH release. In sharp contrast, we have found that beta-endorphin (10-6 M) negates the stimulatory effect of phenylephrine on LHRH neurosecretion and furthermore induces a rebound increase of LHRH release following washout of beta-endorphin. These observations suggest that exposure of LHRH neurons to stimulatory or inhibitory neurotransmitter agents induces a differential redistribution of the releasable pool of LHRH following stimulation or inhibition. Hypothetically, this may be necessary to maintain a steady state condition in LHRH synthesis. Supported by HD 24426 and Southern Illinois University.

176.5

BETA-ENDORPHIN (B-E) and GnRH INNERVATION OF GnRH NEURONS. W.-P. Chen*, J.W. Witkin and A.J. Silverman. Columbia Univ., P&S, N.Y., N.Y. 10032.

Endogenous opiates play a role in the tonic inhibition of gonadotropin secretion, presumably via a modulation of GnRH release. To determine if this effect could be mediated by a direct synaptic input from B-E terminals we studied the organization of B-E input to the GnRH neuron quantitatively. A double label EM procedure that utilizes DAB and TMB (Olucha et al., '85, Norgren and Lehman, '88) as chromogens was employed. 4 month old male rats were perfused with 4% paraformaldehyde and sections immunostained for both peptides. The two antigens could be distinguished by the morphology of the reaction product; flocculent for DAB, sharp crystals for TMB. Each of ten preoptic area/diagonal band GnRH neurons were analysed in 20 serial sections. 22 synapses were found innervating these cells of which 2 were B-E +, 1 was GnRH +. In the same tissue sections numerous GnRH dendritic profiles were present and found to receive 31 synapses of which 7 contained B-E, 7 GnRH. Most, if not all, of these terminals had symmetrical synaptic densities but physiological analysis is required to determine their inhibitory or excitatory characteristics. These results suggest (1) that B-E may act directly on GnRH neurons and need not act via changes in catecholamine release and (2) B-E and GnRH may have equally important roles to play in the direct modulation of GnRH secretion. HD 10665, China Medical Board (WPC).

176.6

SEX DIFFERENCES IN THE RESPONSES OF HYPOTHALAMIC LUTEINIZING HORMONE-RELEASING HORMONE AND CATECHOLAMINE SYSTEMS TO OVARIAN HORMONES AND NALOXONE: IMPLICATIONS FOR SEXUAL DIFFERENTIATION. W.R. Crowley. Dept. of Pharmacology, University of Tennessee College of Medicine, Memphis, TN 38163

Normal male rats do not respond in adulthood to ovarian hormone treatments that stimulate preovulatory-like surges of luteinizing hormone (LH) in normal females. The present experiments tested whether sex differences exist with respect to important neural events that are antecedent to the LH surge. In estrogen-primed females, but not males, progesterone induced a sequential accumulation and decline of LH-releasing hormone in the median eminence prior to the LH surge, and increased turnover rates of norepinephrine (NE) and epinephrine (EPI) in the medial basal hypothalamus during the time of the LHRH accumulation. To test whether direct blockade of opiate action would produce equivalent neuroendocrine and neurochemical responses in males and females, gonadectomized, estrogen-primed rats of both sexes were treated with the opiate antagonist naloxone. In contrast to females, males did not respond to naloxone with either LH release or increases in NE or EPI turnover. These data indicate that the adrenergic systems that control the neurosecretion of LHRH are insensitive to ovarian hormones in adult male rats, and further, that this may be due in part to sex differences in the control of adrenergic transmission by opioid systems.

176.7

LHRH NEURONS HAVE LIMITED RESPONSIVENESS TO NOREPINEPHRINE (NE) IN ESTROGEN-TREATED OVARIECTOMIZED RATS. R.D.Hartman, S.L.Petersen and C.A.Barraclough. Dept. Physiol., Univ. Maryland, Sch. of Med., Baltimore, MD 21201.

We previously reported that electrical stimulation (ES) of the locus coeruleus or the A1 noradrenergic cell groups did not alter basal LH secretion. One explanation is that LHRH neurons in this animal model have limited responsiveness to NE. To test this hypothesis, we infused NE into the third ventricle of chloral hydrate anesthetized rats. LH levels increased 144% within 10 min and rapidly returned to baseline. In contrast, MPN electrochemical stimulation (ECS) induced a LH rise of 637% and when NE was given icv 30 min later a further 340% increase in LH occurred. A second study, examined the effects of bilateral MPN microinfusions of NE (20 ug, 1ul/1min) and failed to obtain any change in basal LH release over 90 min. To avoid LHRH neuronal damage which occurs after ECS, we bilaterally ES the MPN for 5 min and LH rose about 90% within 15 min. If MPN-ES was performed for 5 min and 1 min later NE was infused into the MPN, LH release increased 330% and release was sustained over 45 min. Taken together the data suggest that LHRH neurons are severely limited in their responsiveness to NE. For LHRH neurons to acquire responsiveness to endogenous NE release seems to require the removal of some inhibitory influence which may serve to protect these neurons from extraneous NE secretion except for specific hours during the day of the LH surge. NIH grant HD-02138.

176.8

NEUROENDOCRINE GNRH AND DOPAMINE NEURONS IN THE MONKEY HYPOTHALAMUS IDENTIFIED BY RETROGRADE TRACING AND IMMUNOSTAINING. P.C. Goldsmith, K.K. Thind and J.E. Boggan*. Reproductive Endocrinology Center and Dept. of OB/GYN & Repro. Sci., Univ. Calif., San Francisco, CA 94143.

In order to localize hypophysiotropic neurons in the monkey hypothalamus, 4 juvenile cynomolgus were each given 3 microinjections (30-70 ul each) of the retrograde tracer WGA-apoHRP-gold into the infundibular stalk. Five to 15 days following surgery, the brains were fixed by perfusion, and vibratomed at 40 um in the frontal plane. Every 12th section was immunostained (IMM+) with rabbit anti-GnRH or anti-tyrosine hydroxylase for dopamine (DA) neurons using the PAP technique with DAB. Neuroendocrine neurons (NEU+) were easily identified as brown, DAB-labeled cell bodies containing more than 3 dark blue, tracer-filled lysosomes. Neuron counts in camera lucida maps from a single representative series of sections are compiled here by anatomical region:

GnRH	MPOA	LH	SON	VHT3	VHT2	VHT1
NEU+	19	9	6	2	11	20
IMM+	73	27	11	8	19	28

DA	A11	A12	A14	SCN	SON	ZI
NEU+	134	33	198	63	9	0
IMM+	213	47	436	144	44	240

Significantly more GnRH NEU+ occurred in the medial ventral hypothalamic tract (VHT1 71%) than laterally (VHT3 25%), while the SON (55%), LH (33%) and MPOA (26%) had intermediate amounts. DA NEU+ were more numerous in A12 (70%) and A11 (63%) than anterior periventricular regions (A14 45%; SCN 44%), but were absent from zona incerta (ZI). These results verify the greater NEU+ importance of VHT GnRH and DA neurons near the infundibulum, and suggest 50-75% of more distant IMM+ neurons in primates function otherwise.

Supported by NIH HD10907 and the Andrew W. Mellon Foundation.

176.9

ESTROGENIC REGULATION OF proGnRH mRNA IN THE RAT PREOPTIC-ANTERIOR HYPOTHALAMIC REGION. M. Jakubowski*, M.Blum, P. Seeburg* and J.L. Roberts. (SPON: L. Fricker) Fishberg Center for Neurobiology, Mt. Sinai School of Medicine, CUNY, New York, NY 10029.

The role of ovarian secretions on the content of pro-gonadotropin-releasing hormone (proGnRH) mRNA in the preoptic-anterior hypothalamic (POA-AH) continuum was studied. Female Sprague-Dawley rats, maintained under 12:12 h light/dark cycle (lights on at 07:00 h), were sacrificed by decapitation between 10:00 and 12:00 h. Dissections of the POA-AH were homogenized individually and fractionated. RNA of the cytoplasmic fraction was purified using the proteinase K procedure followed by phenol/chloroform extractions and ethanol precipitation, yielding approximately 0.5 µg total RNA per mg wet tissue. Cytoplasmic proGnRH mRNA was quantitated using solution hybridization/RNase protection assay, with the protected fragments separated on 5% polyacrylamide gel. The level of sensitivity was 40 fgm of a 370-b-long-protected RNA fragment, and the levels measured in individual tissue dissections ranged between 450 and 2100 fgm, depending on the amount of total RNA loaded. The mean ± SEM fgm of protected proGnRH mRNA/µg total RNA was 57.4 ± 2.8 in regularly cycling rats, 42.6 ± 5.9, 26.6 ± 5.0, 38.0 ± 4.7 and 29.8 ± 4.6 in rats sacrificed 2, 9, 16, and 24 day after ovariectomy, respectively, and 47.0 ± 4.3 and 45.6 ± 3.1 in 24-day ovariectomized rats treated with estardiol for 1 or 4 days, respectively (7 rats/group; $p < 0.05$, by one-way ANOVA across the 7 groups). The results suggest that the content of proGnRH mRNA in the rat POA-AH region decreases in the absence of ovarian steroids, and increases following estrogenic stimulation.

176.10

EFFECTS OF SUPRACHIASMATIC NUCLEUS (SCN) OR ANTERIOR MEDIAL PREOPTIC NUCLEUS (AMPO) LESIONS ON GONADOTROPIN RELEASING HORMONE(GnRH) AND PROGnRH LEVELS IN THE FEMALE RAT. Y.J. Ma*, M.J. Kelly and O.K. Ronnekleiv, Dept. of Physiology, OHSU, Portland, OR 97201

Experiments were designed to investigate the changes in proGnRH and GnRH levels after bilateral electrolytic lesions of the SCN or AMPO in the female rat. These lesions produced persistent estrus (PE), and were done as described (Neuroendocrinology 43: 566, 1986). The rats were sacrificed 2-3 months later, the brains were frozen, microdissected and radioimmunoassays were done to measure proGnRH and GnRH. In PE rats, proGnRH in the preoptic area (POA) was dramatically reduced (0.91 ± 0.1 fmoles/mg tissue) vs. proestrous controls (7.99 ± 1.1 fmoles/mg) ($P<0.01$). In contrast, GnRH levels in the POA were not significantly different in PE rats (15.6 ± 2.1 fmoles/mg) vs. controls (15.3 ± 0.5 fmoles/mg). Also, in basal hypothalamus, GnRH levels were not significantly different (94.2 ± 10.6 fmoles/mg) vs. controls (96.3 ± 8.7 fmoles /mg). Thus, lesions of SCN or AMPO cause PE without a reduction in GnRH, while proGnRH levels are reduced, suggesting that these nuclei may control GnRH synthesis and/or release. (Supported by HD 16793 & 19905)

176.11

DISTRIBUTION OF proGnRH AND GnRH IN THE HYPOTHALAMIC-PREOPTIC AREA OF THE FEMALE GUINEA PIG. J.E. Thornton, M.J. Kelly and O.K. Ronnekleiv, Dept. of Physiol., OHSU, Portland, OR 97201.

To gain insight into the cellular biosynthesis and processing of gonadotropin releasing hormone (GnRH), we have examined the localization of proGnRH and GnRH in the adult female guinea pig. Ovariectomized females were injected with 25 μg EB or oil, were perfused 38 h later with 4% paraformaldehyde, and 50 μm vibratome sections were made. ProGnRH was localized with ARK-2 antiserum, and GnRH was localized with EL-14 antiserum, using avidin-biotin-HRP immunohistochemistry (Neuroendocrinology (1987), 45: 518). Specificity was determined by preabsorption of the antisera with their respective antigen. ProGnRH immunopositive perikarya were seen throughout the septal-preoptic-hypothalamic area, with the majority of cells in the preoptic area. Staining was confined to cell bodies and to a few proximal fibers. GnRH was also seen in cell bodies and the distribution and number of GnRH cells was similar to that of proGnRH cells ($r=.89$, $p<.05$). However, in contrast to proGnRH, GnRH was also seen in many small distal fibers and nerve terminals. These data suggest that, similar to the rat and monkey, proGnRH processing to GNRH in the female guinea pig appears to be initiated in the cell soma. (Supported by HD16793 and HD19905).

176.12

DISTRIBUTION OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH)-CONTAINING NEURONS WITHIN THE BRAIN OF A MUSTELID, THE EUROPEAN FERRET. Y.P. Tang and C.L. Sisk, Neuroscience Program and Dept. of Psychology, Michigan State University, East Lansing, MI 48824.

LHRH-containing neurons were identified immunocytochemically in three adult male European ferrets (Mustela putorius furo). Anti-LHRH LR-1 (obtained from Dr. R. Benoit) was used as primary antibody and LHRH cells were visualized in 40 um coronal sections using the Vecastain ABC kit and diaminobenzidene as chromagen. Every other section from rostral forebrain through the mammillary bodies was reacted, counterstained with cresyl violet, and examined microscopically. The total number of LHRH cell bodies within the ferret brain was estimated to be 500-700 (2x absolute number of identified cell bodies). LHRH cell bodies were diffusely distributed in structures ranging as far anteriorly as septum and diagonal band of Broca, in medial preoptic area, and in structures extending as far caudally as the arcuate nucleus and median eminence. Approximately 20% of the somata were located rostral to optic chiasm and 80% were within or caudal to the chiasmatic region. Most hypothalamic LHRH somata were located at the base of the brain. The distribution of LHRH cell bodies in the ferret thus resembles that of primates in that a significant proportion of the somata are within retrochiasmatic mediobasal hypothalamus.

176.13

PULSATILE RELEASE OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) DURING SEXUAL MATURATION IN THE FEMALE RAT. H.F.Urbanski and S.R.Ojeda. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Blocks of hypothalamic tissue (POA-MBH) were obtained from female rats at 6, 12, 18, 24, 30 and 36 days of age and individually perifused for 5 hr using the Endotronics Acusyst system. The perifusate was collected in 5-min fractions (375 μl) and the LHRH content determined by RIA. In most cases the in vitro release pattern of LHRH was found to be pulsatile though pulse amplitude was quite variable, both within and between age groups. On the other hand, the LHRH interpulse interval (40-50 min) was similar in all of the groups and was lower than the LH interpulse interval observed in vivo (ca 35 min). These findings demonstrate that the hypothalamus of developing female rats can release LHRH in a pulsatile manner in vitro in the absence of extrahypothalamic inputs. Moreover, they show that the intrinsic rhythmicity of the hypothalamic pulse generator is established very early in life and is therefore unlikely to be a limiting factor for puberty to occur. Consequently, LHRH-dependent changes in gonadotropin secretion associated with sexual maturation are likely to depend upon alterations in extrahypothalamic inputs to the LHRH-releasing system. (Supported by NIH Grants HD-09988 and RR-00163)

176.14

PULSATILE SECRETION OF LHRH INTO THE HYPOPHYSEAL PORTAL BLOOD: EFFECT OF OVARIECTOMY AND ESTRADIOL REPLACEMENT. M. Ching, F.J. López*, M.G. Wisniewski*, and A. Negro-Vilar. Reprod. Neuroend. Sect., LMIN, NIEHS, NIH, Research Triangle Park, NC 27709.

Recently, we reported that LHRH secretion was reduced at 10-12 days after orchidectomy (ORDX) when compared with intact or ORDX rats given testosterone replacement (Abstr. #608, Endocr. Soc. 69th Mtg., 1987). The present study extended these observations to the adult female rat and evaluated the differences in LHRH secretion between intact metestrous (MET), 10 day-ovariectomized (OVEX) and OVEX-estradiol (E_2)treated rats. The collection and processing of portal blood for LHRH analysis was described recently (Brain Res. 443:325, 1988). Mean portal plasma LHRH levels were significantly reduced in OVEX rats and restored to MET levels with E_2 therapy. As observed in male rats, LHRH secretion occured in a pulsatile pattern. LHRH pulse frequency and duration were unaffected by the treatments. Analysis of area under the pulse revealed 2 populations of LHRH pulses in portal blood: small mass (SM) and large mass (LM) pulses. LM pulses occurred usually after 15.00 h and were absent in OVEX rats. They represented 14% of all pulses during MET and 29% after E_2 treatment ($P<0.02$ vs the OVEX rats). Evidently, E_2 can induce the appearance of LM pulses in the portal blood in the late afternoon. In conclusion, OVEX induces a decrease in mean LHRH levels and a disappearance of LM LHRH pulses. Both changes are reversed by E_2 treatment.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION II

177.1

REPRODUCTIVE PHOTOREFRACTORINESS IN MALE RHESUS MONKEYS: APPEARANCE OF ENDOGENOUS CIRCANNUAL RHYTHMS. E.A. Libré*, C.L. Chik* and G.R. Merriam, Developmental Endocrinology Branch, NICHD, NIH, Bethesda, Maryland 20892.

In seasonally breeding primates such as the rhesus monkey, short days activate and long days suppress reproductive function. When other mammals with a similar pattern of seasonality, such as the sheep, are subjected to constant long days over an extended period of time, gonadal function eventually reactivates. This phenomenon of "photorefractoriness" has not been documented in primates. In the present study, we analyzed the reproductive function of 5 male rhesus monkeys subjected to continuous long days (16h light:8h dark) for a period of 96 weeks. Responses were compared to 4 controls housed under alternating 16 week cycles of long and short days (8h light:16 h dark). Measurements of body weight and testis volume were obtained at 2 week intervals, and blood was drawn for testosterone, prolactin and gonadotropins. The data were examined for cyclicity using least squares sine wave analysis. Under continuous long day conditions, gonadal function was initially suppressed. After ≃12 weeks, there was an escape from suppression, with an increase of mean testis volume from 15 cc to 21 cc, followed by repeated cycles of gonadal regression and recovery, with a periodicity of 49 ± 3 weeks. In contrast, the control animals' testes grew under short days and regressed under long days, displaying a 36 ± 3 week cycle. These findings indicate that the phenomenon of photorefractoriness exists in primates. When housed under continuous suppressive lighting conditions, the animals eventually show a reactivation of the reproductive function, with emergence of an endogenous circannual cycle that is independent of photoperiodic cues.

177.2

EFFECT OF OVARIECTOMY AND ESTRADIOL TREATMENT ON PLASMA LH IN HYPOGONADAL (HPG) FEMALE MICE WITH PREOPTIC AREA (POA) GRAFTS. M.J. Gibson and A.J. Silverman. Dept. of Medicine,Mount Sinai Sch of Medicine,New York,NY 10029.

POA grafts containing GnRH cells implanted into the IIIrd ventricle of adult hpg mice, genetically unable to produce GnRH, correct their hypogonadism. Fibers from grafted GnRH cells project to the median eminence of host brains; increased gonadotropin release and gonadal development is seen. Hpg females with POA grafts (HPG/POA) generally do not have ovulatory cycles but may show an LH surge and ovulation after mating. 1/4 to 1/3 of HPG/POA also exhibit positive feedback with increased plasma LH and/or ovulation after progesterone administration. The present studies were planned to evaluate negative feedback responses.

HPG/POA mice were ovariectomized (OVEX) 3, 6,or 9 months after graft surgery and subsequently received sc 17beta-estradiol(E2) implants. Blood for LH RIA was obtained prior to and post OVEX, and post implant. Age-matched normal females were studied 3 months post OVEX. HPG/POA mice (including individuals previously showing positive feedback) did not show the significant increase in plasma LH after OVEX or decrease after E2 implant seen in normal mice. Negative feedback, previously reported absent in male HPG/POA mice, may require early steroid priming, or the establishment of certain afferents to GnRH cells.

177.3

INTACT GONADOTROPIN RESPONSE TO STEROID FEEDBACK AND ALTERED PROLACTIN CONTROL IN FEMALE RATS WITH ARCUATE NUCLEUS MALDEVELOPMENT AS A RESULT NEONATAL GLUTAMATE.

Ruth L. Callahan* and Richard W. Clough, Department of Anatomy, Southern Illinois University School of Medicine, Carbondale, IL 62901 (D. King,Sp.)

Positive and negative feedback of the gonadal steroids on gonadotropin secretion are mediated through the pituitary gland and the central nervous system (CNS) in rats and other species. Deafferentation and lesion studies have suggested that positive feedback is subserved through the "medial preoptic area" whereas negative feedback is mediated through the mediobasal hypothalamus (MBH), particularly the arcuate nucleus. This abstract reports that maldevelopment of the MBH, as a result of neonatal treatment with MSG, a neurotoxin to developing arcuate neurons, results in many endocrinopathies including obesity and reduced somatic development; however, MSG-treated female rats remain responsive to the positive feedback effect of 10 ug/kg estradiol on luteinizing hormone secretion in the prepubertal period and display normal compensatory ovarian hypertrophy, an indirect measure of the negative feedback of steroids on gonadotropin secretion. In contrast, the tuberoinfundibular dopamine system controlling secretion of prolactin is dramatically altered in MSG-treated female rats. In this regard there is an apparent loss of MBH neurons expressing tyrosine hydroxylase, a catecholamine synthesizing enzyme. Concomitantly, the ability of anesthesia and intraventricular beta-endorphin to stimulate prolactin release in female rats is abolished or severly attenuated in glutamate-treated rats. Although recovery or sparing of arcuate function in regards to negative feedback in MSG-treated rats needs to be considered in more detail, these studies suggest that a reanalysis of the role of the arcuate-MBH in negative feedback of steroids on gonadotropin secretion is warranted. Supported by HD-24426.

177.4

INCREASED SERUM GONADOTROPIN LEVELS FOLLOWING GONADECTOMY ARE NOT ASSOCIATED WITH CHANGES IN PITUITARY RESPONSES TO GnRH IN VITRO. P.C. Fallest*, E.S. Hiatt*, J.B. D'Agostino* and N.B. Schwartz* (SPON:J. Levine). Dept. of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

We sought to determine if increased responsiveness of the anterior pituitary to pulsatile GnRH contributes to the acute rise in serum gonadotropins following gonadectomy. Pituitaries obtained from intact male and female (metestrous) rats and rats gonadectomized 2 or 6 days earlier were cut into eighths and perifused with Medium 199 at a flow rate of 10 ml/hr. The GnRH pulse protocol consisted of 5 brief (~1 min) GnRH pulses (peak amplitude: 50 or 500 ng/ml) delivered 1/h. Five minute fractions were collected for LH and FSH RIA. The response to each GnRH pulse was calculated as the net LH or FSH secreted above baseline. Two-way ANOVA indicated that both baseline and total secretion of FSH increased in female pituitaries following gonadectomy. There was no effect of gonadectomy, however, on the net secretion of LH and FSH in either sex. These results suggest that at 2 and 6 days after gonadectomy, the increases in serum LH and FSH are not mediated by changes in the sensitivity of the rat anterior pituitary to GnRH. Supported by PHS Grants PO1-HD21921 and T32-HD07068.

177.5

BILATERAL TRANSECTION OF THE LATERAL OLFACTORY TRACT (LOTX) PREVENTS SHORT PHOTOPERIOD INDUCED TESTICULAR REGRESSION IN GOLDEN HAMSTERS. D.R. Pieper*, C. Lobocki and S.W. Newman*, Providence Hospital, Southfield, MI + U. Mich, Ann Arbor,MI

Removal of the olfactory bulbs (BX) increases tonic serum gonadotropin levels and prevents the testicular regression associated with short photoperiod. If LOTX mimicked the effect of BX on gonadotropin secretion, this, combined with previous data showing no effect of vomeronasal organ removal (VNOX), would suggest that the olfactory bulb influence on reproductive hormone secretion was due to main olfactory pathway neurons in LOT. Sixteen 23 day old male golden hamsters underwent either LOTX or sham (SH) surgery and were maintained on 14L:10D for another 4 wks. All hamsters were then transferred to a 10L:14D photoperiod for 11 wk. The most caudal damage of any of the lesions was at the level of the mid olfactory tubercle. All 8 of the hamsters in the SH group underwent testicular regression but only 1 of 8 in the LOTX group regressed. The serum LH and FSH levels at the end of the study were much higher in the LOTX group ($p < 0.01$ for both LH and FSH). These results combined with the earlier data on VNOX, suggest that MOB input to the pyriform cortex, amygdala or entorhinal cortex is involved in the influence of the olfactory bulbs on gonadotropin secretion and the effects of short photoperiod. NSF DCB8509789 (DRP) + NINCDS NS 20629 (SWN)

177.6

HORMONAL AND HYPOTHALAMIC NEUROCHEMICAL RESPONSES TO DORSAL RAPHE STIMULATION STUDIED USING MICRODIALYSIS PROBES IN RATS. J.H. Johnson. Dept. of Anatomy, Medical College of Va., Virginia Commonwealth University, Richmond, VA 23298.

We have shown that electrical stimulation of the dorsal raphe nucleus of the rat inhibits the release of luteinizing hormone (LH) while stimulating release of prolactin (Prl). The effects on LH appear to involve serotonergic and adrenergic links. Thus we undertook a study of medial hypothalamic and preoptic neurochemical correlates of these events. Ovariectomized rats were prepared with stereotaxically placed guide cannulae above the arcuate nucleus and medial preoptic area, and received jugular catheters as well. Experiments were performed in unanesthetized animals after insertion of a microdialysis probe (Carnegie Medicin, 2 mm membrane exposure) into each guide. Modified saline solution was perfused through the probes at a rate of 5 μl/minute. Fractions of dialysate (50 μl) and blood (0.3 ml) were collected every 10 minutes during a 60-min. control, a 50-min. stimulation, and a 50 min. recovery period. Dialysates were stored frozen prior to assay using HPLC with electrochemical detection. Plasma LH was measured by double antibody radioimmunoassay. Significant changes in LH and in dialysate contents were identified using ANOVA followed by a contrast procedure. LH decreased during stimulation, while reversible increases were observed in hypothalamic MHPG, DOPA, and DOPAC, and in preoptic EPI, MHPG, DOPAC and HVA. Hypothalamic 5HTP decreased during stimulation, and remained depressed during the recovery period. The importance of these changes to release of LH is under further study. (Supported by NIH grant HD12165).

177.7

MORPHOMETRIC ANALYSIS OF TH-IMMUNOREACTIVE NEUROSECRETORY NEURONS IN THE ARCUATE NUCLEUS OF THE RAT AFTER OVARECTOMY. J.-H. Rho, R.B. Simerly, and L.W. Swanson. Neural Systems Laboratory, The Salk Institute and Howard Hughes Medical Institute, La Jolla, CA 92037.

The effects of estrogen replacement, after ovarectomy of female adult rats, on tyrosine hydroxylase (TH)-immunoreactive neurosecretory neurons in the arcuate nucleus were characterized by an intracellular method to characterize changes in their detailed morphology. Two weeks after ovarectomy, and implantation of either a 25 mg. pellet of 17 β-estradiol (E2) or a control pellet (CP) subcutaneously, neurosecretory neurons were first labeled by an intravenous injection of fast blue and then selectively penetrated in fixed tissue slices and filled intracellularly. TH-containing neurons were further distinguished from other neurosecretory cells by immunostaining the tissue with antiserum to (TH). From two CP replaced rats, 77 neurosecretory neurons, of which 19 stained intensely with TH antiserum and 45 others stained less intensely, were visualized and reconstructed graphically to quantitatively analyze their morphological features. These neurons had extensive dendrites that are smooth proximally, with a sparse complement (6.5/100 microns) of spines, and varicose distally. These spines varied in length from 0.5 to 3 microns and often had a bulbous head. Their soma were small and ovoid, with a mean cross-sectional area of 75 sq. microns. From two E2 replaced rats, the morphology of 61 neurosecretory neurons were analysed, of which 36 neurons stained weakly for TH. These neurons appeared morphologically similar to CP neurons, with similar types of spines and a mean somal cross-sectional area of 73 sq. microns. However, the number of spines was somewhat less (4.2/100 microns) on these neurons. This preliminary evidence suggests that gonadal steroids, in addition to regulating TH synthesis, may also affect the morphology of neurons in the arcuate nucleus.

177.8

ADRENERGIC AND NORADRENERGIC REGULATION OF PULSATILE LH RELEASE. R.V.Gallo, A.Bona-Gallo*, and D.O'Sullivan*.Dept. Physiol. Neurobiol., Univ. Connecticut, Storrs, CT 06268.

This study examined the roles of both norepinephrine (NE) and epinephrine (EPIN) in regulating pulsatile LH release in 8-day OVX rats. Rats were injected ip with vehicle or drug at -27,-20,-5 and -3h prior to a 3h blood sampling period. Hypothalamic-preoptic area (HPOA) levels of NE and EPIN were determined by HPLC. Compared to controls, FLA-63 (25 mg/kg, DBH inhibitor) given at -3h produced 50 and 22% declines in HPOA NE and EPIN, resp., and reductions in pulse amplitude and frequency. LY134046 (LY; 50 mg/kg,PNMT inhibitor) given at -27,-20 and -5h, or -27, -20, -5 and -3h, produced no change in NE, 88 and 86% declines in EPIN, resp., and reductions in pulse frequency only. Either LY treatment protocol produced the same decline in EPIN and pulse frequency. Thus EPIN levels were maximally decreased by 3 LY injections. When rats were given LY at -27,-20 and -5h, and FLA-63 at -3h, compared to rats treated with LY alone there was no further decrease in HPOA EPIN (82% decline), a 46% decline in NE, and a further reduction in both pulse amplitude and frequency. This further suppression of LH release must be due to a reduction in HPOA NE levels since no further decrease in EPIN levels occurred. These data demonstrate within the same animal that NE and EPIN are both stimulatory to pulsatile LH release. NE stimulates the amplitude and frequency, and EPIN stimulates the frequency of pulsatile LH secretion. Supported by NIH HD 17728.

177.9

ENDOGENOUS OPIOID INHIBITION OF LUTEINIZING HORMONE (LH) SECRETION IN THE ABSENCE OF OVARIAN STEROID FEEDBACK IN THE DEVELOPING SHEEP. F. J. P. Ebling and D. L. Foster*. Consortium for Research in Developmental and Reproductive Biology, and Departments of Obstetrics and Gynecology, and Biology, The University of Michigan, Ann Arbor, MI 48109.

Puberty in the sheep results from an increase in the *frequency* of episodic LH secretion. The aims of the current study were to determine whether endogenous opioid regulation of LH pulse frequency changes during sexual maturation, and to determine whether opioid inhibition of LH secretion is dependent on the presence of ovarian steroids. The experimental approach was to determine the effects of an opiate antagonist (naloxone) on the pattern of LH secretion in ovariectomized female sheep at both pre- and postpubertal ages. Lambs (n=8) were ovariectomized at 3 weeks of age, and treated with a constant release 17β-estradiol implant to provide an invariable steroid negative feedback signal. At a prepubertal age (15 weeks) the implants were removed. Three weeks later, blood samples were collected at 6-min intervals (hours 0-4 of experiment) to establish the pattern of endogenous LH secretion. The lambs were then sampled during hourly treatment with naloxone (1 mg/kg BW i.v., hours 4-8 of experiment). As expected, pretreatment LH pulse frequency was high in the absence of steroid negative feedback (4.2 ± 0.3 pulses/4h, mean ± sem). However, naloxone treatment further increased LH pulse frequency (to 6.3 ± 0.5 pulses/4h). The estradiol implants were replaced so that the experimental protocol could be repeated at a postpubertal age in the same animals, but at an equivalent length of time after removal of steroids. Therefore, the estradiol implants were removed at 35 weeks of age, and blood sampling and naloxone treatment carried out again at 38 weeks of age. Pretreatment pulse frequency was higher at this postpubertal age (6.0 ± 0.6 pulses/4h) than during the prepubertal period; naloxone again induced an increase in LH pulse frequency (to 7.8 ± 0.6 pulses/4h). LH pulse amplitude was not affected by the naloxone treatments at either age. These responses to naloxone demonstrate that opioid mechanisms inhibit pulsatile LH secretion in the absence of ovarian steroids. Furthermore, the disinhibitory effects of naloxone on LH secretion at both pre- and postpubertal ages do not support the hypothesis developed in the rat (*Endocrinology* 113:596, 1983) that a decrease in opioid inhibition is responsible for the increase in gonadotropin secretion at puberty. Rather, they suggest that endogenous opioid mechanisms tonically inhibit LH secretion, perhaps contributing to the homeostatic regulation of the reproductive neuroendocrine axis. *(Supported by NIH grants HD-18258 and HD-18394).*

177.10

DIFFERENCES IN THE STIMULATORY AFFERENT REGULATION OF TUBEROINFUNDIBULAR DOPAMINERGIC (TIDA) NEURONAL ACTIVITY IN MALE AND FEMALE RATS. A.C. Barton, K.T. Demarest, K.J. Lookingland and K.E. Moore. Dept. of Pharm./Tox., Mich. State Univ., E. Lansing, MI 48824

In female rats TIDA neurons are tonically activated by afferent input from the rostral hypothalamus. The present study tested whether a similar afferent input exists in the male rat. TIDA neuronal activity was estimated by measuring rates of synthesis and turnover of DA in the median eminence (ME). Complete and retrochiasmatic (RC) deafferentations of the mediobasal hypothalamus (MBH) were made to isolate completely TIDA neurons from the rest of the brain or to interrupt neuronal connections from rostral brain regions. Both deafferentations decreased TIDA neuronal activity in female but not male rats. Rat prolactin (icv) increased DA synthesis in the ME of both sham- and RC-deafferentated female and male rats. These results suggest that afferents originating rostral to the MBH tonically stimulate TIDA neurons in female but not male rats, and that the action of prolactin is not blocked by RC deafferentation. TIDA neuronal activity may be influenced by the stimulatory effect of estrogen and inhibitory effect of testosterone. Ovariectomy decreased DA synthesis in the ME but RC deafferentation did not cause a further decrease. These results suggest that RC deafferentation and ovariectomy remove tonic stimulatory input to TIDA neurons which is mediated through a common afferent pathway. These afferents may not function in male rats since RC deafferentation did not reverse the castration-induced increase in DA synthesis in the ME. (NIH grant MH42802)

177.11

STREPTOZOTOCIN-INDUCED DEFICITS IN SEX BEHAVIOR AND NEUROENDOCRINE FUNCTION IN MALE RATS. R.W.Steger* (SPON: W. Yau). Physiology Dept, Southern IL Univ Sch of Medicine, Carbondale, IL 62901.

Diabetes is often associated with sexual dysfunction and abnormalities in gonadotropin and gonadal steroid secretion. Diabetes is known to affect central neurotransmitter metabolism but little is known about diabetes-induced changes in neurotransmitter turnover in hypothalamic areas known to control anterior pituitary hormone secretion and/or sex behavior. Adult male rats were tested for copulatory performance and then made diabetic by injecting them with streptozotocin (STZ; 50mg/kg) or citrate buffer. At 1 week after STZ, only 5 of 10 rats ejaculated in a 30 minute test of copulatory behavior compared to 10 out of 12 control rats. At 3 weeks, 3 of 10 STZ rats and 11 of 12 control rats reached ejaculation. Four weeks after the induction of diabetes, the animals were injected with saline or alpha methyltyrosine (250mg/kg) and killed 1 hour later. Plasma glucose levels in the STZ rats were elevated 4-fold over control levels . Plasma levels of testosterone (T), prolactin and luteinizing hormone were all depressed in the STZ rats. Norepinephrine (NE) turnover in the median eminence (ME), medial basal (MBH) and anterior hypothalamus (AH) and in remaining brain areas was significantly reduced by STZ treatment. Dopamine turnover in the ME was unaffected by STZ while it was increased in the MBH and decreased in the AH and remaining brain. In a second series of experiments, testosterone injections were shown to reverse the effects of STZ on sexual behavior. They also reversed STZ-induced changes in ME and MBH but not AH NE turnover. In conclusion, it appears that diabetes-induced changes in copulatory behavior are due to reductions in T levels which may be secondary to catecholamine-induced changes in pituitary LH and prolactin release. (Supported by the American Diabetes Association)

177.12

STEROID-INDUCED CHANGES IN HYPOTHALAMIC AND PITUITARY ENDOPEPTIDASE-24.15. A.R. Pierotti*, A. Lasdun*, J.L. Roberts, M. Orlowski* and C.J. Molineaux. (SPON: G Cohen.) Dept. of Pharmacology and Fishberg Ctr. for Neurobiology, Mount Sinai Sch. of Med., CUNY, New York, N.Y. 10029

Degradation of LHRH within the hypothalamus and/or the anterior pituitary (AP) by endopeptidase-24.15 may play a significant neuroendocrine role in modulating the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Previous studies in this laboratory have shown that LHRH is cleaved at the Tyr^5-Gly^6 bond by purified endopeptidase-24.15, and that the cleavage of LHRH by membrane preparations of hypothalamus and pituitary is inhibited by active-site-directed inhibitors of endopeptidase-24.15. These and other data strongly indicate that endopeptidase-24.15 is the enzyme directly responsible for LHRH degradation in the hypothalamus. The release of LHRH from neurons in the mediobasal hypothalamus (MBH) is regulated by negative feedback from gonadal target organs. In the present studies we have measured the activity of endopeptidase-24.15 in the MBH and AP, with the aim of determining whether the enzyme is regulated by gonadal steroids in these tissues.

Endopeptidase-24.15 activity was measured in soluble factions of hypothalamus and AP in pre- and post-pubertal male and female rats. The enzyme activity significantly dec4reased in the hypothlamus with age in both males and females and in the AP of male rats. Thus, a decrease in endopeptidase-24.15 may in part be responsible for the increase in LHRH release from the median eminence which occurs at puberty. The activity of endopeptidase-24.15 was measured in OVX rats treated with estrogen and progesterone. Steroid-treated rats showed a significant increase in the activity of endopeptidase-24.15 relative to OVX controls. These findings are consistent with the hypothesis that endopeptidase-24.15 is regulated by gonadal steroids and may, in part, mediate the negative feedback effects of steroids upon AP LH release.

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177.13

CNS SPECIFICITY OF Tor 23 IN THE RAT S. Wright,* D.T. Stephenson*, and P.D. Kushner (SPON: L.E. Muske) A.L.S. and Neuromuscular Research Foundation, Pacific Medical Center, San Francisco CA 94115

The monoclonal antibody, Tor 23, made to Torpedo synaptosomes, has been shown to bind to the surface of a subset of neurons in the rat CNS (J. Neurosci.,1988). In this study we have examined other tissues including the peripheral nervous system, endocrine structures and other organs to determine if the Tor 23 epitope is present elsewhere. PNS structures tested include the sciatic nerve and ventral and dorsal roots of the spinal cord. Endocrine structures tested were the adrenals, intestine, ovaries, pancreas, parathyroid, pineal gland, pituitary (anterior and posterior), placental tissue and the thyroid gland. Other organs examined were the liver, kidney, lung and spleen. All tissues were negative for Tor 23 except the anterior pituitary in which a discrete population of cells was positive. These cells were only a small fraction of total cells present and the binding appeared to be cytoplasmic (in contrast to the surface staining in the CNS). Costaining with antibodies to various pituitary hormones showed Tor 23 binding to be to the luteinizing hormone (LH) positive cells (as defined by an antibody to the beta-subunit of LH) of the anterior pituitary although Tor 23 does not appear to bind the LH molecule itself. Thus Tor 23 binding in the rat is specific to CNS tissues with the exception of the anterior pituitary. This finding merits further study to explore this heretofore undescribed and potentially exciting neural-endocrine connection.

177.14

INHIBIN β- AND SOMATOSTATIN-28-IMMUNOREACTIVE PROJECTIONS FROM THE NUCLEUS OF THE SOLITARY TRACT TO OXYTOCINERGIC CELL GROUPS. P.E. Sawchenko, S.W. Pfeiffer*, P.M. Plotsky, V.J. Roberts, E.T. Cunningham Jr., R. Benoit, M.R. Brown, and W. Vale. The Salk Institute, La Jolla, CA, 92037.

While several prominent neural inputs to vasopressinergic components of the magnocellular neurosecretory system have been identified, major pathways subserving afferent control of oxytocin (OT) secretion have remained elusive. Light-level immunohistochemical analyses suggested a preferential innervation of OT-immunoreactive (ir) neurons in magnocellular compartments of the paraventricular (PVH) and supraoptic (SO) nuclei by inhibin β- (Iβ-) and somatostatin-28- (SS-28-) ir fibers. Combined retrograde transport-immunohistochemical studies indicated that both projections arise principally from non-catecholaminergic cells centered in the caudal part of the nucleus of the solitary tract (NTS), but extending also into dorsomedial aspects of the medullary reticular formation. Preembedding immunoperoxidase staining at the EM level established that Iβ- and SS-28-ir are contained within presynaptic elements in the PVH and SO. Concurrent immunoperoxidase and autoradiographic (using ^{125}I-labeled secondary antisera; J. Histochem. Cytochem., 34:707, 1986) localization at the ultrastructural level identified terminals ir for each peptide in conventional synaptic contact with OT-ir elements (primarily dendrites) in the PVH and SO.

Both SS-28 and Iβ have been found to stimulate oxytocin secretion after central administration. Because the region of the NTS from which these peptidergic pathways arise appears to receive somatosensory (J. Comp. Neurol., 255:438, 1987), as well as visceral, afferents, these projections constitute candidate substrates for parturition- and suckling-related controls of OT secretion.

177.15

DISTRIBUTION OF INHIBIN SUBUNITS IN THE RAT BRAIN AND PITUITARY. V.J. Roberts, H. Meunier*, J. Vaughan*, C. Rivier, W. Vale, and P.E. Sawchenko. The Salk Institute, La Jolla, CA 92037.

Two related glycoproteins, inhibin and activin, are produced and secreted by the gonads and act at the pituitary to regulate FSH secretion. Recent studies, however, have revealed that the mRNAs encoding the subunits of inhibin (α/β_A and α/β_B) and activin (β/β) are also expressed in other tissues including the brain and pituitary. The aim of this study was to examine the distribution of these polypeptide subunits in the brain and pituitary and explore their regulation by ovarian factors.

Inhibin subunits were localized using antisera that read preferentially the α-, β_A-, or β_B-subunit. In brain, both β-subunits were localized in a group of cells centered in the nucleus of the solitary tract (NTS). Fibers and terminals consistent with known NTS efferents were also stained. Hypothalamic oxytocin-containing neurons appear to comprise prominent targets of immunoreactive (ir) fibers. In the pituitary, the α- and β_B-subunits were colocalized in the cytoplasm of most all FSH- and LH-ir cells. Ovariectomy dramatically increased the size and number of gonadotropes immuno-positive for these subunits. The levels of the α and β_B mRNAs were also greater following ovariectomy. These results suggest that (1) inhibin-related peptides, in the brain, may mediate reproductive-related functions at the neuronal level, and (2) these peptides, produced by gonadotropes, may play an endocrine role in the modulation of gonadal secretions and/or have a paracrine function in the anterior pituitary.

STRESS, HORMONES AND AUTONOMIC NERVOUS SYSTEM I

178.1

EFFECT OF STRESS AND ADRENALECTOMY ON LONG-TERM POTENTIATION IN RAT HIPPOCAMPUS. T.J. Shors, S.Levine, and R.F. Thompson. Dept. Psychol., Univ. Southern Calif., LA,CA 90089, Dept. Psychiatry, Stanford Univ., Stanford,CA 94305.

Stress impairs long-term potentiation (LTP) in the rat hippocampal slice (Foy et al., Behav. Neural Bio.,48:138,1987). To investigate the possible role of the stress hormone, corticosterone, LTP was measured in adrenalectomized (ADX) rats immediately following restraint/shock. Field potentials were recorded in vitro from CA1 after tetanic stimulation of the Schaffer collaterals. Percentage of pre-tetanus amplitude at 30 minutes was 191% for controls, 142% for ADX, 111% for stress, and 124% for ADX/stress. Both stress and ADX alone resulted in a significant decrease in LTP. The difference between ADX and ADX/stress was significant ($p<0.05$), but the difference between stress and ADX/stress was not. Therefore, even though there was decreased potentiation with ADX, stress resulted in a further decrease. This further reduction suggests that neurogenic factors may be either responsible for or interacting with the adrenal component to impair LTP in response to stress.

(Supported by NICH&HD grant HD02881).

178.2

HIPPOCAMPAL DAMAGES ASSOCIATED WITH PROLONGED AND FATAL SOCIAL STRESS IN VERVET MONKEYS. H. Uno, R. Tarara*, J.G. Else*, M.A. Suleman and R.M. Sapolsky. Reg. Primate Res. Ctr., Univ. Wisconsin, Madison, WI 53715; Institute of Primate Res., Nairobi, Kenya; and Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305

Although the incidence of spontaneous gastric ulcer in nonhuman primates is very low, macaques are highly susceptible to gastric ulcer induced by psychosomatic stress. Eight adult vervet monkeys *(Cercopithecus aethiops)* succumbed to cachexia associated with multiple gastric ulcers in the captive social groups at the Institute of Primate Research, Nairobi, Kenya. These animals appeared to have suffered atypically high rates of social harassment and attack from peers, suggestive of social subordinance. All animals were cachectic with persistent diarrhea, anorexia, dehydration and died as early as 1 month and 6 months to 4 years. We observed microscopic changes of the brain from all 8 ulcer-laden animals as well as 6 healthy, euthanized animals in the same colony. The neural degeneration representing shrinkage, disarrangement, and loss of the pyramidal neurons was prominent in the CA3 and CA1 regions. Ultrastructurally, edematous swelling of the dendritic branches and disappearance of the synaptic terminals of the mossy fibers were noticed. Numerous swollen oligodendrocytes were seen in the overall hippocampus, cerebral cortex, midbrain, and striatal ganglia. Numbers of the pyramidal neurons in CA1 and CA3 were reduced substantially in stressed animals. Degenerative changes and depletion of the neurons were more prominent in males than females. These hippocampal changes were similar to those found in our earlier experiments with dexamethasone in prenatal rhesus monkeys. The adrenal cortex of ulcer-laden animals showed a moderate degree of hyperplasia. The prolonged and fatal social stress appeared to induce hippocampal damage as well as gastric ulcers and severe generalized maladies in adult monkeys. (Supported by NIH grant RR00167.)

178.3

SEX DIFFERENCE IN BINDING AFFINITIES OF TYPE I AND TYPE II GLUCOCORTICOID RECEPTORS IN RAT HIPPOCAMPUS. B.B. Turner and M.S. Ansari*. Dept. of Physiology, Coll. of Med., East Tenn. State Univ., Johnson City, TN 37614.

We previously reported a sex difference in the affinity of 3H-dexamethasone (3H-Dex) and 3H-corticosterone (3H-Cort) binding in hippocampus, with males having the greater affinity (Brain Res. 343:16, 1985). We have also found that in vivo injection of a tracer dose of 3H-Cort results in greater nuclear uptake in males (Soc. Neurosci. Abstr. 13:1027, 1987), suggesting either greater receptor density or greater affinity of Type I receptors in males. We report here that the affinity of Type I receptors is much greater in males than in females. No difference in receptor number was found between sexes for either Type I or Type II receptors.

Rats were killed 12 h post-adrenalectomy and bound steroid was separated on LH-20 columns. Using 3H-Dex and unlabelled RU-28362, the Kd obtained for the Type I receptor was 0.53 $\pm$ 0.05 nM in males vs. 1.30 $\pm$ 0.25 nM in females, $p<0.025$. Using 3H-RU 28372, the Kd for Type II receptors was 1.41 $\pm$ 0.03 nM in males vs. 1.30 $\pm$ 0.04 nM in females, $p<0.025$. The Type I receptor affinity difference was confirmed using 3H-Cort and hydroxylapatite. Under basal conditions, the occupancy of Type I receptors and consequent spill-over to Type II receptors may be greater in males, thus limiting the range of the male stress response. Supported by NIH grant NS-22158.

178.4

CORTICOSTERONE HYPERPOLARIZES PYRAMIDAL NEURONS OF THE HIPPOCAMPUS IN VITRO. J.B. Aldenhoff, and S. Hennig*, Dept. Psychiatry, Univ. of Mainz, Untere Zahlbacher Str. 8, 6500 Mainz, FRG

Different types of receptors for glucocorticoids occur in the brain with particular density in the hippocampus (1) suggesting neuromediator function. However, except earlier findings (2) mainly genomic actions have been considered.

Hippocampus slices from rats (100-200g) were superfused with oxygenated Ringer solution at 35°C in a perfusion chamber (3). Intracellular recordings and current injections were made with a bridge amplifier and glass microelectrodes filled with 2 M KCl (resistance 30 to 40 MOhm). Pyramidal neurons with stable resting potentials (> -45 mV) were used. Corticosterone 10^{-6} to 10^{-9} Mol (solved in ethanol 0.1-0.0001 %.) was applied to the superfusate for 15 to 20 min. Within 3 to 5 minutes after application, neuronal activity ceased and a hyperpolarization of about 10 mV occurred. Membrane resistance decreased as well as the post-stimulus hyperpolarization. Both, effects of corticosterone on Cl-currents as well as on K-currents could underly this action. Hypothetically, corticosterone might serve as negative feedback mechanism for the excitatory action of Corticotropin-releasing factor such as described before (3).

1)De Kloet & Reul(1987)Psychoneuroendocrinology,12,83-105.
2)Pfaff et al. (1971) Science 172, 394-395.
3)Aldenhoff et al. (1983) Science 221, 875-877
Supported by the DFG (Al 239/1-1).

178.5

GLUCOCORTICOID-STIMULATED 35 S-LABELING OF A PROTEIN FROM THE RAT HIPPOCAMPUS. L.K. Schlatter* and L.A. Dokas. Departments of Biochemistry and Neurology, Medical College of Ohio, Toledo, OH 43699.

Following treatment of rats with 5 mg of corticosterone (s.c.), hippocampal slices in vitro show increased labeling from [^{35}S]-methionine of a protein with an apparent molecular weight (M_r) of 35,000 and IEP of 6.6. These characteristics are similar to those of glycerol-3-phosphate dehydrogenase. Since responses to an injection of this magnitude may be pharmacological, several experiments have been done to determine whether stimuli that increase endogenous levels of corticosterone have the same effect on labeling of the 35,000 M_r protein. One hour after various stresses (immobilization, cold, ether or sham-injection), when plasma levels of corticosterone are elevated, labeling of the 35,000 M_r protein is increased. Injection of ACTH also stimulates the synthesis of this protein. A dose-response curve for corticosterone with adrenalectomized rats shows that the protein's synthesis is maximally increased when the injected dosage causes serum corticosterone to increase to levels seen during stress. Injections of the corticosterone receptor agonist RU 28362 and the mineralocorticoid aldosterone into adrenalectomized animals differentially stimulates the synthesis of the 35,000 M_r protein. The increase appears to be due to short-term glucocorticoid increases such as those observed during stress. Supported by the Van Ness-Thompson Foundation and AFAR.

178.6

THE EFFECT OF SHOCK PREDICTABILITY ON HORMONAL STRESS INDICANTS. D. Pitman*, B.Natelson, T. Pritzel,* J. Ottenweller* and R.McCarty. (SPON: S.D. Cook). Primate Neurobehavioral Unit, VAMC and Dept of Neurosciences, New Jersey Medical School, East Orange, NJ 07019.

Male Sprage-Dawley rats were implanted with femoral artery catheters and subcutaneous flank electrodes for shock delivery. The rats were then placed into an apparatus we described earlier (Neurosci Abs 13: 1293, 1987) which allows for undisturbed blood sampling and complete experimental control. Groups of rats were given either 100%, 66%, 33%, or 0% predictable shock by varying the number of times a light (CS) preceded each of 42 daily shocks (US) in a 21.5 h session. Each morning for six consecutive days, animals were sampled and then given a CS-US pair. Area under the corticosterone (cort) response curve (AUC) following the CS-US pair partially habituated by day 3 in all of the shocked groups (p's<0.05). In the 100% group, the AUC increased on day 6 of the experiment (p<0.05). This suggests sensitization of the cort response to the probe in this group. On the 6th day, basal cort levels varied directly with predictability levels, p<0.01;R=.47. The pattern of plasma catecholamine (cat) responses was the opposite of the cort responses; on day 6, the 100% group manifested a decrement in response to the CS-US probe (p<0.05), while the 0% group continued to respond to the probe (p<0.05). Using basal cort as the stress indicant, these data suggest chronic stress may track shock predictability. Additionally, the way the adrenal responded to highly predictable shock varied by the end of the experiment; the cort response was actually greater than levels after animals had habituated while the cat response was a decrement following shock. Supported by VA research funds.

178.7

HABITUATION OF THE ADRENOCORTICAL STRESS RESPONSE. B. Natelson, D. Pitman*, E. Grover*, and J. Ottenweller*. Primate Neurobehavioral Unit, VAMC and Department of Neurosciences, New Jersey Medical School, East Orange, NJ 07019

Frequency and intensity of stressor presentation are known to influence the rate at which the adrenocortical stress response (ACSR) habituates over time. This study was designed to further evaluate stressor intensity as a variable. Chronically catheterized rats bearing subcutaneous shock electrodes were placed in an apparatus developed to permit remote blood sampling without the need to touch the rat (Neurosci Abs 13: 1293, 1987). Rats were assigned to low and high shock groups or a sampling- only group which was treated as were the shocked groups but was never shocked. On days 1,2,4,6, and 8 of the study, animals were sampled, given a single shock probe, and sampled again. Thereafter, animals in the low and high shocked groups were given a daily 3 h shock session consisting of 18 shocks. The ACSR of rats receiving high shock probes was greater than that of low shock and no-shock groups (p's<0.05) on the first day of the experiment. Animals in this group then showed evidence of sensitization on the 8th probe day (p<0.05). Low shocked animals showed partial habituation to the shock probe by the fourth day and then a return of ACSR to levels seen following the first probe. Samples taken 1 h after sessions finished revealed comparable AC levels in both shocked groups on the fourth day. However, AC levels on days 6 and 8 were greater in low shocked than in high shocked rats (p's<0.01). Rats receiving high shock had heavier adrenals, and a greater ACSR to ACTH than the other groups. These data indicate that parametric analysis of habituation of ACSR is possible. Supported by VA research funds.

178.8

BEHAVIORAL CHANGES ELICITED BY CHRONIC STRESS IN RATS. J.E. Ottenweller*, B.H. Natelson, S.D. Drastal*, D.L. Pitman* (SPON: J. Jacoby). Primate Neurobehavioral Unit (127A), VA Medical Center, East Orange, NJ 07019.

We recently reported that basal plasma corticosterone levels were elevated in repeatedly stressed rats and in rats that were simply moved into the same room as stressed rats (P&B 43[1], 1988). We have now replicated these results with 10 daily 2 hour sessions of intermittent tail shock. Before and during the 10 days of stress, activity patterns were monitored with tilt cages and afterwards with Wahmann running wheels. During stress, shocked rats and those in the room with them decreased their activity in the 4 hours before the stress sessions were to begin, and not at other times of day. Thus, these rats appeared to develop a stress-induced, anticipatory fear state that suppressed activity in the 4 hours before stress sessions. This anticipatory suppression of activity was not evident on the last 3 days of stress, but reappeared in shocked rats on the first 2 days in running wheels. 3 hours after the last stress session, shocked rats showed a prolonged latency to drop from a wire suspended 18 inches off the ground. This effect was present 4 days (but not 7 days) later. The response to novelty was measured after the hanging wire test by placing these rats in running wheels, and shocked rats ran less in the first hour than either other group. We believe these behavioral disruptions and elevated basal glucocorticoids characterize a state of chronic stress. Supported by VA Medical Research Funds.

178.9

EFFECT OF BILATERAL ADRENALECTOMY ON LEARNED HELPLESSNESS INDUCTION AND 5-HT RELEASE IN RATS. W. Kornrich, F.A. Henn and E. Edwards. Dept. of Psychiatry, SUNY. Stony Brook, NY 11794

Rats exposed to intermittent inescapable shock develop a transient behavioral deficit when later tested in a shock escape paradigm. We have recently demonstrated that deficient rats or "learned helpless" rats can be distinguished by an abnormal hypothalamic-pituitary-adrenal axis. The present experiments examine the effects of bilateral adrenalectomy on the induction of learned helplessness in rats. Hippocampal 5-HT release was examined as the biochemical marker showing some correlation with the effect of adrenalectomy on LH behavior. We have found that rats having bilateral adrenalectomies exhibit a significantly higher number of failures than sham operated controls and non-operated controls (11.07 ± .63 ADX vs 5.67 ± .75 sham and 6.8 ± .44 non-operated controls). The release of ^{3}H-serotonin from hippocampal slices one day after shock escape testing was significantly lower in ADX rats with pronounced LH behavior compared to non-escape deficient sham controls. Oral corticosterone replacement did not reverse the behavioral deficit, but hippocampal 5-HT release in ADX + CORT replacement rats was comparable to sham controls and non-operated controls. These results implicate the serotonergic system as a possible mediator of the accentuated behavioral deficit observed in ADX rats (supported by grants BNS-8614098 and MH4404801 to E. Edwards).

178.10

STRESS-RELATED EFFECTS OF LONG-TERM CALORIC RESTRICTION ON PLASMA CORTICOSTERONE AND VASOPRESSIN IN THE FEMALE RAT: A NEW METHODOLOGICAL APPARATUS. J.K. Nishita, E.H. Ellinwood, Jr., W.J.K. Rockwell*, J.C. Ritchie*, and C.M. Kuhn. Depts. of Psychiatry and Pharmacology, Duke Univ. Med. Cntr., Durham, NC 27710.

A new method for studying stress of prolonged caloric restriction in the rat was used. In this paradigm weaning rats were required to crawl back-and-forth across Plexiglass tubes in order to obtain food and water that was placed on opposite sides of a barrier. If a rat could not squeeze through the tube passageway, it was denied access to either food or water. We have previously shown that the onset and severity of caloric restriction can be controlled by changing the diameter of the tube passageway (Small tube (ST)=3.3 cm, Large tube (LT)=4.2 cm; Nishita et al., 1987) and these rats learn to reduce their daily food and water intakes to compensate for the ST and LT conditions.

Because our paradigm could also impose significant stress, we measured plasma corticosterone (CORT) and vasopressin (VP) levels in rats housed for 90 days in ST and LT conditions and in littermate controls housed without tube conditions. Only ST rats (n=8) showed elevated CORT (27.5 ug/dl) and VP (17.9 pg/ml) compared to LT littermates (12.3 ug/dl and 5.1 pg/ml, respectively) and controls (9.8 ug/dl and 5.6 pg/ml, respectively).

178.11

ANORECTIC EFFECT OF CRF DECREASES WITH REPEATED ADMINISTRATION.

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CRF(icv) causes acute anorexia, and CRF levels are elevated in the CSF of patients with depression and anorexia nervosa. Therefore, it has been hypothesized that repeated, stress-induced elevations of CRF cause the symptom of chronic anorexia seen in these disorders. However, the effects of repeated CRF(icv) administration on feeding and body weight have not been studied. In this study, rats were treated daily with CRF (1.1 nmole icv) or a daily 2-hour restraint stress period. Controls received daily icv injections of saline. Nocturnal food intake (2,4,12, and 22 hours) and body weight were measured over 5 consecutive days. Both CRF (icv) and restraint caused significant reductions in short-term food intake. There was a significant treatment X day interaction which was due to a decreased anorectic effect of CRF and restraint. CRF treatment decreased 4-hour intake to 10% of control on day 1 but only decreased intake to 50% of control on day 5. Restraint-treated animals showed smaller but similar changes over time. A second experiment with a lower dose of CRF (0.22 nmole) showed similar results. In both experiments, saline-, CRF- and restraint-treated animals showed no differences in daily weight-gain increments. Thus, repeated exposures to CRF or restraint result in an adaptation to their anorectic effects. Further research will examine the effects of CRF administered continuously or with different frequencies.

178.12

MORPHINE INCREASES ACTH AND B-ENDORPHIN PLASMA LEVELS DURING HYPOTHERMIA.

C. Alcaraz*, M. Lovitz*, H. Turndorf* and M.M. Puig, Dept. Anesthesiology, N.Y.U. Medical Center, New York, NY 10016.

The effect of intravenous morphine (MS) on plasma levels of ACTH, B-endorphin (B-END), histamine (HIS) and catecholamines (CA) was determined in two groups of dogs at 37 and 30°C. Animals were anesthetized with isoflurane and MS (1 mg/kg IV) administered two hours after the desired temperature was obtained. ACTH, B-END, HIS and MS were determined by RIA and CA levels by REA. Cooling from 37 to 30°C significantly increased CA plasma levels. MS produced a significant increase in ir ACTH, B-END and HIS, and a reduction in CA levels at 30 but not at 37°C. At all sampling times, MS plasma levels were significantly higher at 30 than at 37°C. Our results show that mild hypothermia enhances the effects of MS on hormonal release.

	37°C		30°C	
	Pre	Post	Pre	Post
MS (ng/ml)	0	353±64	0	648±65a
HIS (ng/ml)	12.5±2	17.8±3	12±1	21.8±3b
B-END (pmol/l)	32.3±4	34±5	27±2	44.6±2b
ACTH (pg/ml)	106±5	143±24	140±18	296±52b
CA (pg/ml)	733±67	647±98	1310±280a	320±47b

Post: Values obtained five minutes after MS. a, $p<0.05$ when compared to 37°C group and b, $p<0.05$ when compared to pre-values in the same group. (Student's t-test)

178.13

AN EXTRACTION PROCEDURE FOR PLASMA ACTH.

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The need for a specific, sensitive, reproducible assay for ACTH in human plasma capable of detecting relatively small changes in levels following emotional stimuli in human subjects led to the development of an extraction procedure for use in conjunction with a readily available ACTH radioimmunoassay (RIA) kit (INCSTAR Corp.).

The extraction was performed by adding 500 ul of plasma and 500 ul of water to 150 mg of (methanol pre-rinsed) PrepPAK-500/C18 (Waters) in 12X75 mm tubes. After standing for 30 min with mixing and centrifugation at 6000 rpm, the supernatants were removed by aspiration. The adsorbent in each tube was rinsed three times with 1 ml of distilled water and ACTH was eluted with two 1 ml rinses of acetone:water (50:50). Following evaporation to dryness at 45°C under nitrogen, the concentration of ACTH in each tube was determined by RIA.

Recovery of ^{125}I-ACTH (Peninsula Labs.) added to plasmas (endogenous range 5-76 pg/ml) was 64.7 % with no indication of deviation from the mean at high or low plasma levels. Sensitivity of the assay was 2pg/ml. Plasma samples from volunteer subjects were assayed directly and as well as after the extraction procedure for comparison purposes. In some subjects there was a good correlation between the extracted vs non-extracted values while in others there were significant differences indicating that routine extraction is necessary to insure the accurate measurement of ACTH in human plasma using this antiserum.

178.14

COCAINE-INDUCED CARDIOVASCULAR (CV) AND PITUITARY-ADRENAL STIMULATION: ROLE OF DOPAMINE (DA) AND CORTICOTROPIN RELEASING HORMONE (CRH).

J.A. Kiritsy-Roy, S.M. Standish*, R.D. Whitmore*, M. Smith*, J.B. Halter* and L.C. Terry. Dept. of Neurology, Inst. of Gerontology, Univ. of Michigan and Veterans Admin. Med. Center, Ann Arbor, MI 48105.

Cocaine is a potent CV stimulant but its mechanism of action is unclear. It appears that the reinforcing properties of cocaine relate to inhibition of DA uptake, and cocaine self-administration is blocked by D-2, but not D-1 blockers. Recently, Rivier and Vale (Brain Res. 422:403, 1987) reported that cocaine induces ACTH secretion by a CRH-dependent mechanism. These studies examine the role of CRH in the CV effects of cocaine, and also determine if the selective D-1 antagonist, SCH 23390 (SCH), or the D-2 antagonist, eticlopride (ETC) modify CV or pituitary-adrenocortical responses to cocaine.

Experiments were conducted using freely behaving rats with a cannula in the carotid artery for blood sampling and CV recording. Arterial pressure (AP) and heart rate (HR) were monitored continuously and blood samples (0.5 ml) were drawn before and at intervals following treatment with cocaine (25 mg/kg ip) in combination with saline, SCH (0.1 mg/kg ip), ETC (0.075 mg/kg ip), CRH antiserum (1.0 ml ia), or pre-immune serum. Concentrations of ACTH and corticosterone (CS) were determined in plasma by radioimmunoassay.

Cocaine increased systolic and diastolic AP by 31±4 and 24±4 mmHg and decreased HR by up to 60±10 bpm. These effects were not altered by pretreatment with CRH antiserum, SCH or ETC. Cocaine also increased plasma ACTH from 47±9 to 585±60 pg/ml and CS from 13±4 to 38±5 ug%. Although CRH antiserum completely antagonized ACTH responses to cocaine, CS responses were not blocked. Both SCH and ETC blocked the ACTH response to cocaine. Conclusions: 1) CRH, D-1 and D-2 receptors mediate ACTH releasing but not CV effects of cocaine and 2) cocaine may stimulate CS secretion directly.

178.15

CLASSICAL CONDITIONING OF A CRF MODULATED ELEVATION OF ARTERIAL BLOOD PRESSURE.

M.Kreutz#, D.Hellhammer, H.Lehnert and R.Murison#, Universities of Bochum, Trier, Mainz (F.R.G), and Bergen (Norway)

It has recently been shown that an i.c.v. application of CRF results in an elevation of arterial blood pressure. To investigate the role of CRF as a possible mediator of a psychologically induced elevation of blood pressure, we tried a classical conditioning of these autonomic effects of CRF. Six adult male Wistar received implants of guided cannulas into the lateral ventricles, and chronic catheders into the abdominal aortis. A Stathan pressure transducer combined with a Siemens Sirecust recorder were used for monitoring of arterial blood pressure. After recovery from surgery, the animals were exposed to 8 daily sessions, where they received 4 conditioning and 4 control trials in an alternating way. As the CS, the rats received an i.a. injection of .2 ml of a 0.154 molar saccharine solution, and, at the same time, 2 ul i.c.v. hCRF (150 pmol) as the UCS. During the control sessions the animals received same volumes of a Ringer solution (icv) and isotonic saline solution (i.a.), respectively. A significant increase of arterial blood pressure from 93.1 to 103.8 mm Hg was observed 30 min after CRF, while no changes occured after placebo application. Two test sessions were performed one and three days later, where the rats received the CS, but the UCS was replaced by Ringer solution (i.c.v.). Under these conditions, CS presentation alone resulted in a significant increase of blood pressure from 92.0 to 97.3 mm Hg, suggesting a classical conditioning of CRF effects on blood pressure. (Supported by a twinning grant of the European Science Foundation to D.H. and R.M.)

178.16

CORTICOTROPIN-RELEASING FACTOR (CRF) MEDIATES LOCUS CERULEUS (LC) ACTIVATION BY HEMODYNAMIC STRESS.

Rita J. Valentino and Richard G. Wehby*. Department of Pharmacology, George Washington University Medical Center, Washington, D.C. 20037.

CRF has been hypothesized to act as a CNS neurotransmitter, being released by stressful stimuli in brain circuits to initiate a multicomponent response to stress. The LC, may be a target for CRF neurotransmission because CRF-containing fibers have been localized in LC, and exogenous CRF alters LC discharge. To test this hypothesis, the effects of a hemodynamic stressor, i.v. nitroprusside infusion (NIT), on LC discharge of halothane-anesthetized rats were compared to those of i.c.v. CRF. Like CRF, NIT increased LC spontaneous discharge rate and attenuated LC discharge evoked by sciatic nerve stimulation. To determine whether endogenous CRF mediates these effects of NIT, the ability of dexamethasone or α helical CRF_{9-41}, a CRF antagonist, to attenuate NIT effects was determined. Dexamethasone, which decreases hypophyseal CRF release by NIT, did not alter NIT effects on LC discharge. In contrast, α helical CRF_{9-41} (50 µg, i.c.v.) completely blocked increases in LC spontaneous discharge produced by NIT. The results indicate that certain hemodynamic stressors activate the LC and this is mediated by extrahypophyseal CRF. These findings support a neurotransmitter role for CRF in the LC. Supported by NIH Grants DA 03695 and MH 40008.

178.17

EFFECT OF CHRONIC STRESS ON THE LEVEL OF CORTICOTROPIN-RELEASING FACTOR (CRF) mRNA IN RAT BRAIN T.Imaki*, J.L.Nahon*, C.Rivier, P.Sawchenko and W.Vale. Clayton Foundation Laboratories for Peptide Biology, Developmental Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

CRF plays a physiologically important role in the response to stress. However, the molecular mechanisms which control CRF biosynthesis during stress are not known . We examined the effect of chronic stress on the level of CRF mRNA in the rat brain by Northern blot analysis and *in situ* hybridization. Male Sprague-Dawley rats were exposed to intermittent footshock stress (1.5 mA. 1 sec duration, 60 times during 30 min, twice daily for 1, 3 or 7 days). Twenty-four hours after the last shock session, the rats were decapitated and RNA was extracted from 8 different brain regions by Glowinski's method. By Northern blot analysis, CRF mRNA (1.4kb) was detected in brainstem>olfactory bulb>hypothalamus>cortex>midbrain. CRF mRNA levels increased about 1.6 times over control values after 3 days of stress, while no significant change was seen in extrahypothalamic area after stress. Using *in situ* hybridization, CRF mRNA-positive grains were detected predominantly in the parvocellular division of the paraventricular nucleus (PVN). CRF mRNA levels in the PVN selectively increased after stress. Maximum induction was seen after 3 days of stress; CRF mRNA levels had returned to control level after 7 days of stress. These results suggest that chronic stress induced in increase in CRF mRNA levels in PVN.

178.18

SEROTONERGIC REGULATION OF CORTICOTROPIN-RELEASING FACTOR (CRF) NEURONAL ACTIVITY IN VIVO. P.E. Bond*, M.J. Owens, G. Bissette and C.B. Nemeroff (SPON: M. Bowman). Duke Univ. Med. Ctr., Durham, NC 27710.

Corticotropin-releasing factor (CRF) is the principal physiological regulator of the hypothalamic-pituitary-adrenocortical (HPA) axis. Previous studies have demonstrated a potassium-induced, calcium-dependent CRF release from hypothalamus, amygdala, midbrain, and striatum. In the present studies, we examined the effects of acute and chronic treatment with several drugs which act on serotonergic systems in order to elucidate which serotonergic receptor subtypes alter CRF neuronal activity.

Rats were treated with a single (acute) or daily (chronic) injections of the following serotonergic drugs: ketanserin (a $5HT_2$-specific antagonist), quipazine (a 5HT agonist), 4-bromo-2,5-dimethoxyamphetamine (DOB, a $5HT_2$-specific agonist), or vehicle. The rats were decapitated and blood was collected for measurement of plasma ACTH and corticosterone concentrations by radioimmunoassay. Anterior pituitary glands were removed and CRF receptor affinity (K_D) and density (Bmax) were measured. Brains were removed and 18 brain areas were microdissected. Brain samples were assayed for CRF concentration, CRF mRNA levels and autoradiographic analysis of CRF receptor density.

Acute and chronic treatment with quipazine and DOB produced an increase in plasma concentrations of corticosterone and ACTH. In contrast, ketanserin did not alter plasma ACTH or corticosterone concentrations. In agreement with the above findings, acute quipazine decreased the concentration of CRF in the median eminence, although not significantly. Chronically, quipazine produced pituitary CRF receptor down-regulation.

These preliminary data suggest that hypothalamic serotonergic neurons activate the HPA axis perhaps via both $5HT_1$ and $5HT_2$ receptor mechanisms. (Supported by NIMH MH-42088)

178.19

RELEASE OF CORTICOTROPIN-RELEASING FACTOR (CRF) FROM THE BOVINE MEDIAN EMINENCE (ME) IN VITRO. T.H. WELSH*, M.R. SUTTON*, D.D. ZALESKY*, P.G. HARMS*AND N.H. MCARTHUR. Texas A&M Univ., College Station, TX 77843

The effect of norepinephrine (NE) on CRF release from the ME was studied. Individual ME halves from heifers (n=10) were perifused for 400 min with Krebs-Ringer bicarbonate medium (500-µl chambers; 5.5 mM glucose, 95% O_2:5% CO_2; 37°C; 1 ml/10 min fractions for CRF RIA). ME halves were challenged with NE (0.5 or 5 mM) or 5 mM clonidine for 30-min periods at 130 and 220 min of perifusion. The initial 0.5 mM NE challenge did not affect CRF release whereas the second 0.5 mM NE challenge increased CRF release 2-fold. The initial 5 mM NE challenge increased CRF release (25±6 pg/ml pre-NE to 140±6 pg/ml 30 min post-NE). Similarly, the second 5 mM NE challenge increased CRF release (from 9±2 up to 121±13 pg/ml). Clonidine (an α_2 agonist) treatment did not alter CRF release. Potassium (60 mM) challenge increased (∿10-fold) CRF release from NE and clonidine treated ME. In summary, NE stimulated CRF release from the bovine ME in vitro. This model system may facilitate the study of neurotransmitter regulation of hypothalamic release of CRF.

178.20

STRESS-INDUCED GH FALL IS INDEPENDENT OF CRF IN NEONATAL RATS. C.M. Kuhn, L. Nathan* and S.M. Schanberg*. Dep't of Pharmacology, Duke University Medical Center, Durham, NC 27710.

Corticotropin releasing factor (CRF) is postulated to organize stress responses, including the decline of growth hormone (GH). However, in 3-4 day old rats, separation from the mother (MS) causes a fall of GH but no rise in corticosterone (CS). To determine if this fall in GH is mediated by CRF, two strategies were used. Serum GH was measured in 4-10 day old rats after CRF injection intraventricularly (ivt). Also, the ability of the CRF antagonist alpha helical CRF 9-41 (CRF-A) to block the separation-induced GH fall was studied. CRF (.001-1 ug) did not suppress, and actually stimulated GH 88% at 0.1 ug ($p<.001$) in 4 d.o. pups, although it produced a significant 4-5 fold rise in CS. Adrenergic agonists increased CS but did not suppress GH. Finally, CRF-A (50 ug ivt) did not block the fall in GH during MS. These results suggest that during ontogeny, stress-induced inhibition of GH secretion precedes stimulation of CRF release, and raise the possibility that stress-induced GH inhibition can occur independently from CRF. Supported by MH13688 and NIDA 02739

STRESS, HORMONES AND AUTONOMIC NERVOUS SYSTEM II

179.1

ALTERED GLYCEMIC AND NEUROCHEMICAL RESPONSES TO HYPOXIC STRESS IN STREPTOZOTOCIN-DIABETIC RATS. L.L. Bellush & W.N. Henley*, Depts. of Psychology and Zoological & Biomedical Sciences, Athens, Ohio 45701

Diabetic (D) and nondiabetic (N) rats were exposed to hypobaric hypoxia (simulated altitude=12,000 ft) for 24 hrs and compared with normoxic control(n=7). All D rats had lower DOPAC/DA ratios in hypothalamus (HYP) and striatum (STR), and lower 5HIAA and 5HIAA/5HT ratios in HYP, brainstem (BST), STR and frontal cortex (FCX), than N rats. They also excreted more urinary NE than N. Hypoxic D and N rats had significant (17%) reductions of NE in HYP and signigicant reductions of DOPAC/DA ratios in HYP and STR. Hypoxia had no effects upon 5HIAA or 5HIAA/5HT ratios in D rats, while it reduced both in N rats, with significant interactive effects in BST. Hypoxic D and N rats had reduced food and water intakes. Hypoxic D rats also experienced significant elevations in plasma glucose concentration and NE excretion. Taken together, these results indicate that diabetic rats show enhanced vulnerability to hypoxic stress, making it a useful tool for exploring mechanisms of metabolic decompensation associated with stress in diabetes.

179.2

CARDIORESPIRATORY, PLASMA CATECHOLAMINE AND BEHAVIORAL RESPONSES FROM STIMULATION OF THE PERIAQUEDUCTAL GREY REGION IN THE RABBIT. C.G. Markgraf, D.R. Liskowsky, P.M. McCabe, R.W. Winters*, and Neil Schneiderman. Dept. of Psychology, Univ. of Miami, Coral Gables, FL 33124.

Stimulation of the periaqueductal grey region (PAG) of the midbrain in cats and in rats is known to elicit the 'defense' reaction. This response is characterized by a constellation of behavioral and cardiovascular changes that are thought to prepare the animal for 'fight or flight'. Although stimulation of PAG and regions of the hypothalamus produce the defense reaction in cats and rats, identification of the neuroanatomical substrates of the defense reaction in rabbits has been more problematic. The present study assessed cardiovascular, respiratory, hormonal and behavioral changes elicited by electrical stimulation of the ventrolateral PAG of New Zealand albino rabbits.

The electrical stimulus was a 10 second stimulus train (100 hz, 100-250 uA). Heart rate (HR), arterial blood pressure (BP), hindlimb blood flow (BF), respiration rate and volume, and catecholamine levels were measured in pentobarbital anesthetized animals. Behavioral changes were assessed in unanesthetized, unrestrained animals by passing current through stimulating electrodes that were chronically implanted in the ventrolateral PAG.

Stimulation of the ventrolateral PAG produced a response profile in anesthetized animals that was, for the most part, characteristic of the defense reaction. Electrical stimulation led to increases in BP (20-40 mmHg), BF (0.6-1.25 cc/min), norepinephrine and epinephrine levels, and in the rate and volume of respiration; these changes were accompanied by bradycardia (20-60 beats/min). In unanesthetized rabbits, ventrolateral PAG stimulation elicited increased motor activity, which is consistent with HR increases that are characteristic of the defense reaction in freely behaving animals. Supported by NIH grants HL 07426 and HL 36588.

179.3

STRESS-INDUCED RENIN AND CORTICOSTERONE (CORT) SECRETION IS MEDIATED BY CATECHOLAMINE TERMINALS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN). K.D. Richardson Morton, L.D. Van de Kar, M.S. Brownfield#, S.A. Lorens, J.H. Urban (SPON. W.H. Simmons). Dep. Pharmcol. Loyola U. Chgo., Med. Maywood, IL 60153 and #U. Wisconsin, Madison.

Cell bodies in the PVN mediate stress induced increases in renin and CORT secretion. In order to determine if catecholamine terminals in the PVN play a role in the neuroendocrine response to conditioned fear (CER), 6-hydroxydopamine (6-OHDA; 8.0 μg in 1.5 μl vehicle) was injected bilaterally into the PVN. Damage to noradrenergic nerve terminals in the PVN was verified by dopamine β-hydroxylase immunohistochemistry. The PVN 6-OHDA lesions prevented the CER induced increases in plasma renin activity (PRA), plasma renin concentration (PRC) and CORT levels. Subsequently, the β-receptor blocker sotalol (2.0 μg in 0.5 μl) was injected bilaterally into the PVN, 5.0 min before placement in the CER chamber for 10 min. Sotalol prevented the stress induced increase in CORT levels, but did not block the CER induced increases in PRA and PRC. These results suggest that catecholaminergic terminals in the PVN mediate the effect of stress on both renin and CORT secretion. The CER induced increase in CORT secretion, furthermore, appears to be mediated by β receptors, whereas stress-induced increases in renin secretion are mediated by another type(s) of catecholamine receptor.

179.4

INCREASES IN URINARY EPINEPHRINE ASSOCIATED WITH REALISTIC TRAINING EXERCISES. M.A. Oleshansky, F.J. Manning and C.F. Tyner*. Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

The stress associated with training exercises at the Chemical Decontamination Training Facility (CDTF) at the USA Chemical School, Ft. McClellan, Alabama was assessed by measurement of overnight urinary catecholamines. Military trainees at the CDTF undergo three mornings of detection and decontamination exercises while wearing a standard issue protective suit. The first morning of training takes place outdoors and simulants are employed; the second and third mornings of training take place in a controlled indoor environment and trainees are exposed to small amounts of toxic chemical warfare agents. For the purpose of this study, overnight urines were collected from 125 voluntary subjects during training with toxic agents and from 125 subjects who underwent training with simulants on all three days.

Overnight urinary epinephrine values were significantly higher on each of the three nights before the toxic agent training exercises as compared with values from the nights before the simulant exercises. Enlisted soldiers and officers who were new to the Army had higher epinephrine values during toxic agent training than seasoned non-commissioned officers and senior officers. These findings suggest that anticipation of toxic agent training was sufficient to stimulate epinephrine secretion and that prior experience influences the stress-related increases in urinary epinephrine excretion associated with toxic agent training.

179.5

ONE STRESSFUL EVENT SENSITIZES THE INCREASED FRONTAL CORTICAL DOPAMINE (DA) AND PLASMA CORTICOSTERONE ACTIVITY, BUT ATTENUATES THE ANALGESIC RESPONSE TO STRESS 10 DAYS LATER.
A. R. Caggiula, S. M. Antelman*, E. Aul*, S. Knopf* and D. J. Edwards Depts. of Psychol., Behav. Neurosci., Psychiat., and Physiol./Pharmacol., Univ. of Pittsburgh, Pittsburgh, Pa. 15260

A 40 sec. footshock (1.6 mA), administered to male rats, induced analgesia, as reflected by a significant increase in latency of tail withdrawal from a 55.5^0 C water bath. Animals exposed to a 1 hr. shock session (15, 0.5 sec. shocks) 10 days earlier showed a significant attenuation of shock-induced analgesia. Animals were sacrificed immediately after testing, and plasma corticosterone and regional brain catecholamines determined. Animals exposed to shock 10 days before testing exhibited higher corticosterone levels than all other groups. NE levels in the frontal cortex, and DA and DOPAC levels in frontal cortex and nucleus accumbens were not altered in any group. However the DOPAC/DA ratio in the frontal cortex was increased by analgesia testing, and this increase was enhanced only by the combination of shock 10 days before testing and shock immediately before the test. These results are consistent with previous reports from this laboratory which indicate that assessment of an animal's acute response to a stressor can only be understood by taking into account its past history of stress. Similar studies dealing with the effects of stress on the immune system are currently underway.
Supported by MH24114

179.6

REDUCTION IN NOREPINEPHRINE WITH FLA-63 BLOCKS EXCESSIVE WHEEL RUNNING AND GLANDULAR ULCERATION DURING ACTIVITY-STRESS. N.S. Morrow and S.W. Kiefer. Department of Psychology, Kansas State University, Manhattan, KS 66506.

The role of norepinephrine (NE) in triggering excessive wheel running and gastric ulceration during restricted feeding was examined. Rats were initially matched by body weight and divided into 3 activity groups (n=10 each) and 1 home-cage group (n=10). During the 18 day testing period, each group was injected (ip) twice daily with either FLA-63 (a dopamine-B-hydroaxylase inhibitor) or saline in the 7 hr preceding the feeding session. Two activity groups were fed for 1 hr each day and injected with either FLA-63 (10 mg/kg) or saline; the third activity group was fed ad lib and injected with FLA-63. The fourth group also was injected with FLA-63 but lived in home cages during restricted feeding. Results indicated that activity rats injected with FLA-63 during restricted feeding ran less and had fewer ulcers than rats injected with saline; there were no differences in food intake, body weight, or survival between rats injected with FLA-63 or saline. Neither the home-cage nor free-feeding rats became ulcerated during testing. FLA-63 had neglible effects on food intake and body weight. It is suggested that NE contributes to diurnal increases in running and that adequate stores of NE are necessary for the survival of rats during activity-stress procedures.

179.7

SOUND STRESS-INDUCED INCREASES IN TRYPTOPHAN HYDROXYLASE (TrpH) ACTIVITY BLOCKED BY ADRENALECTOMY (ADX). V.B. Singh*, K.C. Corley, T-H. Phan*, M. Kalimi* and M.C. Boadle-Biber. Dept of Physiology, Virginia Commonwealth University, Richmond, VA 23298.

Acute sound stress (SS)(2 sec, 110 dB sound pulses, VI-1 min for 120 min) produces a 50% increase in in vitro TrpH activity, that is reversed with alkaline phosphatase or disappears in vivo if the rats are killed 1 hr after the SS. When repeated 3 days/week for 1, 2 or 6 weeks, SS produces a persistant 45% increase in enzyme activity measured 24 hr after the final SS (Boadle-Biber et al., Fed. Proc. 46:3780, 1987: Soc. Neurosci. Abstr. 13:370.20, 1987). We now report that the adrenal gland is required for both the reversible and stable increases in TrpH activity. Male Sprague Dawley rats (175-200g) were adrenalectomized (ADXD) 4 days prior to exposure to an acute SS or 1 week of repeated SS (100 msec, 110 dB sound pulses, VI-1 min, for 1 hr). This modified SS protocol increased TrpH activity in sham-operated and normal rats. However enzyme from midbrain or cerebral cortex of ADXD rats showed neither the reversible nor the stable increases in activity in response to acute or repeated SS respectively. Dexamethasone (300 or 500μg/ day i.p. in DMSO for 3 days) 4 days after ADX restored the response in ADXD animals and did not alter the response in sham operated rats. Aldosterone (5μg/day, pellet s.c) was ineffective. Supported by NIH NS14090 to M.C.B-B.

179.8

STRESS CAN AFFECT DIFFERENTIALLY THE HUMORAL AND CELLULAR IMMUNE RESPONSES. J. Amat* and A. Torres* (SPON: E. Jaffe). Inst. Med. Exp., Univ. Central Ven., Apartado 50587, Caracas, Venezuela.

Stress is generally reported to depress or enhance the immune response depending on certain characteristics of the stressor. Since the immune response involve a variety of cellular elements and mechanisms, we decided to explore the effects of a single type of stress on some of the principal types of immune responses. Sprage Dawley (SD) and Wistar (W) female rats, and SD/W hybrids, of 180-250 g were used. Inescapable footshock stress was administered for 4 consecutive days. Immediately after the last shock session the rats, and their controls, were subjected to one of the following protocols: 1) Intraperitoneal injection of 10^7 sheep red blood cells (RBC) followed by tail blood extraction, every three days for 20 days, for testing anti sheep RBC seric antibodies, 2) Removal of the spleen (SD strain) and isolation of limphoid cells for hindlimb footpad injection in unstressed hybrids, whose popliteal ganglia were isolated and weighted 7 days later (graft vs host assay), 3) subcutaneous injection of a standarized dose of syngenic Walker carcinoma cells. It was found that the anti sheep RBC titer of stressed rats was significantly lower than that of controls. However, the graft vs host response and tumor rejection were higher in stressed animals. Therefore, it appears that stress may affect differentially the humoral and cellular immune responses.

179.9

ACUTE STRESS LOWERS SERUM CHOLESTEROL EVEN IN RATS ON A CHOLESTEROGENIC DIET. G.D. Coover and B.W. Santi*. Dept. of Psychology, Northern Illinois Univ., DeKalb, IL 60115.

Male rats aged 200 days were fed a mash diet for 10 days before assessing total- and high density lipoprotein-cholesterol (total-c and HDL-c) in jugular vein serum before and after a 3-hr stress period. Subgroups were given a cholesterogenic diet (lab chow plus 1% cholesterol and .5% cholic acid), and a 160-min session of moderate footshocks (2 s each on a VI 2-min schedule). Rats receiving the high cholesterol diet for 10 days exhibited a 98% increase in serum total-c (control mean = 79 mg/dl) along with a 38% decrease in HDL-c (control mean = 51 mg/dl).

The jugular sample/footshock stress decreased total-c by decreasing lower density lipoprotein-cholesterol (lDL-c). In the rats fed plain lab chow diet, the 12% decline in total-c ($p<.01$) and non-significant decrease in HDL-c replicate results with younger (90-day) rats (Coover & Murry, Soc. Neurosci. Abstr., 1987). Present results also show that a jugular blood sample alone does not cause any change in lDL-c, while causing a small (3 mg/dl) decrease in HDL-c, even in rats on the cholesterogenic diet.

Rats on the cholesterogenic diet exhibited a 20% decrease ($p<.001$) in total-c during the 3-hr stress of a jugular blood sample plus footshock. The effects of acute stress are clearly at odds with the expected effects of chronic stress (Bryant, Story & Yim, Life Sciences, 1987, 41, 545. (Supported in part by BRSG S07 RR07176, NIH.)

179.10

ULTRASONIC VOCALIZATIONS ARE PART OF THE RAT'S CONDITIONED EMOTIONAL RESPONSE. R.J. Frysztak*, L. Plopa & E.J. Neafsey Dept. of Anatomy, Loyola Univ Med Ctr, Maywood, IL 60153

Six male Sprague-Dawley rats were conditioned to a tone paired with a footshock. The following day heart rate (HR), respiration (R), and ultrasonic vocalization (USV) were recorded continuously during three trials in which only the tone was presented. Animals responded initially to the tone with increases in HR (+15 bpm) and R rate. Behaviorally, the rats "froze" during and after the tone. USV began after a variable latency period (5 to 45 sec after tone offset), and R rate and pattern changed dramatically with the onset of USV. R rate at rest was 1.5 Hz; during the tone the rate increased to 2.7 Hz; during USV the rate became highly variable, but averaged <1 Hz. USV varied in both intensity and duration (0.5 to 2 sec). HR increased 20 bpm from baseline during USV, and HR variability was also markedly increased. Freezing was paired with USV, such that during USV there was no movement. The average number of USV per trial decreased over the 3 trials (from 220 to 65), as did the total time period for USV and freezing (5:56 to 2:48). The number of USV/burst also decreased over the 3 trials (11.2 to 3.75). USV in rats has been reported following footshock (Tonue, Psychoneuroend 11. 1986) and during the natural stress of being introduced into the home cage of a dominant rat (Fokkema, LS 38. 1986). To our knowledge this is the first report of classically conditioned USV to the CS alone. (Funded by Potts Estate Fund Grant 842-04).

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SYMPOSIUM. NEW INSIGHTS INTO THE FUNCTIONS OF THE BASAL FOREBRAIN CHOLINERGIC SYSTEM. R.T. Richardson, Johns Hopkins Univ. (chairperson); M.M. Mesulam, Beth Israel Hospital; M. Steriade, Univ. of Laval; R. Dykes, Univ. of Montreal; D. Olton, Johns Hopkins Univ.

Recent advances in understanding the anatomical and physiological properties of the basal forebrain cholinergic system (BFCS) indicate how it may be specifically involved in various functions including cortical activation and memory formation. **Dr. Mesulam** will provide an overview of the anatomical connections of the BFCS emphasizing the strong interconnections with limbic-related areas. **Dr. Steriade** will review evidence for a projection from the BFCS to the reticular thalamic nucleus. He will also discuss the role of basal forebrain and brainstem cholinergic afferents in thalamocortical activation. **Dr. Dykes** will review recent studies of the effects of ACh applied directly to neurons. He will emphasize the long-term duration of the changes in cortical neurons produced by application of ACh or stimulation in the basal forebrain. **Dr. Richardson** will summarize recent studies of the activity of single BFCS neurons in behaving animals. He will discuss the implications of the responses of BFCS neurons to stimuli associated with reinforcement. **Dr. Olton** will conclude the symposium with a review of the effects of BFCS manipulations on several behaviors, particularly on memory and attention.

182

NEURONAL GENE EXPRESSION: PHYSIOLOGICAL ACTIVATION, TRANSCRIPTIONAL CONTROL AND DNA BINDING PROTEINS.
M.J. Iadarola, NIH (chairperson) **W.S. Young III**, NIMH; **J. Douglass***, Oregon Health Sci. Univ.; **M. Comb***, Mass. Gen. Hospital; **M. Montminy***, Salk Inst. and **D. Levens***, NIH.

Many genes important to nervous system function and structure have been isolated and their protein products characterized. This has provided a foundation of structural information at the genetic, cellular and neuroanatomical levels. Relatively recent developments built upon this foundation have led to new insights into the mechanisms by which neuronal gene expression is regulated and the in vivo situations in which regulation occurs. This symposium will examine control of neural gene expression from experimental, conceptual and methodological standpoints. An initial analysis of enhanced expression due to specific physiological or environmental stimuli will progress to an examination of DNA enhancer/promotor sequences responsible for tissue- or stimulus-specific expression. The precise nucleotide sequences of several of these regulatory regions, termed cis-acting DNA elements have been identified. They are known to mediate enhanced gene expression due to activation of adenylate cyclase and/or phosphatidyl inositol second messenger systems. These sequences are also recognition sites for specific DNA binding proteins or trans-acting factors. The enhancer elements can be composed of multifunctional domains that interact with several specific proteins. Several proteins that mediate or participate in trans-activation have been isolated and the dynamic process that regulate their binding and thereby the expression of the target gene will be discussed. Experimental evidence suggests that phosphorylation and cooperative interaction between several such proteins are important factors in governing enhanced transcription and ensuring specificity. Supported by Genentech, Inc. and Bethesda Research Laboratories.

AMINO ACIDS: GABA AND BENZODIAZEPINES IV

183.1

BENZODIAZEPINE RECEPTOR SUBTYPES IN THE RAT SPINAL CORD: MODULATION BY γ-AMINOBUTYRIC ACID (GABA). M.G. Corda, B. Longoni* and G. Biggio. Department of Experimental Biology, Chair of Pharmacology, University of Cagliari, Italy.

In the present study we evaluated the relationship between GABA receptors and benzodiazepine (BZD) recognition site subtypes in the rat spinal cord. Scatchard analysis of saturation data indicated that Type I sites labelled with ^{3}H-2oxoquazepam (^{3}H-2-OQ) and ^{3}H-ethyl-β-carboline-3-carboxylate (^{3}H-βCCE) were approximately 20% of the sites labelled by ^{3}H-flunitrazepam (^{3}H-FNT) (Bmax, fmoles/mg prot.: ^{3}H-FNT 0.23 ± 0.04; ^{3}H-2-OQ 0.05 ± 0.01; ^{3}H-βCCE 0.04 ± 0.01). The "in vitro" addition of GABA (10^{-8} to 10^{-4} M) stimulated both ^{3}H-FNT and ^{3}H-2-OQ binding in a concentration-dependent manner. The maximal enhancement, produced by 10^{-4} M GABA, was 48 and 85% above control values for ^{3}H-FNT and ^{3}H-2-OQ binding, respectively. We also evaluated the effect of BZDs on the binding of ^{3}H-GABA to spinal cord membranes, as compared with brain areas that contain a higher proportion (≥ than 40%) of Type I BZD recognition sites. Diazepam and 2-OQ (10^{-7} to 10^{-5} M) increased the specific binding of ^{3}H-GABA in membranes from the cerebral cortex, cerebellum and hippocampus, but not in spinal cord membranes. The results indicate that: a) both Type I and Type II sites in the spinal cord are linked to GABA receptors, and b) the stimulation of ^{3}H-GABA binding by BZDs is present only in areas that contain more than 40% of Type I BZD recognition sites.

183.2

QUAZEPAM AND 2OXOQUAZEPAM ARE THE MOST EFFECTIVE BENZODIAZEPINE (BZ) RECEPTOR LIGANDS AS INHIBITORS OF ^{35}S-TBPS BINDING IN THE RAT BRAIN. A. Concas*, M. Serra*, T. Atsoggiu* and G. Biggio. Dept. of Exp. Biol., Univ. of Cagliari, Italy.

The effect of several BZ receptor ligands on ^{35}S-TBPS binding sites associated to the GABA-dependent chloride channel was studied in unwashed membrane preparations from rat cerebral cortex. Quazepam and 2oxoquazepam were the most effective inhibitors of ^{35}S-TBPS binding producing 100% inhibition at concentrations near 30 μM, an effect similar to that induced by GABA (100 μM) and muscimol (10 μM). In contrast, other BZs were much less potent. In fact the maximal inhibition (23 ± 2% of control) was observed in the presence of 100 μM diazepam or flunitrazepam. Flurazepam inhibited ^{35}S-TBPS binding in the presence of 300 nM to 10 μM concentrations; concentrations higher than 10 μM induced an opposite effect. Zolpidem and Cl 218872, two non BZ compounds having high affinity for the Type I BZ recognition site inhibited ^{35}S-TBPS binding with less efficacy than BZs (49 and 40% of control at 100 μM, respectively). The rank order of efficacy for the above compounds as inhibitors of ^{35}S-TBPS binding was muscimol > quazepam >GABA=2oxoquazepam> flunitrazepam=diazepam> Cl 218872> zolpidem> flurazepam. The different efficacy of these BZ receptor ligands on ^{35}S.TBPS binding may reflect:a)a different interaction with the Type I and/or Type II BZ receptors, b)a different coupling of these receptors with the Cl channel. This mechanism may be responsible of their different pharmacological profile.

183.3

DEVELOPMENTAL HETEROGENEITY OF THE POSTSYNAPTIC GLYCINE RECEPTOR IN RAT SPINAL CORD. **C.-M. Becker*, W. Hoch*, and H. Betz. ZMBH, Universität Heidelberg, Im Neuenheimer Feld 282, D-6900 Heidelberg, Fed. Rep. of Germany**

The inhibitory glycine receptor from spinal cord is a glycoprotein of ≈250k composed of three polypeptides of 48k, 58k, and 93k. The ^{3}H-strychnine binding site is located on the 48k subunit.

^{3}H-strychnine binding is low in neonatal rat spinal cord. A search for glycine receptor-associated antigens, however, revealed the presence of high levels of mAb 4a epitopes which have been assigned to the 48k polypeptide. This neonatal mAb 4a antigen sediments as a protein complex of ≈250k. It could be separated from adult glycine receptor by immunoprecipitation using another mAb. Furthermore, it bound to a 2-aminostrychnine affinity column and was eluted from this column by lower concentrations of glycine than the adult receptor. These data indicate that neonatal spinal cord contains a glycine receptor species of low strychnine binding affinity. Cell cultures of embryonic mouse spinal cord almost exclusively express the neonatal glycine receptor.

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183.4

TAURINE INDUCED CHANNELS IN MEMBRANE PATCHES EXCISED FROM CULTURED SPINAL CORD NEURONS. D.A. Mathers* and Y. Wang* (Spon: D.F. Schwarz). Department of Physiology, University of British Columbia, Vancouver, B.C., V6T 1W5 Canada.

The monocarboxylic amino acid taurine is present at high levels in the mammalian brain. It has also been shown to exhibit marked anticonvulsant activity in human epilepsy. Available evidence suggests that taurine may activate chloride-dependent membrane conductances controlled by the inhibitory transmitters gamma-aminobutyric acid (GABA) and glycine. To investigate this hypothesis, we compared the properties of chloride channels induced by taurine (40 uM), GABA (1.5 uM) and glycine (1.5 uM) in outside out patches excised from mouse spinal cord neurons growing in primary culture. Experiments took place at 21-23 °C using a List EPC-5 patch clamp amplifier. Taurine induced single channel currents reversed at the equilibrium potential for chloride ions. Compared to events triggered by GABA, taurine induced currents showed less tendency to occur in a burst-like manner. Substate conductance levels were observed for all three amino acids. However, taurine induced events showed a significantly greater tendency to enter the larger conductance states than currents activated by GABA in the same membrane patches.

183.5

BEHAVIORAL CHARACTERIZATION OF GABA/BENZODIAZEPINE (BZ) RECEPTOR SUBTYPES USING A MODIFIED VOGEL CONFLICT PARADIGM. C.Wambebe, A. Berkovich, A. Guidotti and E. Costa., FGIN, Georgetown Univ., Washington, D.C. 20007.

Rat diazepam binding inhibitor (DBI 1-86) extracted from rat brain is a putative precursor of neuropeptides which function as negative allosteric modulators of various $GABA_A$ receptor subtypes. TTN (DBI 17-50), EPN (DBI 26-50) and ODN (DBI 33-50) induced proconflict activity when injected intraventricularly in rats. The nature of the $GABA_A$ receptor involved in the proconflict behavior was unvailed by pretreatment with specific antagonists for various BZ recognition sites. Flumazenil, (a ligand for GABA receptors with beta carboline, $GABA_{A1}$, and anxiolytic BZ, $GABA_{A1}$-$GABA_{A2}$, recognition sites) antagonized the proconflict effects of ODN and that of the anxiogenic beta carbolines (FG 7142) but was ineffective aganist TTN and 4-chloro-diazepam, (Ro 5-4864), a ligand for peripheral BZ sites coupled to brain $GABA_{A3}$ receptors. On the contrary, isoquinoline carboxamide, PK 11195, (an antagonist of modulator sites of $GABA_{A3}$ receptors) blocked the proconflict effects of Ro 5-4864 and TTN but not that of FG 7142 or ODN. Thus, the proconflict activity induced by natural processing products of DBI is mediated through a specific action on different BZ recognition sites that characterize $GABA_A$ receptor subtypes.

183.6

SODIUM-INDEPENDENT ^{35}S-CYSTEINE BINDING TO FROZEN/THAWED AND EXTENSIVELY WASHED SYNAPTOSOMAL-MITOCHONDRIAL FRACTIONS. C.H. Misra. Dept. of Psychiatry and Behavioral Sciences, U.T. Medical School, Houston, Texas 77030.

Previous studies in our laboratory have demonstrated the existence of a specific-independent cysteine binding to synaptic membrane preparation of rat brain (Misra, J. Neurochem., 48S, 1987). This binding however has been done in Tris-HCl medium (containing 2.7 mM $CaCl_2$, 1.2 mM $MgCl_2$, 5 mM KCl, 256 mM sucrose and 50 mM Tris-HCl) buffer, pH 7.4. In an extended study it has been found that the binding increased about one and a half times, when the binding was carried out only in Tris-HCl buffer pH 7.4. Therefore the experiments were conducted in Tris-HCl buffer, pH 7.4 using lysed crude synaptosomal-mitochondrial (P_2) fraction from rat brain tissue, which has been frozen/thawed. Binding reached maximum at 15 minutes, slightly increased thereafter and then remained constant for approximately 90 minutes, whereas the frozen/thawed synaptic membrane preparation reached maximum at 15 minutes, slowed down, and increased at a slow space for 90 minutes. The significance of the binding constants (K_D and B_{max}) of different treatment of synaptosomal-mitochondrial preparations and their displacement constants (IC_{50}) with cysteine, and its analogues and other neuroexcitatory amino acids will be discussed.

183.7

ω-CONOTOXIN GVIA BLOCKS GABA RELEASE FROM CHICK CEREBRAL NEURONS. C.L. Thompson* and E.M. Barnes, Jr. Dept. of Biochem., Baylor Col. of Med., Houston, TX 77030.

Cultured cortical neurons were loaded for 60 min with [^{3}H]GABA (1 μM, 2 μCi/ml) in Hepes-buffered saline. After washing and a 15 min incubation in fresh saline, the cells were transferred to release media containing 40 mM K. Depolarization-evoked [^{3}H]GABA release was determined after 5 min and corrected for the efflux which occurred in normal saline (5 mM K). K-evoked release accounted for 7-9% of the [^{3}H]GABA pool and represented more than twice that observed in 5 mM K. Addition of EGTA (1 mM) and Ca omission blocked over 75% of K-evoked GABA release. ω-Conotoxin GVIA (50 nM) produced a 50% inhibition of GABA release, while nitrendipine (10 μM) gave less than a 15% reduction. However, after 10 min in 40 mM K, nitrendipine produced a 40% inhibition.

We have shown previously that midazolam (0.1 μM) induces a ^{36}Cl uptake into cortical neurons which, although found in the absence of exogenous GABA, is mediated by GABA-A receptors. ω-Conotoxin (50 nM) blocked over 80% of the midazolam-dependent ^{36}Cl uptake, supporting the notion that synaptic release of endogenous GABA is required. The results suggest that synaptic GABA release requires the participation of ω-conotoxin-sensitive Ca channels.

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183.8

CYCLIC-AMP ACCELERATES GABA-A RECEPTOR DESENSITIZATION. E.M. Barnes, Jr., M.H. Jalilian Tehrani and J.J. Hablitz (SPON: P. Kellaway). Depts. of Biochem. and Neurology, Baylor Col. of Med., Houston, TX 77030.

At a holding potential of -60 mV, the GABA-gated current into chick cortical neurons declines during continued application of agonist. This desensitization process in controls fit a single expotential with a time constant of 7-10 s. After 6 min exposure to 50 μM forskolin, the peak amplitude of GABA-induced currents declined and a fast component of desensitization (τ = 1 s) appeared, while the slow component was essentially unchanged. This effect of forskolin was reversed following washout. Similar, but less robust, effects were produced by 8-Br-cAMP. Desensitization could also be measured by GABA-gated ^{36}Cl flux. A 20 s incubation with 10 μM GABA (5 mM K external) produced a 29% inhibition of subsequent GABA-gated ^{36}Cl uptake (40 mM K). This inhibition was increased to 64% by addition of 100 μM forskolin; this effect was blocked by 100 μM 2',5'-dideoxyadenosine. A similar acceleration of desensitization was produced by 8-Br-cAMP or by isobutylmethylxanthine. These compounds failed to produce desensitization in the absence of exogenous GABA. The results suggest that a cAMP-dependent phosphorylation converts the GABA receptor into a more rapidly desensitizing form.

Supported by NIH grants DK 17436 and NS 11535.

183.9

INHIBITORY MODULATION OF GABA-GATED CHLORIDE INFLUX BY RO5-4864 AND DELTAMETHRIN IN RAT BRAIN SYNAPTONUEROSOMES. L.L. Devaud and T.F. Murray, College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Both pyrethroid insecticides and the convulsant benzodiazepine Ro5-4864 may act to impair GABAergic neurotransmission. To further characterize the mechanism of action of these compounds, we investigated the effects of Ro5-4864 and deltamethrin on the functional coupling of $GABA_A$ receptors to chloride channels by measuring GABA stimulation of $^{36}Cl^-$ influx into rat brain synaptoneurosomes. The stimulation of $^{36}Cl^-$ uptake by GABA (3-300 µM) was significantly antagonized by a concentration of 100 nM Ro5-4864. This dose of Ro5-4864 reduced the E_{max} for GABA-gated $^{36}Cl^-$ influx by approximately 20% without affecting the EC_{50} value for GABA (control EC_{50}=29.7±2.8 µM, in the presence of Ro5-4864 EC_{50}=26.0±1.8 µM). The Type II pyrethroid deltamethrin (DM) also decreased the E_{max} for GABA stimulation of $^{36}Cl^-$ uptake without altering the potency of GABA. DM (0.01-10 µM) elicited a concentration dependent inhibition of GABA-gated $^{36}Cl^-$ influx with a maximal inhibition of 67% occurring at a dose of 10 µM. The IC_{50} value for DM as an inhibitor of GABA-gated $^{36}Cl^-$ influx was 1.24±0.16 µM, which is in excellent agreement with the IC_{50} value of 1.37±0.11 µM for DM as an inhibitor of [^{35}S]TBPS binding to rat cerebral cortical membranes. The noncompetitive inhibition of GABA-gated $^{36}Cl^-$ uptake by both Ro5-4864 and deltamethrin may underlie the pro-convulsant actions of these compounds.

183.10

ENHANCEMENT OF GABA MEDIATED TRANSMEMBRANE CHLORIDE EXCHANGE BY DEUTERIUMOXIDE. J. Kardos & D. J. Cash, Dept. of Pharmacodynamics, Cent. Res. Inst. for Chem. Hungarian Academy of Sciences, Budapest, Hungary H-1525 POB17 and Neurochemistry Unit, Missouri Inst. of Psychiatry, Univ. of MO-Columbia, Sch. of Med., St. Louis, MO 63139.

GABA receptor mediated chloride ion-exchange and receptor desensitization were measured using $^{36}Cl^-$ isotope tracer and quench flow techniques with membrane suspension prepared from rat cerebral cortex suspended in buffer solutions in the presence and absence of D_2O. The chloride exchange was measured by following the uptake of $^{36}Cl^-$ by membrane vesicles in the suspension in the presence or absence of GABA. Reaction times in the sub-second time region were achieved by using quench-flow technique. Specific chloride channels were opened on rapid mixing with GABA and closed on mixing with a solution containing bicuculline methiodide which terminates the GABA mediated $^{36}Cl^-$ uptake. The chloride exchange takes place in two phases terminated by two desensitization processes in different time regions with half times of 230 ms and 2-5 s in 40 µM GABA. These different phases have been attributed to two distinguishable receptors with different activities and different rates of desensitization. In the presence of D_2O the $^{36}Cl^-$ uptake in the faster phase of ion-exchange was increased due to an increase in the initial rate of ion-exchange of the more predominant, faster desensitizing receptor. Deuterium isotope effect on the slower desensitizing receptor was less pronounced. This enhancement reflects differences in dehydration of chloride ion in D_2O.

183.11

THE ß-CARBOLINE DERIVATIVES ZK 93426 AND FG 7142 DO NOT INDUCE ABSTINENCE SIGNS IN DIAZEPAM-DEPENDENT CATS. G. Biggio, M. Corda and O. Giorgi. (Spon.: M.R. Melis) Department of Experimental Biology, Chair of Pharmacology, University of Cagliari, Italy.

Diazepam-dependent cats were treated with different benzodiazepine recognition site ligands 24 h after the last dose of chronic treatment with diazepam (7 mg/Kg, i.p. at 08.00 and 20.00 h, for 21 consecutive days). The benzodiazepine derivatives Ro 15-4513 (a partial inverse agonist), Ro 15-1788 (an antagonist), and the pyrazoloquinoline derivative CGS 8216 (a partial inverse agonist) precipitated abstinence signs when administered at the dose of 10 mg/Kg, i.p. Abstinence signs included tremors, increased muscle tone, irritability, fear, pupillary dilation and vocalizations. On the contrary, the ß-carboline derivatives ZK 93426 (an antagonist) and FG 7142 (a partial inverse agonist) failed to precipitate abstinence signs in diazepam-dependent cats when given at doses (10 mg/Kg, i.p.) that prevented the acute effects of diazepam. These results indicate that the ability to induce withdrawal signs in diazepam-dependent cats depends on the chemical structure of the challenge drug (i.e., benzodiazepine or pyrazoloquinoline), since ß-carboline antagonists like ZK 93426 and partial inverse agonists like FG 7142 lack this property.

183.12

AN ELECTROENCEPHALOGRAPHIC STUDY OF THE BUTYL-BICICLOPHOSPHOROTHIONATE TERTIARY. P.Lorenzini*, D.DeMedici*, L.Mele* and M.Massotti (SPONS:R.del Carmine). Lab di Farmacologia, Istituto Superiore di Sanità, Roma, Italy, 00161.

Butyl-bicyclophosphorothionate tertiary (TBPT) binds with high affinity to picrotoxinin recognition sites in membrane preparations from mammalian brain tissue (Life Sci.,33:2363,1983).

TBPT elicits convulsions in mouse (ED_{50}, 60 µg/kg ip and 50 µg/kg iv), rat (28 µg/kg iv) and rabbits (41 µg/kg iv).

In rats and rabbits, electroencephalographic changes occur which can be divided into two and three dose-responses stages, respectively as reported in the table

	Slow-Waves (Optic Ctx)	Spike-and-Waves (Sensorimotor Ctx)	"Grand-Mal"
Rats	---	5-20	>20
Rabbits	10-20	20-30	>30

Ctx=Cortex The values are expressed as µg/kg i.v.

These changes are similar to those observed after bicuculline, inverse benzodiazepine agonists, picrotoxin and pentilentetrazol (Pharmac.Biochem.-Behav., 23:661,1985).

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PROCESS OUTGROWTH, GROWTH CONES, AND GUIDANCE MECHANISMS II

184.1

INHIBITION AND STIMULATION OF NEURITE OUTGROWTH BY SEROTONIN IN NEURONS CULTURED FROM EMBRYONIC AND ADULT *HELISOMA*. J.I. Goldberg, L.R. Mills and S.B. Kater. Dept. of Zoology, University of Alberta, Edmonton, Alberta, T6G 2E9 and Program in Neuronal Growth and Development, Colorado State University, Fort Collins, CO 80523.

While a variety of neurotransmitters have been implicated in the regulation of neurite outgrowth, it is not known whether the same mechanisms operate during both embryonic development and adult neuroplasticity. We now examine the regulation of neurite outgrowth by serotonin in parallel embryonic and adult dissociated cell culture systems. In both classes of neurons (ie. developing and adult regenerating), serotonin can evoke quite different responses: growing neurites can be inhibited and stable neurites can be stimulated to re-initiate outgrowth. In embryo cultures, addition of 50 µM serotonin resulted in cessation of outgrowth in 44.2% of the advancing neurites (N=52). In matched control cultures, spontaneous cessation occurred in only 12.5% of neurites (N=48; p<.001). In adult cultures, 35.5% of the advancing neurites were outgrowth inhibited by serotonin (N=45), while spontaneous cessation occurred in 8.6% of the control neurites (N=58; p<.001). Non-advancing neurites were also examined to test for stimulation of outgrowth by serotonin. In embryo cultures, serotonin re-initiated outgrowth in 44.7% of the non-advancing neurites (N=47), in comparison to spontaneous re-initiation in 21.6% of the control neurites (N=37; p<.05). Similarly in adult cultures, serotonin re-initiated outgrowth in 23.3% of the non-advancing neurites (N=77), in comparison to spontaneous re-initiation in 7.5% of the control neurites (N=53; p<.05). These data demonstrate that serotonin has stimulatory or inhibitory effects on neurite outgrowth in a large number of embryonic and adult neurons. Whether these opposite effects occur in different sets of neurons, or in the same set of neurons during different states of outgrowth, remains to be determined.

This research was supported by the Alberta Heritage Foundation for Medical Research and the NIH.

184.2

AFFERENT GROWTH CONES INTERACT *IN VITRO* WITH TARGET CELLS FROM DEVELOPING CEREBELLUM

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Previous observations of developing cerebellum *in situ* indicate that single afferent axons initially form contacts with several target cell populations before "choosing" a single target cell type. We have devised a culture system to investigate the mechanism of this target cell selection and to define the behavior of growth cones during this process. In a modification of the microcultures used in our lab to analyze neuron-glia contacts, explants from the brainstem sources of cerebellar afferents (pontine nuclei for mossy fibers, inferior olive for climbing fibers) are co-cultured with dissociated and purified populations of cerebellar target neurons. The target cells and the explants are initially plated on either side of a partition that is later removed. Separating the explants from the target cells makes it easier to distinguish explant from target cell neurites, and allows us to test for trophic or tropic interactions.

Video enhanced DIC time-lapse microscopy permits high resolution observation of the behavior of single, identified explant growth cones interacting with identified target cells. Growth cones from pontine axons encountering their appropriate target granule cell growth cones exhibit initial contacts of long duration. Both growth cones have continuous filopodial and lamellipodial activity, yet cease to extend. In contrast, when granule cell growth cones interact with their own type, initial contacts are of shorter duration and neurites continue to extend. EM and immuno-histochemical studies are in progress to correlate the video observations with synaptogenesis. Supported by NIH grant NS-15961.

184.3

THE LOCATION OF CYTOSKELETAL ELEMENTS AND ORGANELLES PREDICTS THE SITE OF GROWTH CONE EMERGENCE FROM A GRASSHOPPER PIONEER NEURON OBSERVED IN SITU WITH CONFOCAL IMAGING. Frances Lefcort and David Bentley. Neurobiology Group and Department of Zoology, University of California, Berkeley, CA 94720.

In embryonic grasshopper limb buds, the Til pioneer neurons arise from a mother cell (PMC; Keshishian, 1980), located in the limb tip epithelium, which undergoes a proximo-distally oriented mitosis shortly before pioneer axonogenesis. The growth cone of the proximal daughter cell (Tilp) emerges perpendicularly to the apparent cleavage plane. Using a confocal microscope and fluorescent probes, we have characterized the arrangement of several cytoskeletal elements and organelles from early prophase of PMC through axonogenesis of Tilp. The proximal spindle pole of the PMC (later in Tilp) is defined by the location of (i) telophase chromosomes labeled with propidium iodide, (ii) spindle microtubules labeled with a monoclonal anti-tubulin antibody (gift of Dr. David Asai, Purdue), (iii) centrosomes labeled with anti Drosophila-centrosome serum antibody (gift of Doug Kellogg, UCSF), and (iv) cleavage furrow and mid-body F-actin labeled with rhodamine-phalloidin. After cytokinesis, a brightly labeling tubulin-containing focus at the cell pole, which may be a centrosome or MTOC, persists and is seen later to lie within the base of the nascent growth cone. Initially, the Golgi apparatus, labeled with FITC-WGA, is distributed perinuclearly. At the onset of axonogenesis, transient, focal FITC-WGA label also appears in the base of the nascent growth cone. Formation of the PMC cleavage furrow marked by F-actin is followed by initiation of axonogenesis. To test the hypothesis that displacement of cortical actin into the cleavage furrow may be associated with the initation of axonogenesis at the cell pole, embryos were exposed to 0.1 ug/ml cytochalasin D. Exposure after the onset of axonogenesis does not block further axon extension; however, exposure before PMC cytokinesis blocks axonogenesis while allowing expression of other neural phenotypic characteristics.

184.4

EFFECTS OF ENZYMATIC REMOVAL OF THE BASAL LAMINA ON PIONEER NEURONS IN GRASSHOPPER EMBRYOS. Maureen L. Condic and David Bentley. Neurobiology Group and Dept. of Zoology, University of California, Berkeley, CA 94720

Several proteolytic enzymes were tested for their effects on the basal lamina and the disposition of the Til neurons in embryonic grasshopper limb buds. Embryos of Schistocerca americana were incubated at 30°C for 2.5 hrs with either elastase (0.04%), chymotrypsin (0.005%), ficin (0.02%), papain (0.025%), trypsin (0.01%) or saline. Elastase, ficin and papain completely removed the basal lamina when viewed in SEM. Chymotrypsin and trypsin had little or no effect on the structural integrity of the basal lamina. Removal of the basal lamina at 32% of development when the Til axons have extended over the intrasegmental epithelium, but are not in contact with other immature neurons resulted in retraction of the growth cones to the cell somata. This suggests that the Til axons are under tension in vivo and that the cells are dependent on the basal lamina to remain extended over this region of the limb. Removal of the basal lamina later in development (34%) when the Til growth cones are in contact with either nascent neurons or limb segment boundaries resulted in the relocation of the somata proximally. This suggests that neural-neural and neural-segment boundary interactions are at least in part due to adhesive cell-cell interactions that are independent of the basal lamina. The ability of the Til neurons to extend axons in limbs from which the basal lamina had been removed by enzymatic digestion was also investigated. Embryos at 30-32% were treated with either elastase or ficin as described, then rinsed extensively for 30 minutes and cultured for 12-16 hours in the absence of enzyme. The Til neurons extended axons after enzymatic treatment at rates approximately equal those seen in control animals. Til growth cones extended after the removal of the basal lamina frequently exhibit lamella and extend numerous filopodia. The growth cones recognized and were reoriented by immature neurons and limb segment boundaries. The effect of removing the basal lamina on Til pathfinding is currently under investigation.

184.5

DIRECTIONAL CUES INFLUENCE, BUT DO NOT DETERMINE STEREOTYPED NAVIGATION OF A GROWTH CONE ARRAY IN THE EMBRYONIC LEECH. J. Jellies & W.B. Kristan, Jr., Dept./Biology, UCSD, La Jolla, CA, 92093.

The oblique muscle of the leech, Hirudo medicinalis, is laid down upon the multiple parallel processes of an identified cell, the Comb- or C-cell. Each segment contains a bilateral pair of mirror-image cells. The ≈70 growth cones on each C-cell navigate along precise trajectories with relatively constant spacing. Injecting C-cells with HRP revealed occasional (<0.1% of processes) deviations in trajectory. Deviating processes were most often oriented along the mirror-image path destined to be followed by the processes of the contralateral C-cell, implying the presence of directional cues. The earliest C-cell processes orient orthogonally, implicating the grid of circular and longitudinal muscle cells in this role. The 1^0 circular muscle cells (22/hemisegment) can each be identified by position and morphology. To examine the influence of these cells in guiding C-cell processes, one pair of them was dye-filled then photoablated during embryonic day 10. When examined later, most C-cell processes had crossed the lesion site normally. There was, however, an approximately 60 fold greater number of navigational errors across the lesion compared to normal. Thus, while the ablated circular muscle cells may provide navigational cues, these must act in concert with other factors to guide C-cell growth cones. Supported by NIH grants NS20746 & NS25916 (WBK).

184.6

THE EFFECT OF NEURAL CREST DELETIONS ON THE DEVELOPMENT OF SPECIFIC MOTONEURON PROJECTIONS IN THE EMBRYONIC CHICK HINDLIMB. C. Lance-Jones and J. W. Yip. Depts. of Neurobiology, Anatomy and Cell Science and Physiology, Sch. of Med., Univ. of Pittsburgh, Pittsburgh, PA 15261.

Schwann cells may play a role in motoneuron axon outgrowth, yet their precise function in this process is unknown. One possibility is that Schwann cells arising from neural crest at specific sites on the neuraxis are uniquely able to recognize specific targets and guide motoneurons to them. To test this idea we excised dorsal neural tube including the neural crest from posterior thoracic (T) through the limb-innervating lumbosacral (LS) regions in stage 14 chick embryos and then examined the specificity of motoneuron projections to selected hindlimb muscles at stage 35-36. The operation gave rise to embryos that lacked dorsal root ganglia (DRG) from posterior T segments through LS4,5,6,7, or 8. Separate analyses of quail/chick neural tube chimeras suggested that these deletions would remove all Schwann cells that would normally be found along nerves originating from the DRG-deleted segments. Retrograde HRP labelling of motoneuron pools to muscles normally innervated by motoneurons in LS1-3 indicated that in all cases the pools were normally positioned. These data suggest that LS motoneurons do not require Schwann cells from corresponding axial levels to make correct projections. Supported by NSF: BNS-8518864 and NIH: NS23916.

184.7

PROTEASE INHIBITORS INTERFERE WITH AXONAL OUTGROWTH OF IDENTIFIED MOTONEURONS IN LIVE ZEBRAFISH EMBRYOS. B. Debu and J.S. Eisen, Institute of Neuroscience, University of Oregon, Eugene OR 97403.

During axonal elongation and pathfinding some growth cones release proteases that modify their local environments, a process that may be important in neurite outgrowth. To examine whether such proteolysis might play a role in axonal elongation in embryonic zebrafish, we injected protease inhibitors in vivo and examined axonal pathfinding by identified neurons. In zebrafish each myotome is innervated by 3 identified motoneurons. The motor growth cones initially follow a common pathway, later extending along cell-specific pathways to mutually exclusive regions of muscle. Injection of leupeptin, a serine protease inhibitor, resulted in abnormalities in motor axonal outgrowth. In 16/25 fish the motor axons in injected segments extended along the common pathway, but did not extend along cell-specific pathways. Some axons appeared to stop growing, while others extended branches in inappropriate regions of muscle. Control injections of saline or trypsin inhibitor did not have observable effects on motor axonal outgrowth or pathfinding. These results are consistent with a role for proteolysis in axonal outgrowth by zebrafish motoneurons. Supported by the NIH, NSF and the Chicago Community Trust.

184.8

DEVELOPMENT OF MUSCLE INNERVATION IN *DROSOPHILA* EMBRYOS AND LARVAE. J. Johansen, M.E. Halpern and H. Keshishian. Department of Biology, Yale University, New Haven, CT 06511.

In *Drosophila* embryos and larvae each segment contains a precise array of no greater than 31 muscle fibers per side, with each fiber individually identifiable. This is a particularly favorable arrangement for examining motoneuronal exploration and the development of innervation. Previously, we had shown that by the end of larval life (the third instar) many of the muscle fibers have specialized and stereotypic arrays of motor endings, often with characteristic axonal trajectories over the fiber surfaces. Double labelling experiments with neuron-specific antibodies (anti-HRP), together with an antiserum to glutamate, indicate that the entire innervating motoneuronal population in the third instar expresses glutamate as a transmitter (a subset coexpress proctolin and probably octopamine). Using both neuron and neurotransmitter-specific probes we have followed the establishment of these precise and stereotyped projections during embryogenesis and find that: 1. When innervation begins the muscle fibers are already organized into their segmental pattern. 2. That in early stage 16 embryos the glutamate transmitter appears to be expressed in growth cones before the muscles are contacted, and 3. that elements of the muscle fiber-specific axonal trajectories are recognizable from the earliest innervation by growth cones from the developing nerve. A detailed description of synaptogenesis and of the development of the stereotypic glutamatergic muscle innervation during embryogenesis and throughout the larval stages will be presented. (Glutamate antiserum was a gift of Dr. P. Petrusz).

184.9

DIRECTING AXONAL REGENERATION BY DIFFUSIBLE FACTORS FROM MUSCLE FIBERS AND NERVE TUBES.
D.P. KUFFLER and B.STOLZ*. Dept. of Pharmacology, Biocenter, Univ. of Basel, Basel, Switzerland.

Earlier studies in this laboratory have shown that the outgrowth of regenerating frog muscle axons can be directed by denervated lengths of nerve tube and the non-synaptic regions of muscle fibers. In the present experiments denervated nerve tubes and muscle fibers were placed inside filters which isolated the target cells but allowed the diffusion away of molecules they released. Target cells inside filters were still able to direct the outgrowth of regenerating axons. These results show that the target cells release a diffusible factor/s that causes regenerating axons to change their direction of growth. Furthermore this influence is effective over millimeters.

The present findings set the stage for tissue culture experiments in which the phenomena can be analyzed in terms of molecular mechanisms. So far Schwann cells, muscle fibers and a variety of adult spinal neurons have been successfully cultured. Our aim is to combine these cells to establish whether directed outgrowth occurs and to characterize the factors involved.

184.10

RAT CNS-MYELIN AND OLIGODENDROCYTES IMPAIR THE GROWTH OF GOLDFISH RETINAL AXONS *IN VITRO*
M.Bastmeyer, J.Vielmetter, M.Schwab and C.A.O.Stuermer, Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Tübingen, FRG and Brain Res. Inst. Zürich, Zürich, CH.

Mammalian oligodendrocytes and CNS myelin are non-permissive substrates for mammalian neurites (Schwab and Caroni , 1988). Responsible for the growth inhibiting effect are two proteins. They are absent from fish CNS myelin (Caroni and Schwab, 1988).

We tested whether goldfish retinal axons are sensitive to the growth inhibiting components of the mammalian myelin and oligodendrocytes. Using time-lapse videomicroscopy we monitored the contacts of goldfish retinal axons 1) with mammalian oligodendrocytes and 2) with myelin. Repeated contacts with myelin were provided by a patterned substrate. It consisted of laminin and rat or fish myelin in alternating stripes of 40-50μm width in its first half and of only myelin in its second half.

1. When fish axons encountered O1-positive rat oligodendrocytes 50% of the axons either degenerated, retracted, branched or curved around the cells. 2. On the patterned substrate of laminin and rat myelin, axons preferred the laminin stripes. When their growth cones met the myelin at the end of the stripes, axons degenerated, performed U-turns, or they reduced their velocity dramatically but kept their growth cones active. When goldfish instead of mammalian myelin was used axons crossed frequently over both substrates, grew onto and elongated on the myelin at the end of the stripes.

Thus, rat CNS myelin and oligodendrocytes are non-permissive substrates for goldfish retinal axons. Whether the avoidance response of fish axons is caused by the inhibitory proteins associated with mammalian myelin and oligodendrocytes will be decided in ongoing experiments.

184.11

DIRECTIONALLY SPECIFIC AXONAL REGENERATION IN THE ADULT LAMPREY SPINAL CORD.
D.I. Lurie* and M.E. Selzer. David Mahoney Inst. of Neurol. Sciences and Dept. of Neurology, U. of Penn., Sch. of Med., Phila., PA. 19104.

Larval sea lampreys recover from spinal transection by a process involving directionally specific axonal regeneration (Mackler et al., J. Neurosci., 6:1814, 1986). Is this also true of adult lampreys? Thirteen Petromyzon marinus adults were transected at the level of the 5th gill and allowed to recover for 10 wks. Muller and Mauthner axons were then impaled with microelectrodes and injected with HRP. Wholemounts of brain and spinal cord were prepared and regenerating neurites traced with camera lucida. Twelve animals recovered coordinated swimming. In these, 72 of 116 (62%) neurites from 25 axons regenerated beyond the scar. Of the neurites which had grown beyond the scar, 93% were correctly oriented, i.e. caudalward and ipsilateral to the parent axon. A 13th animal showed no behavioral recovery. Mechanical deformity prevented apposition of the cut ends of the cord and no axons regenerated across the site of injury. Retransection in 2 additional animals eliminated the recovered swimming. Thus, behavioral recovery in adult sea lampreys involves directionally specific axonal regeneration. (NIH grants NS14837 and NS25581).

184.12

LARGE-SCALE COMPUTER SIMULATIONS OF AXONAL GROWTH: PARAMETERS WHICH RESULT IN "TWO-CENTER EFFECTS" LIKE THOSE FOUND IN DRG CULTURES.
G.E.Schneider and W.F.Davis*. Dept. Brain & Cognitive Sciences, MIT, Cambridge, MA 02139; Davis Assoc., Newton, MA 02165. (Spon.: W.A. Rosenblith)

Results of computer simulations of the simultaneous growth of many axons following specified rules of development will be presented. The program allows the user great flexibility in defining boundaries, cell populations and densities and initial distributions, initial growth directions, rates of extension, and various parametrized reactions. Reactions used in this study included "wander" for specifying properties of non-rectilinear elongation, and "retraction" for specifying the consequences of contact between a growing axon tip and another axon. Simulations of up to 988 elongating axons were run. Output of each simulation was used to create plot files for producing displays of the results at specified simulated times after initiation of growth. Resulting displays were plotted, or were used to create motion-picture sequences of small time segments of selected simulations.

Initial parameters, and their statistical variability, for the wander and retraction reactions were chosen with the aid of data from published tissue culture studies. Two populations which follow identical growth rules were specified, representing a pair of explants. Neuronal densities, retraction probability, retraction distance (d) and the post-retraction growth-direction algorithm were varied. With sufficiently high densities of outgrowth, both major types of "2-center effects"--P. Weiss's fasciculated "attraction" effect and G. Dunn's non-fasciculated "repulsion effect"--were obtained by using different settings of d, e.g. 2.5 µm for "attraction" and 30 µm for "repulsion". (Fasciculation occurred with the smaller values of d without any rule to force parallel growth.) A critical role for this parameter has not previously been proposed. [Support: NSF grant BNS 85-16984; IBM.]

DOPAMINE RECEPTORS II

185.1

SUBSTRATE DEPENDENT INTERACTIONS WITH THE DOPAMINERGIC SYSTEM OF THE ERGOLINE FCE 23884.
Buonamici* M., Achilli* G., Caccia* C., Cervini* M.A., Pegrassi* L. and Rossi* A.C. Farmitalia Carlo Erba, R&D, C.N.S. Line, 20014 Nerviano, Italy.

FCE 23884, an ergoline derivative, was investigated for central dopaminergic activity in several animal models. In normal animals it displayed a variety of pharmacological effects reminiscent of dopamine antagonism i.e., selective suppression of avoidance responding in rats, dose-related blockade of amphetamine-induced toxicity in aggregated mice and prevention of apomorphine-induced stereotypic behaviour either in mice or rats.

In vitro binding studies of the compound showed its affinity for both D-1 and D-2 receptors (nM range) and in vivo experiments led to a marked increase of DA turnover and of serum prolactin levels. These results obtained in normal animals are suggestive of antagonistic effects to DA-ergic neurotransmission afforded by FCE 23884.

Conversely, FCE 23884 showed a strong agonist DA-ergic activity in two denervated animal models. In rats bearing unilateral nigrostriatal 6-hydroxydopamine lesions the compound induced dose-related contralateral turning (0.05-0.1 mg/kg s.c.).

In preliminary studies in non-human primates with severe MPTP-induced Parkinson-like syndrome, FCE 23884 completely reversed the akinesia and incoordination of movement (0.05-0.25 mg/kg s.c.). The compound FCE 23884 could be of therapeutic interest in Parkinson's disease due to its unusual ability to stimulate denervated supersensitive DA-receptors while depressing normosensitive ones.

185.2

A FULL REPETITIVE JAW MOVEMENT (RJM) RESPONSE WITH ONLY TWENTY PERCENT OF THE D_1 RECEPTORS.
H.Rosengarten , J.W.Schweitzer , A.J.Friedhoff. Department of Psychiatry Millhauser Laboratories,NYU School of Medicine,New York N.Y. 10016

We have previously demonstrated that repetitive jaw movements (RJM) in rats can be produced through activation of dopamine D_1 receptors with SKF 38393 in a dose dependent manner and facilitated through D_2 receptor blockade.These movements are antagonized by the specific D_1 antagonist SCH 23390 or by specific D_2 receptor stimulation.In the present study we have further explored quantitative relationships between D_1 receptor activation and RJM.The D1 receptors were inactivated with the irreversible antagonist EEDQ while the D_2 receptors were protected with the selective D_2 antagonist eticlopride . This treatment resulted 24 hours later in 78% destruction of the D_1 receptors and only 10% of the D_2 receptors in the rat caudate . EEDQ treated and control rats were tested for the frequency of SKF 38393-inducible RJM .Although only 22% of caudate D_1 receptors were present following exposure to EEDQ , a full RJM response was elicited by a high dose of SKF 38393 . From these findings it appears that there is a functional reserve in D_1 mediated RJM in rat.The implications of these findings will be discussed.

185.3

ROTATION INDUCED BY THE D2 AGONIST LY 171555 FOLLOWING UNILATERAL IRREVERSIBLE INACTIVATION OF STRIATAL DOPAMINE RECEPTORS BY N-ETHOXYCARBONYL-2-ETHOXY-1,2-DIHYDROQUINOLINE (EEDQ). O. Giorgi and G. Biggio. Department of Experimental Biology, Chair of Pharmacology, University of Cagliari, Italy.

A novel rotational model was used to study the interactions between D1 and D2 dopamine (DA) receptors. Male Sprague-Dawley rats (280-320 g) were unilaterally injected with the irreversible DA receptor blocker EEDQ into the head and body of the striatum (10 mmoles/ 1 μl / 3 min, x 2) and the effects of different agonists and antagonists for D1 and D2 DA receptors on circling behavior were evaluated 24 h thereafter. EEDQ treatment decreased the density of striatal D1 (-51%) and D2 (-46%) DA receptors labeled with ^{3}H-SCH 23390 and ^{3}H-Spiperone, respectively. LY 171555 (1 mg/Kg, i.p.) induced ipsilateral rotation (141 ± 11 turns/rat/15 min, N = 10) in animals with a 40 to 60 % decrease in the Bmax of striatal D1 and D2 DA receptors, but not in rats in which these receptors were reduced by 15 to 25 % after EEDQ. In contrast, SKF 38393 (10 mg/Kg, i.p.) failed to induce circling behavior. Rotations elicited by LY 171555 were antagonized (-88%) by L-sulpiride (100 mg/Kg, i.p.) and by SCH 23390 (-70% at 1 mg/Kg, i.p.). The inhibitory effect of SCH 23390 was reverted by SKF 38393 (40 mg/Kg, i.p.). These results indicate a permissive role of D1 DA receptors for the expression of D2 DA receptor-mediated effects and are consistent with the existence of a DA receptor reserve (i.e., "spare" receptors) in the rat striatum.

185.4

DIFFERENTIAL RECEPTOR RESERVE AT PRE-VS. POSTSYNAPTIC D2 DOPAMINE (DA) RECEPTORS ACCOUNTS FOR THE AUTORECEPTOR SELECTIVITY OF DA AGONISTS. E. Meller, A. Enz* and M. Goldstein. Dept. of Psychiatry, NYU Medical Center, New York, NY 10016 and Preclinical Research, Sandoz, Ltd., Basel, Switzerland.

We have recently demonstrated the presence of a large receptor reserve at DA autoreceptors in rat striatum mediating local negative feedback inhibition of neurotransmitter synthesis (Meller et al., Mol. Pharmacol. 31:592-598, 1987), and proposed that a differential receptor reserve at pre- vs. postsynaptic D2 receptors could account for the autoreceptor selectivity of DA agonists. We now report the results of studies examining the relationship between receptor occupancy and response at postsynaptic striatal D2 receptors regulating cholinergic activity.

N-Propylnorapomorphine (NPA) dose-dependently increased rat striatal ACh levels (ED_{50}=18 ug/kg). After irreversible DA receptor inactivation with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 6 mg/kg), neither the ED_{50} nor the slope of the dose-response curve for NPA was altered, whereas the maximal response was reduced to 56% of control. Analysis of the results yielded a linear relationship between receptor occupancy and response (i.e. no receptor reserve). The results support the hypothesis that a differential receptor reserve at pre- vs. postsynaptic DA receptors in rat striatum accounts for the autoreceptor selectivity of DA agonists. Supported in part by PHS grants NS 23618, MH 02717 and MH 35976.

185.5

DIFFERENT RECEPTOR RESERVE TO R(-)NPA FOR NIGRAL AND VTA DOPAMINE (DA) NEURONS. R.F. Cox and R.L. Waszczak. Pharmacol. Sect., Northeastern Univ., Boston, MA 02115.

Differences in autoreceptor density between nigral (SN) and VTA DA neurons were suggested by previous studies. To explore this possibility, i.v. dose-response curves (drc) were constructed for inhibition of SN and VTA DA cell firing by R(-)NPA before and after partial DA receptor inactivation with EEDQ (6 mg/kg in ethanol). For SN cells, EEDQ caused a 3-fold parallel rightward shift of the R(-)-NPA drc but no change in maximal response relative to vehicle-treated controls. For VTA cells, this EEDQ dose caused not only a greater (8.6-fold) drc shift, but also reduced maximal response by 25%. Furchgott analysis showed a steep, hyperbolic occupancy-response (O-R) relationship for R(-)NPA in SN, with 50% and full responses occurring at 3.5% and 28% occupancies. The O-R curve for VTA cells was less steep, with 50% and full responses at 10% and 78.5% occupancies. While these analyses reveal a 3-fold larger receptor reserve to i.v. R(-)NPA for SN than VTA cells (72% vs 21.5% reserve), K_A's were similar (7.7 and 5.5 ug/kg) indicating no difference in affinity between the regions.

In related ongoing studies, the same dose of EEDQ seems to cause greater declines in responsiveness to iontophoretically applied DA than were seen for i.v. NPA. Additional work will focus on whether this difference can be attributed to differing sizes of receptor pools available to i.v. vs iontophoresed agonists, to different intrinsic efficacies of DA and NPA, or both. (Supported by NS 23541)

185.6

D-1 SPECIFIC AGENTS MODULATE DOPAMINERGIC TERMINAL EXCITABILITY. M. Diana, S.J. Young* & P.M. Groves. Dept. of Psychiatry , U.C.S.D., La Jolla, 92093.

Recent studies provide evidence for D1 receptors on dopamine neurons within substantia nigra pars compacta suggesting that these receptors may also be present on dopaminergic axon terminals in the neostriatum. We have examined the action of locally applied D1 specific drugs on the terminal excitability of dopaminergic terminals in the neostriatum.

Infusion cannulae and an adjacent bipolar stimulating electrode were implanted in the neostriatum or medial forebrain bundle(MFB) of urethane anesthetized rats. Terminal excitability was assessed in terms of the current neccessary to evoke an antidromic response from nigral dopaminergic neurons before and after drug infusion.

Striatal infusion (300nl., 5min.) of 10uM R-SKF38393, a D1 specific agonist, produced a decrease in excitability which was reversed by subsequent infusion (300nl., 5min) of 10uM SCH23390, a D1 specific antagonist. These effects did not depend on D1 receptors on striatal neurons as they occurred following destruction of these cells with kainate. Since infusions of SCH into the MFB produced a slight decrease in excitability of MFB axons while SKF produced no change, a nonspecific axonal effect can be excluded. Experiments involving dopamine depletion will also be presented. The results are consistent with the possibility that D1 receptors exist on dopaminergic terminals in the striatum. (Supported by NIDA grants DA 02845 and RSA DA 00079.)

185.7

D_1 DOPAMINE RECEPTORS IN FRONTAL CORTEX OF NON-HUMAN PRIMATES. B.K. Madras, C.E. Pfaelzer*, M. Fahey*, D. Canfield*, R.D. Spealman. Harvard Medical School and New England Regional Primate Research Center, Southborough, MA 01772.

In order to compare D_1 dopamine receptors in frontal cortex with caudate-putamen (Madras et al. 1988), we characterized D_1 dopamine receptors in washed frontal cortex membranes of non-human primates (Macaca fascicularis). D_1 receptors were labeled with [^{3}H]-SCH 23390 using (S)-butaclamol (1 μM) to define non-specific binding. Membranes bound [^{3}H]-SCH 23390 with high affinity (Kd: .41±.076 nM). The density of these sites was 7.8±2.3 pmoles/g (n=4) which is 25-30% of the density detectable in monkey striatum. Na^+ ions (120 nM) increased receptor affinity 2.7-fold and decreased apparent density by 45%. In striatum only affinity was affected by Na^+. A serotonergic component of [^{3}H]-SCH 23390 binding was suggested by the shallow displacement curves of ketanserin and cinanserin which were resolvable into two sites; the high affinity site represented approx. 25% of the sites. Dopamine receptor agonist and antagonist binding affinities were similar to those found in striatum. These results indicate that [^{3}H]-SCH 23390 labels primarily, but not exclusively, D_1 dopamine receptors in monkey frontal cortex. Supported by USPHS Grants DA00499, DA03774 and RR00168.

185.8

ADENYLATE CYCLASE CHANGES IN CAUDATE OF 6-OHDA-LESIONED RATS. L.L. Watkins,* H.E. Criswell, R.A. Mueller and G.R. Breese. UNC Sch. Med., Chapel Hill, NC 27599.

Adult rats treated neonatally with 6-OHDA (n.6OH) exhibit hyperlocomotion following systemic administration of the D_1-DA receptor agonist SKF 38393 (JPET 234:447, 1985). This investigation examined whether changes in caudate adenylate cyclase activity (ACA) accompany this 6-OHDA treatment. Crude homogenates from caudate nucleus of control and n.6OH rats were incubated with D_1-DA agonists (SKF 38393 or SKF 82526) or a D_2-DA agonist (RU 24213) in the absence or presence of forskolin or NaF. Caudate homogenates from n.6OH rats exhibited no change in ACA to the D_1-DA agonists, but showed increased inhibition of ACA to the D_2-DA agonist. Forskolin (20 μM) and NaF (10mM) stimulation of AC was 20 and 40% lower, respectively, in n.6OH rats than in controls. Forskolin showed a biphasic effect on receptor-mediated changes in caudate ACA in control rats. Low forskolin (.01 to 5 μM) facilitated D_1-DA agonist stimulation of ACA and high forskolin (10 to 50 μM) facilitated D_2-DA agonist inhibition of ACA. In caudate of n.6OH rats, forskolin enhanced only D_2-DA agonist inhibition of ACA. The decreased responsiveness to AC stimulation by forskolin and NaF, the failure of forskolin to enhance D_1-DA receptor-mediated ACA, and the increased inhibition of ACA by D_2-DA agonists provides evidence that D_2-DA, but not D_1-DA receptors linked to AC, are hyperresponsive in n.6OH rats. (Supported by NS-21345 and HD 23042.)

185.9

17β-Estradiol Regulation of DA Receptor Interactions with G-proteins. Robert E. Hruska. Univ. Buffalo, NY*

17β-Estradiol produces a variety of pre-synaptic and post-synaptic effects on the nigro-striatal DA system. These effects include an increase in the densities (without affinity changes) of the D1 and D2 DA receptors by a process which appears to be located post-synaptically and be time-dependent and dose-related. While these actions have been demonstrated biochemically and behaviorally after in vivo administration, no direct in vitro effects of 17β-estradiol have been demonstrated on these receptors unless high μM concentrations are used. This report demonstrates that nM concentrations of 17β-estradiol in vitro alter the GTP-dependent shift of agonist displacement curves at the D2 DA receptors isolated from rat striatal tissue. GTP produces a well-known shift of the DA displacement curve to lower affinity. At a concentration of 1.0 nM, 17β-estradiol prevents the GTP shift. This action is concentration dependent (half-maximal effect at 0.1 nM), stereospecific (1000-fold greater concentration of 17α-estradiol required), and limited to the D2 subtype (the GTP shift at the D1 subtype was not altered). These results suggest that 17β-estradiol can act directly and rapidly on the interaction of D2 DA receptors with the specific Gi-protein. This is observed in vitro at nM concentrations and suggests that this could occur in vivo at physiological concentrations.

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185.10

DOPAMINE D_2 RECEPTOR BINDING SUBUNITS: DIFFERENCES IN GLYCOSYLATION ACCOUNT FOR THE EXISTENCE OF HIGH MOLECULAR WEIGHT FORMS. K.R. Jarvie, H.B. Niznik, and P. Seeman. **Dept. Pharmacol., Univ. of Toronto, Toronto, Canada, M5S 1A8.**

Dopamine D_2 receptor binding subunits can be photoaffinity labeled with [^{125}I]NAPS and visualized autoradiographically following sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). [^{125}I]NAPS has been shown, in several species, to incorporate into a band of Mr=94,000. In contrast, in porcine striatal homogenates, [^{125}I]NAPS labels a protein with an estimated molecular mass greater then that previously observed. In porcine striatal homogenates, [^{125}I]NAPS labels two bands (Mr=140,000 and 94,000). Exoglycosidase treatment (neuraminidase) caused the two labeled bands to migrate as one band with an increased mobility (Mr=51,000). Complete N-linked deglycosylation with Glycopeptidase F (PNGaseF) resulted in a shift in the labeled band to a Mr=44,000. This value coincides with the estimate for the molecular mass of the PNGaseF-treated D_2 binding subunit of canine brain. These data indicate that variable glycosylation may account for the observed differences in the mobility of the dopamine D_2 receptor binding subunits on SDS-PAGE gels.

This work was supported by the Medical Research Council of Canada (MRC) and the Ontario Mental Health Foundation (OMHF). KRJ is a recipient of an OMHF Studentship. HBN is a recipient of a MRC Post-Doctoral Fellowship.

185.11

STRUCTURAL ANOMALIES IN THE D_2 RECEPTOR COMPLEX OF DOPAMINE-RESISTANT, PROLACTIN-SECRETING RAT PITUITARY TUMORS. C. Bouvier*, G. Lagacé* and R. Collu. **Research Unit on Biology of Reproduction and Development, Pediatric Research Center, Hôpital Ste-Justine and Université de Montréal, Montréal, Québec, Canada, H3T 1C5**

The structure of the dopamine (DA) D_2 receptor complex of DA-resistant, and prolactin (PRL)-secreting rat pituitary tumors 7315a and MtTW15 was evaluated to determine the reason for tumors insensitivity to DA inhibitory action on PRL release and tumor growth. The size of the receptor complex in active form, as determined by radiation inactivation (RIS) after preincubation with the DA agonist NPA, was found to be abnormally large in the two tumors (RIS, kDa; Normal, 140; 7315a, 210; MtTW15, 280). The GTP-binding protein G_o-α was found to be greatly decreased or absent in the two tumors, both by immunoblot and by pertussis toxin (PT)-induced [^{32}P] ADP-ribosylation. On the other hand, the binding unit of the tumors receptor was found, by photoaffinity labeling with [^{125}I]-N_a-NAPS with or without pretreatment with the alkylating agent N-ethyl-maleimide (NEM), to be normal and to contain SH- groups essential for ligand binding. These groups were found also to be essential for G-protein ADP-ribosylation in normal and tumoral preparations. These data suggest that tumors insensitivity to DA may be due to failure of transmission of the inhibitory message after agonist binding because of multiple anomalies in the receptor complex.

POTASSIUM CHANNELS I

186.1

FOUR cDNA CLONES FROM THE SHAKER LOCUS OF DROSOPHILA INDUCE KINETICALLY DISTINCT A-TYPE POTASSIUM CHANNELS IN XENOPUS OOCYTES. Leslie. C. Timpe, Yuh Nung Jan & Lily Y. Jan, Howard Hughes Med. Inst. and Dept. of Physiol., UCSF, San Francisco, CA 94143-0724.

Genetic evidence suggests that the Shaker gene of D. melanogaster encodes the A channel of larval and pupal muscle, or one of its subunits. At least four distinct messages derive from the Shaker locus by alternative splicing. We have previously shown that two cDNA clones, ShA and ShB, direct the synthesis of active A channels when expressed in Xenopus oocytes. Two additional clones have now been expressed. Specific messenger RNA was synthesized in vitro, injected into oocytes, and the ionic currents were examined with a two electrode voltage clamp. Oocytes injected with either ShC or ShD mRNA have voltage-dependent , transient, outward currents. The ShÇ currents recover very slowly from inactivation at -80 mV. The ShD currents, in contrast, resemble the ShB currents: they recover from inactivation within seconds at -80 mV. The inactivation kinetics of ShB and ShD are not identical, however. The ShD current inactivates more slowly at positive potentials, and recovers from inactivation more slowly at negative potentials, than does ShB. Comparison of the sequences of the four predicted proteins indicates that the large difference in rate of recovery from inactivation between ShA and ShC versus ShB and ShD is due to amino acid differences at the carboxyl terminal. The subtler differences between ShB and ShD result from sequences at the amino terminal. Although it is not known whether the A currents expressed in oocytes accurately reflect the properties of fly A currents, these results indicate that the products of the Shaker locus may be components of several kinetically distinct A channels in flies.

186.2

IMMUNOCYTOCHEMISTRY AND WESTERN -BLOT ANALYSIS OF A-CHANNELS IN DROSOPHILA.

T.L. Schwarz, D.M. Papazian, R.P. Carretto, Y.N. Jan & L.Y. Jan. Howard Hughes Med. Inst., University of California, San Francisco, CA 94143-0724.

Although the Shaker locus, a gene that encodes a family of alternatively spliced potassium channels, has been cloned, little about the protein products is known. Antisera were raised to a protein produced in E. coli by the fusion of a beta-galactosidase gene with a portion of a cDNA (ShA1) . The antigen included residues from a hydrophilic region that is found in all of the predicted variant products of the Shaker locus. On Western blots, the antisera recognized a broad band (M r = 70 - 80 kD) that is consistent with the predicted sizes of ShA, ShB, ShC, and ShD (64 - 74 kD) with the posssibility of some glycosylation. This protein was enriched in a membrane preparation. On sections of fly brains, the sera stained regions of the neuropil in wild-type flies, but not in a mutant that lacks Shaker products. The darkest staining was in synaptic regions and it was not evenly distributed; cell types appear to differ in the levels to which they express these channels. Within the optic lobe, for example, the lobular plate and the margin of the medulla and lobula where they border on the secondary optic chiasm contained particularly densely stained regions. Major axon tracts, such as that in the neck or the optic chiasms showed some staining, but were generally less densely stained then synaptic regions. Electron microscopy may reveal if there is a concentration of the channel in terminals or dendrites.

186.3

DIVERSE TRANSIENT K^+ CURRENTS IN DROSOPHILA NEURONS DELETED FOR THE SHAKER LOCUS PREDICT OTHER GENES HOMOLOGOUS TO SHAKER. K.H. Baker*, L. Salkoff. Dept. of Anatomy and Neurobiology, Wash. U. Sch. of Med., St. Louis, MO. 63108.

Diversity in transient K^+ currents as generated by differential splicing at the Shaker locus appears extensive (Schwarz et al. Nature 331, Pongs et al. EMBO 7, Kamb et al. in press). Apart from the diversity generated by Shaker, we have direct physiological evidence for a diversity of transient K^+ currents encoded by at least one other locus. Molecular evidence is also suggested by a cDNA clone with homology to Shaker cDNA's (Butler et al. Soc. Neurosci. Abst. This issue). In flies whose Shaker locus is deleted (B55/W32) we have characterized at least three types of transient K^+ currents in neurons using the whole-cell voltage clamp. The classes differ with respect to rates of inactivation and recovery from inactivation. In flies deleted for the Shaker locus approximately 60% of cells have transient K^+ currents. In wild-type, a significantly higher percentage had transient K^+ currents indicating that Shaker gene products are expressed in these cells.

186.4

A DROSOPHILA ION CHANNEL ON CHROMOSOME 3 IS SIMILAR TO THE SHAKER POTASSIUM CHANNEL ON CHROMOSOME 1. A. Butler*, A. Wei, N. Scavarda*, and L. Salkoff. Dept. Anatomy and Neurobiology, Wash. U. Sch. Med., St. Louis, MO. 63110.

Based on observations that a multitude of fast transient K^+ currents exist in Drosophila which are not coded by the Shaker locus, (Baker & Salkoff, Soc. Neurosci. Abst., this issue), we postulated the existence of other Drosophila genes homologous to the Shaker potassium channel. We report here the existence of such a gene on chromosome 3L in region 62-63. The gene encodes an ion channel protein containing homology to the gating charge region of both the Shaker K^+ channel and the Drosophila Na^+ channel. However, the overall similarity is closer to the Shaker protein; membrane spanning regions have an average of 50% identity and most changes are conservative. The low DNA sequence homology between these genes in regions of amino acid identity suggests that they are derived from an ancient ancestral form. The different chromosomal locations also supports the hypothesis of ancient divergence.

186.5

GENES CONTROLLING SYNAPTIC TRANSMISSION IN *DROSOPHILA*. M. Stern* and B. Ganetzky (SPON: G. Robertson) Laboratory of Genetics, University of Wisconsin, Madison, WI 53706

One way to identify genes that control ion channel activity and synaptic transmission in the *Drosophila* neuromuscular system is by their interactions in double mutant combination with other ion channel mutations. The *Shaker (Sh)* gene encodes the rapidly inactivating potassium A channel (Tempel et al, *Science* 237: 770, 1987). Mutations in *Sh* interact with several other genes affecting ion channels. Therefore we have screened for mutations that interact with *Sh*. Five mutations, which define at least three genes, each confer reduced viability to *Sh* mutants. Two of these mutants (2-19 and 2-68) display extremely prolonged dorsal longitudinal muscle (DLM) responses to giant fiber stimulation. In all five mutants, single stimulations of larval motor neurons evoke multiple responses. This phenotype resembles that of *eag* mutants (Ganetzky and Wu, *J. Neurogenet* 1:17, 1983). In addition, the 2-60 mutation shows a striking increase in synaptic transmission in combination with *eag*. Possible involvement of these genes on potassium channels is being investigated.

We have also studied the induction of long term facilitation at the larval neuromuscular junction. We have found that *Hyperkinetic (Hk)* mutants exhibit an abnormally rapid induction of long term facilitation. Results from *Hk Sh* double mutants suggest that *Hk* mutations exert their effects on synaptic transmission via I_A, the current affected by *Sh* mutations. Supported by Research Grant NS15390 from the National Institutes of Health, a Klingenstein Fellowship, and postdoctoral fellowships from the NIH and Damon Runyon Walter Winchell Cancer Research Fund.

186.6

CLONING & EXPRESSION OF A PROBABLE POTASSIUM CHANNEL GENE FROM MOUSE BRAIN. B.L Tempel, J. Urban*, D.M. Dorsa*, L.C. Timpe, Y.N. Jan & L.Y. Jan. Howard Hughes Med. Inst. & Depts. of Physiol. & Biochem. Univ. of Calif., San Francisco, CA 94143-0724. *Depts. of Pharmacology & Med., U. of Washington & VA Med. Ctr., 1660 S. Columbia Wy, Seattle, WA 98108.

Potassium channels are a diverse class of ion channels that are important for neuronal excitability and plasticity. The recent cloning of the Shaker locus fron Drosophila has provided a starting point for molecular studies of potassium channels. Predicted Shaker proteins appear to be integral membrane proteins and have a sequence similar to the S4 sequence, which has been proposed to be the voltage sensor in the vertebrate sodium channel. Expression studies in frog oocytes confirm that Shaker encodes a component of a potassium channel (the A channel) that conducts a fast transient potassium current.

Using a probe derived from one of the Shaker cDNA, we have recently isolated clones from the mouse brain that predict a protein which is 65% identical to the predicted Shaker protein. In two stretches of over 50 amino acids the mouse and Shaker proteins are over 95% identical; in the S4-like region they are 100% identical. Because of their remarkable similarity, it is likely that these mouse brain cDNA clones also encode a component of a potassium channel.

In spite of their strong protein homology, the nucleotide sequences of the fly and mouse clones are quite different. For example, in the identical S4-like regions, 77% of those nucleotides that could change (based on codon redundency) are in fact different between fly and mouse. This high level of nucleotide divergence suggests that strong selective pressure has maintained functionally important portions of the proteins. In this light, we observe that the S4-like and predicted membrane-spanning regions are well conserved. In addition, the hydrophillic amino-terminal region preceeding the hydrophobic domain is highly conserved although its functional role is not known.

We are currently studying the expression of this gene in frog oocytes, using Northern analysis of various tissues and by in situ hybridization against sections of the mammalian brain.

186.7

CLONING OF A SHAKER POTASSIUM CHANNEL HOMOLOGUE FROM RAT BRAIN. L.K.Kaczmarek, M.B.Boyle, E.Blumenthal* and J.Marshall*. Dept. Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510 and Unit of Mol. Neurobiol. MRC, Cambridge, U.K.

Tempel et al. (Science **237**:770, 1987) have recently cloned the gene for a *Drosophila* channel that generates the transient A-current, and which is encoded by the Shaker locus. We have synthesized oligonucleotides derived from two different regions (S4 and H4) of the *Drosophila* channel. Northern analysis of 12-day rat brain poly-A^+ RNA showed that these probes bound to RNA species of approximately 6 kB. We have found that when such RNA is fractionated on a sucrose gradient and injected into *Xenopus* oocytes, fractions containing this size RNA cause the expression of the transient A-current in the oocyte.

A size-selected cDNA library was constructed from 12 day rat brain RNA in the vector gt10 and screened with a mixture of the two labelled probes. In a screen of 240000 independent clones, two positive clones were identified and purified. One of these (K41) was found to bind both probes independently and has been partially sequenced. The regions for which sequence has been obtained are highly homologous to the Shaker sequence (59% homology in nucleotides; 66% homology in predicted amino acid sequence). The 5' end of the K41 insert matches the 5' end of the H3 segment of the Shaker sequence. These results suggest that K41 represents a partial clone of the sequence encoding a transient A-potassium channel in rat brain. Hybrid selection and depletion experiments are in progress to test this hypothesis.

186.8

DIFFERENTIATION OF I_{KA} IN AMPHIBIAN SPINAL NEURONS. A.B. Ribera, C.R. Kintner, B.L. Tempel and N.C. Spitzer. Department of Biology, UCSD; The Salk Institute; Department of Physiology, UCSF.

Embryonic *Xenopus* spinal neurons exhibit voltage-dependent Ca, Na, K and Ca-dependent K currents at the time of primary neurite extension early during the first day in culture (O'Dowd et al., 1988). By the second day *in vitro*, all neurons express a third potassium current. This current is activated rapidly, inactivates during a voltage step and at depolarized holding potentials, is sensitive to mM concentrations of 4-aminopyridine, and thus resembles "A" currents of other neurons. The inactivation of this current during a depolarizing voltage step becomes more rapid during the second day. Neurons at both 1 and 2 days exhibit a relatively slow component of inactivation with a time constant of ~40 msec. However, by 2 days, a faster component with a time constant of 9 msec is apparent.

An A current is expressed in *Drosophila* nerve and muscle cells, and both the gene and trancsripts encoding this ion channel have been elucidated (Tempel et al., 1987). A *Xenopus* genomic library was screened for sequences with identity to a cDNA (ShA1) from the fly. The nucleotide sequence of one isolated clone can be aligned with the *Drosophila* sequence over the regions of 4 putative transmembrane domains: S4, H4, H5 and H6. Over a stretch of 390 nucleotides there is a 66% identity; if the analysis is restricted to the H4 segment, the identity is 80%. At the conceptual amino acid level, 79% identity is observed over 130 amino acids. Similar comparison with the mouse sequence (MBK1; Tempel et al., 1988) indicates that over the same region there are 72% and 90% identities at the nucleotide and amino acid levels, respectively. Northern blot analyses, RNase protection assays and *in situ* hybridizations using probes from this genomic clone (XSha) are likely to provide information about the molecular bases for developmental expression of this current as well as the changes in its functional properties.

186.9

MOLECULAR CHARACTERIZATION OF AN ESTROGEN-INDUCIBLE K CHANNEL mRNA FROM RAT MYOMETRIUM. M.B. Boyle, S. Cooperman*, J.S. Trimmer*, N. J. MacLusky, F. Naftolin*, and L.K. Kaczmarek. Depts. Pharmacol., Cell. Molec. Physiol. and Ob. Gynecol., Yale Univ. Sch. Med, New Haven, CT 06510 and Dept. Molec. Med., Tufts-NE Med Center, Boston, MA 02111.

We have previously reported the apparent induction by estrogen of a rat myometrial mRNA species capable of causing expression of a very slowly activating K current in *Xenopus* oocytes. We hypothesize that this mRNA species encodes a K channel whose level of expression varies during the estrous cycle and pregnancy. We have now subjected polyA+ uterine RNA from estrogen-treated animals to size-fractionation on a sucrose gradient. RNA fractions migrating near the 18S ribosomal RNA yielded expression of the slowly activating K current when injected into *Xenopus* oocytes. Using 0.8 µg of this size-selected RNA, we constructed a directional cDNA library consisting of 3 million recombinants in Lambda GEM 4 (Promega). The library was screened by injecting oocytes with sense cRNA made using 8 pools of 20,000 recombinants apiece. One pool yielded RNA capable of inducing the slowly activating K current in oocytes, implying that it contains a cDNA encoding a functional protein. We are now attempting to isolate this clone.

186.10

CHARACTERIZATION AND SOLUBILIZATION OF RAT BRAIN DENDROTOXIN RECEPTORS ASSOCIATED WITH POTASSIUM CHANNELS. R. G. Sorensen. Dept. Physiol., University of Maryland School of Medicine, Baltimore, MD. 21201.

The venom of the green mamba, Dendroaspis angusticeps, contains components (dendrotoxins, DaTx) that block brain voltage-dependent K channels. Two of these components, α-DaTx (selective for a rapidly-inactivating K channel) and β-DaTx (selective for a non-inactivating K channel), have been radioiodinated, and used in ligand binding studies and for receptor solubilization.

Monovalent and divalent cations inhibit [^{125}I]α-DaTx and [^{125}I]β-DaTx binding. The order of potency of monovalent cations for inhibiting the binding of both radiolabelled toxins is: Cs >> Rb > Li > K > Na. Only Cs inhibited toxin binding by 100% (IC_{50} = 5 mM). Dixon Plot analysis indicates that Cs is a non-competitive inhibitor. Of the divalent cations tested, Ba (IC_{50} = 5 mM) was more potent than Ca (IC_{50} = 11 mM). Because Cs and Ba block K channels, the ability of these ions to also inhibit [^{125}I]α-DaTx and [^{125}I]β-DaTx binding suggests that the toxin binding sites are at or near the ion binding site(s) of the channel.

Synaptic membranes were equilibrated with [^{125}I]α-DaTx, and the receptor solubilized as a toxin-bound complex. Approximately 75% of the specifically-bound radioactivity was solubilized, and 33% of the soluble radioactivity remained bound to the receptor. There was little change in the apparent K_D for toxin binding to the soluble receptor.

186.11

Neurotoxic action of β-Bungarotoxin may involve K channel blockade. Christina G. Benishin, Dept. of Physiology, Univ. of Alberta, Fac. of Med.,Edmonton, Alberta T6G 2H7

β-Bungarotoxin (β-BTX) isolated from the venom of Bungarus multicinctus has been previously reported to be a neurotoxic protein. The phospholipase A_2 (PL) activity of β-BTX may account for some, but not all of the neurotoxic actions. Because of the structural similarities between a portion of β-BTX, and other snake toxins reported to block K channels (Benishin et al. Fed. Proc, 46:504, 1987), β-BTX was examined for its ability to block 86Rb efflux from synaptosomes, as described earlier (Bartschat & Blaustein, J.Physiol. 361:419, 1985). β-BTX, when preincubated with synaptosomes for 30 min in the absence of Ca2+, selectively inhibited the slowly-inactivating, voltage-dependent (S) component of 86Rb efflux. The EC50 for inhibition is 3 to 5 nM. Inactivation of the PL activity with p-bromophenacylbromide does not effect the ability of β-BTX to block the 86Rb efflux, suggesting that inhibition is not associated with PL activity. Reduction and carboxymethylation of disulfide bonds dissociated the A and B chains of β-BTX. K channel inhibition is associated with the B (smaller) chain. These results suggest that the neurotoxic action of β-BTX may involve K channel blockade. Additionally, the active fragment of β-BTX may prove to be a useful tool with which to further examine functions of synaptosomal K channels. (Supported by AHFMR Scholarship)

VISUAL CORTEX III

187.1

RED/GREEN OPPONENT-COLOR INPUT TO MOTION AT LOW SPATIAL FREQUENCIES. P. Cavanagh* and S. M. Anstis* (SPON: M. Ptito) Département de Psychologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7 and York University.

The contribution of color to motion was estimated by determining the contrast of a drifting luminance grating that could just null the motion of a superimposed color grating drifting in the opposite direction. Our results showed that color contributed to motion only for very low spatial frequencies, specifically only for the range of spatial frequencies for which chromatic aberration does not generate a suprathreshold luminance artifact in uncorrected viewing (less than 2 cpd). Above this range chromatic aberration ensures that there will always be some response to a chromatic grating in a luminance pathway. The color contribution to motion below 2 cpd therefore fills a functional role in providing a response to stimuli for which a luminance-based motion system would be otherwise unresponsive. The contribution of color to motion also varied substantially as a function of chromatic axis. As much as 20% luminance contrast was necessary to null the motion of chromatic gratings modulated along the red/green opponent-color axis but negligible contrast was necessary for those modulated along the tritanopic axis. Additional tests showed that the contribution of color to motion could not be attributed to a residual response in a luminance pathway resulting from retinal inhomogeneity, response nonlinearities, phase lag, chromatic aberration or interunit variation of equiluminance points. We conclude that there is a substantial contribution of red/green, opponent-color units to the motion pathway and therefore that the separation between magnocellular and parvocellular streams that is clear at early levels of the visual system becomes less so at higher levels.

187.2

THE ROLE OF THE COLOR-OPPONENT (C-O) AND BROAD-BAND (B-B) CHANNELS IN VISION. **Peter H. Schiller, Nikos K. Logothetis and Eliot R. Charles, Massachusetts Institute of Technology, Cambridge, MA 02139.**

Localized ibotentic acid lesions were made in either the parvocellular (PLGN) or magnocellular (MLGN) portions of the monkey LGN to disrupt either the C-O or B-B channels in a small region of the visual field. The consequences of these lesions were examined for a range of visual functions which included color and luminance contrast sensitivity, color, pattern, shape, texture and brightness discrimination, stereopsis and movement perception. The paradigm we used confined stimuli to either the normal or lesioned representations of the visual field: the stimuli were presented on a color monitor, and each trial began with the onset of a central fixation spot, which when properly foveated, was followed by stimuli appearing in either the intact or lesioned portions of the visual field. Foveation of the correct stimulus with a single saccade was rewarded with drops of apple juice. Catch trials minimized guessing. Eye position was monitored with a scleral search coil. PLGN lesions devastated color and pattern discrimination abilities as well as stereopsis, and had moderate to no effects on the detection of low spatial frequency stationary or moving stimuli and on coarse shape perception. MLGN lesions produced deficits in movement perception but had little or no effect on stereopsis and form vision. These findings will be discussed in relation to current ideas about the role of the C-O and B-B pathways in vision.

187.3

CLOSE CORRELATION OF COLOR, ORIENTATION AND LUMINANCE PROCESSING IN V1,V2, AND V4 OF THE BEHAVING MACAQUE MONKEY. T. Yoshioka*, B. M. Dow and R. G. Vautin*. Dept of Physiology, Sch. of Med., SUNY/Buffalo, NY 14226.

A recent report on the primary visual cortex (V1) of the macaque monkey suggests the segregation of non-oriented and oriented zones for the processing of endspectral colors (i.e., blue and red) and midspectral colors (i.e., yellow and green), respectively (Dow and Vautin, J. Neurophysiol. 57: 712, 1987). The phenomenon was further examined in the present study by adding luminance as a third variable, and by recording from cells in areas V2 and V4 as well as V1. Each cell was tested with its preferred stimulus color and shape presented on a color video screen (Chromatics) at a variety of luminance levels (both positive and negative) with respect to an intermediate gray background. The data from all 3 areas (400 well-categorized cells) indicate a strong correlation between hue, luminance, and orientation selectivity, such that cells with strongly positive luminance preferences tend to be orientation selective and to prefer midspectral wavelengths, while cells with preferences for lower (but still positive) luminances tend to be orientation unselective and to prefer endspectral wavelengths. The unoriented endspectral and oriented midspectral systems may be associated, in area V1, with the cytochrome oxidase blobs and interblobs, respectively. Supported by NIH EY02349.

187.4

UNCONFOUNDING ORIENTATION AND DIRECTION COMPONENTS IN THE VISUAL NEURON'S RESPONSE J.Zhang* and R.L.DeValois* (SPON:D.G.Albrecht) Neurobiology Group, University of California, Berkeley, CA 94720

Using moving bars or gratings as stimuli, information on orientation (which has a period of 180°) and direction (which has a period of 360°) are inherently confounded in the response of visual cortical neurons which are known to be selective for both orientation and direction. In an attempt to unconfound these two components, Wörgötter and Eysel (Biol.Cybern.57(1987),349-355) proposed to use (but incorrectly formulated) the Fourier transform technique. Here we formally develop and extend the technique to obtain from the response curve the peak and bandwidth of each underlying component and their relative strengths. We computed the coefficients of 1-D Fourier expansion of the response along a full cycle of 360° and thus transformed the N data points of equal sampling spacing into N/2 order of sine/cosine-term coefficients. The odd harmonics contain only the direction component, while the even harmonics are contributed to by both orientation and direction components, provided that the two components are linearly additive. Under this linear assumption, it can be shown mathematically that the phase and amplitude of all the harmonics are related, respectively, to the peak angle and bandwidth of the individual components. We tested this with 38 cells in monkey V1 collected and presented earlier, and found that more than half of the population follow this assumption strikingly well.

187.5

COMPUTING OPTICAL FLOW IN THE PRIMATE'S VISUAL SYSTEM: A NETWORK MODEL. **C. Koch, T.-C. Wang* and B. Mathur*, Division of Biology, 216-76, Caltech, Pasadena, CA 91125 and Science Center, Rockwell Intl., Thousand Oaks, CA 91360.**

Computing motion on the basis of the time-varying intensity brightness is an important but difficult problem for both artificial intelligence and biological vision. We here map a classical computer vision motion algorithm (Horn & Shunck, 1981) onto a primate's visual system. The aperture problem is solved by a smoothness constraint: the final optical flow should be 1. compatible with the measured data and 2. the smoothest in a certain sense. Direction sensitive cells in V1 use the time derivative of the retinal image convolved with a Difference-of-Gaussians receptive field to compute local velocity estimates (component selectivity). Sixteen cells at each location code for motion in 16 different directions. In a second stage, assumed to be located in the deep layers of area MT, the smoothness constraint is used to compute the smoothest optical flow. The resulting network was implemented using simple threshold neurons and a population coding scheme: the final optical flow is obtained by a weighted average of the vector contributions from the 16 cells coding for different directions of motion in each location.

If a plaid pattern, consisting of two gratings moving at right angles to each other, is projected onto the retina, cells in the model MT respond to the resultant coherent motion (pattern selectivity), in agreement with psychophysical and electrophysiological studies (Movshon, Adelson, Gizzi & Newsome, 1985). Our system also displays motion capture (Ramachandran & Anstis, 1985): the motion of random dots is perceptually captured by a nearby moving grating. Depending on stimulus conditions, our MT cells can respond to motion both perpendicular and parallel to the moving contour, suggesting that the type I and type II cells reported by Albright (1984) in MT represent a continuum of cells.

187.6

ILLUSIONS OF A PARALLEL MOTION ALGORITHM. H. Bülthoff, J. Little* and T. Poggio. Center for Biological Information Processing and Artificial Intelligence Laboratory, MIT, NE43, Cambridge, MA 02139

Computational theory can tell us that in general it is not possible to recover the correct 2-D projection of the true 3-D velocity from changing images. This holds not only for computer vision algorithms but also for biological motion systems. In human psychophysics many different illusions demonstrate this fact quite dramatically. It is therefore not surprising that artificial vision systems are not free of such movement illusions. A new parallel motion algorithm that we have recently developed suffers from several movement illusions (eg., barber pole illusion, motion capture and a psychophysical observation described recently by Nakayama and Silverman (Vis. Res. in press; 1988). Our algorithm consists of patchwise correlation between image features (such as the filtered brightness data or intensity edges) and then choice of the motion corresponding to the peak of the correlation over the patch around the pixel under consideration. The algorithm has been implemented on the Connection Machine by using a regularization technique which exploits the simple assumption that the optical flow is locally uniform. We propose a physiological equivalent to our "Vote for Motion" scheme which is based on a layered structure of neurons. The comparison between the different "frames" (corresponding to the product in the correlation function) takes place in different layers with progressively larger sampling bases (V1). The voting step (corresponding to the patchwise sum in the correlation function) is performed by cells with a larger receptive field that summate neurons in V1 within a patch (supposedly in Area MT). This model is consistent with psychophysical (Adelson and Bergen; 1982) and physiological data in primate visual cortical areas V1 and MT (Movshon *et al.*, 1985).

187.7

A MOVING TEXTURED BACKGROUND MODULATES THE DIRECTION SELECTIVITY OF MT NEURONS IN MACAQUE MONKEY. L. Lagae*, B. Gulyás, S. Raiguel* and G.A. Orban. Lab. Neuro- en Psychofysiologie, K.U.Leuven, Leuven, Belgium.

The modulation of the response to a narrow moving bar by a moving textured background was investigated in the middle temporal (MT, V5) and surrounding visual areas of anesthetized and paralyzed cynomolgus monkeys. The interaction series was run with the bar moving at optimal speed in either direction on the optimal axis and 7 background conditions, including no background motion and motion in either direction on the optimal axis at three speeds : same speed as the bar, two octaves slower and two octaves faster than the bar.

MT cells are strongly influenced by background motion. Their response to the moving bar is suppressed to less than 50% of their control response and the direction selectivity of most of them is modulated by the background motion. Most MT cells exhibited an antiphase conditional direction selectivity : these cells remain direction selective only when the background moves opposite to the preferred direction for the bar. The background moving in the preferred direction of the bar suppresses the response to the bar. In the areas surrounding MT, cells where less modulated by the background motion and those cells influenced were nondirection-selective cells which preferred the bar directions opposite to the background motion (antiphase cells).

The results in MT are in striking contrast to those obtained with similar methods in V1 and V2 of the monkey : only 12% and 38% of the cells have their direction selectivity modulated by background motion in V1 and V2 respectively.

187.8

NEURAL RESPONSES TO TEXTURE PATTERNS IN MACAQUE VISUAL AREA MT. J. Olavarria, E.A. DeYoe, J. Knierim, D.C. Van Essen. Div. Biology 216-76, Caltech, Pasadena, CA 91125.

We have quantitatively studied responses from 116 MT neurons to bar-like figures defined by motion, texture and/or luminance cues. In anesthetized macaques we measured responses to stimuli moved in four orthogonal directions, using a graphics monitor to induce apparent motion by frame to frame displacement. Solid luminance bars or texture bars (containing numerous line elements of identical orientation) could be surrounded by a blank field or by a texture field of the same average luminance. The surround elements were either the same orientation or orthogonal to those in the bar.

As expected, nearly all MT neurons were direction selective for solid bars, and texture bars on a blank field were just as effective. In addition, many cells responded well (88%) and were selective (53%) for the orientation or direction of motion of texture bars on a static texture background. However, both response magnitude and direction selectivity were often reduced by the texture background, and in a few cases the preferred direction of motion was reversed. About half of the cells responded better to a motion bar on a static background than to uniform motion throughout the texture field. Thus, moving texture patterns are usually effective stimuli in MT, but tuning characteristics sometimes depend on the cues used to delineate motion.

187.9

COMPARISON OF NEURAL AND BEHAVIORAL SENSITIVITY TO VISUAL MOTION IN ALERT MONKEYS. William T. Newsome, Kenneth H. Britten* and J. Anthony Movshon. Dept. of Neurobiology, Stanford University Medical School and Dept. of Psychology, New York University.

We have trained rhesus monkeys to discriminate direction of motion in dynamic random dot displays containing a variable proportion of correlated motion. We compared behavioral performance with the responses of isolated neurons recorded from extrastriate cortical area MT while the monkey performed the psychophysical discrimination; the stimulus location and direction of motion were optimized for the neuron being recorded. We performed a signal-detection analysis of neural discharge to estimate the best performance that could be based on the signals from the particular neuron under study.

As motion correlation increased, psychophysical and neural performance improved from chance to near perfection. Threshold correlations were typically between 2% and 10%, and the psychometric functions for both neural and behavioral measures were of comparable steepness. For the majority of cells, the neural correlation thresholds were within a factor of 2 of the behavioral thresholds obtained on the same trials. Within this range, some cells were more sensitive than the monkey, while others were less. We conclude that signals from relatively small numbers of MT neurons could support the monkeys' performance. (Supported by NIH grants EY-05603 and EY-2017).

187.10

ASSOCIATION BETWEEN CORTICAL UNIT ACTIVITY AND PSYCHOPHYSICAL RESPONSE IN ALERT MONKEYS. Kenneth H. Britten*, William T. Newsome, and J. Anthony Movshon, Dept. of Neurobiology, Stanford University Medical School and Dept. of Psychology, New York University.

As described in the preceding abstract, we compared the visual motion sensitivity of rhesus monkeys with the sensitivity of single neurons in extrastriate area MT. For many neurons we observed an association between the monkey's psychophysical responses and the discharge of the neuron under study. For a given stimulus condition (direction of motion, correlation level), trials eliciting judgements that motion was in the neuron's preferred direction tended to contain neural responses that were 5-10% larger than trials eliciting the opposite judgement. As a rule, this was the case for stimuli in both the preferred and null directions, and also for zero correlation stimuli, which contain no net motion.

Using a method derived from signal detection theory, we computed a statistical estimate of the predictability of the monkey's response based on the neuron's discharge; this prediction is better than chance for most neurons, and for some substantially so. These neurons may, therefore, contribute directly to perceptual decisions concerning visual motion. (Supported by NIH grants EY-05603 and EY-2017).

187.11

MOTION RIVALRY USED TO STUDY NEURONAL ACTIVITY UNDERLYING MOTION PERCEPTION. N.K. Logothetis and J.D. Schall, Department of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

Rhesus monkeys were trained in a motion detection task. Horizontal gratings drifting up or down were presented to both eyes independently either in the same direction or in opposite directions. The monkeys were trained to signal whether they perceived up or down motion, and we recorded OKN. When the stimuli were not rivalrous, the monkeys performed correctly; hence, we inferred that when the stimuli were rivalrous, the response corresponded to the perceived motion direction. In agreement with an earlier study (Fox et al., Vis. Res. 18:849 77), we found that the perceived direction corresponded to the direction of the slow phase of OKN under rivalrous conditions. Moreover, when the monkey did not pursue either of the rivalrous gratings, he failed to respond. These results were confirmed with human observers who reported a coherent whole field motion percept only when pursuing one of the rivalrous gratings. We are currently studying the responses of single units in MT while monkeys perform this task to determine if the neuronal activity reflects the physical stimulus or the perceived stimulus.

187.12

"DEFICITS OF VISUAL MOTION ANALYSIS AFTER POSTERIOR RIGHT HEMISPHERE LESIONS." L. Vaina[1,2], M. LeMay[1]*, S. Naili[1]*, P. Amarillio[1]*, D. Bienfang[2]*, C. Montgomery[1]*, Y. Thomazeau[2]*. Intelligent Systems Laboratory, Boston Univ. Depts. of Biomedical Engineering and Neurology, Massachusetts Institute of Technology-Harvard Medical School, Boston, MA. 02215

Several new experimental tasks have been designed to address the measurement and the interpretation of visual motion in patients with focal brain lesions involving the visually responsive cortical areas. We were interested to learn how motion deficits cluster and what correlation may be established with the anatomical locus of the lesion, the neuro-ophthalmological profile of the subject, and with other aspects of visual processing such as contrast sensitivity, acuity and hyperacuity, stereopsis, texture and form. The most significant findings on the detailed study of 5ROcctemp and 5ROccPar patients were: the ability to appreciate speed of motion is strongly coupled with the ability to derive 3-D structure from motion; the perception of 2-D pattern from motion occurs more quickly than the perception of 3-D structure ($p<.02$); ROT group is able to perform coarse segregation through motion (speed), stereopsis, but they are markedly impaired in determining the specific shape of the figure. Acuity and hyperacuity were in all cases good; the ROP group was significantly impaired on tasks involving the interpretation of motion, random dot stereograms, and the processing of low contrast moving patterns.

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY II

188.1

MORPHOLOGICAL DEVELOPMENT OF PRIMATE RETINAL GANGLION CELLS. D. DeJager* and M.A. Kirby. Depts. of Pediatrics and Anatomy, Sch. of Med., Loma Linda University, Loma Linda, CA 92350.

Little is presently understood of the developmental processes that determine the mature dendritic morphology of retinal ganglion cells (RGC). To evaluate this we have employed retrograde transport of horseradish peroxidase (HRP) and cultured retinal wholemount techniques to examine the time course and pattern of dendritic maturation of RGC in the primate (Macaca mulatta).

Fetal monkeys (embryonic day, E60-E150; n=12) were delivered by Cesarean section, deeply anesthetized, and the retinae dissected into oxygenated media. HRP crystals were inserted into the fiber layer and the retinae placed into a 95% O_2-5% CO_2 incubator (37^0 C) for three hours. The retinae were then fixed in aldehyde and processed for HRP. Cells were used for analysis employing a strict criteria that included: (a) an axon that could be followed in the fiber layer toward the optic disk/deposit site (typically 1-2mm), (b) terminal dendritic processes that were densely labeled, continuous, and terminated either in growth cones or high order branches.

At E60 ganglion cells evidenced relatively uniform, small dendritic arbors derived from one or two primary dendrites. When two primary arbors were present these were observed to arborize in opposite portions of the inner plexiform layer. Axons at this age often had small limited branches that rarely entered the optic disk. Over the next several weeks the dendritic arbors continued to increase in size and complexity. By E131-138, ganglion cells had complex arbors with numerous small high order branches; several terminating in growth cones. By E147-150 few growth cones were found, and the majority of cells were similar in morphology to classes described for the adult. Our results suggest that classes of RGC in the fetal monkey can be distinguished morphologically as early as two weeks prior to birth.

188.2

THE NASOTEMPORAL DIVISION OF THE RETINAL GANGLION CELL DECUSSATION PATTERN IN THE FETAL RHESUS MONKEY. Barry Lia, Cara J. Snider* and Leo M. Chalupa, Dept. Psychology & California Primate Research Center, University of California, Davis CA 95616

Primates are distinguished from other mammals by the strict nasotemporal division of their retina: ganglion cells in the nasal hemiretina project contralaterally, while those in the temporal hemiretina project ipsilaterally. In all other mammals, a substantial proportion of cells with contralateral projections is found in the temporal hemiretina. To learn how the unique primate pattern is established, we examined the decussation of retinal ganglion cell projections in 5 fetal rhesus monkeys (*Macaca mulatta*) ranging in age from embryonic (E) days E69 to E129 (gestation: 165 ±2 days). Prior to *in utero* surgery, the optic chiasm and the lateral geniculate bodies were localized in the fetus by ultrasonography of the gravid female. This facilitated the subsequent unilateral injection of horseradish peroxidase into the optic tract.

Analysis of the distribution of labeled ganglion cells in retinal wholemounts revealed that at all fetal ages the retinal decussation pattern is remarkably precise. In the youngest fetus (E69), we found that only 0.3% of the retinal ganglion cells project to the inappropriate hemisphere. This degree of nasotemporal specificity is evident at a time when the projections from each eye are completely intermingled within the lateral geniculate and superior colliculus (Rakic, 1976), and prior to any appreciable loss of retinal ganglion cells (Rakic & Riley, 1983). Our results indicate that the precision of the fetal primate decussation pattern is greater than that of any other mammalian species studied to date, including that of the fetal cat (Lia, Kirby, and Chalupa, 1987). Moreover, there is no 'recapitulation' of the general mammalian plan of decussation.

(Supported by EY03991 from NEI and RR00169 from NIH)

188.3

TRANSIENT PROJECTION OF BETA RETINAL GANGLION CELLS TO THE SUPERIOR COLLICULUS DURING PRENATAL DEVELOPMENT. C.J. Shatz, A.S. Ramoa and G. Campbell. Dept. of Neurobiology, Stanford University School of Medicine, Stanford CA 94305.

The majority of retinal ganglion cells (RGCs) of the beta class project to the lateral geniculate nucleus (LGN) but not to the superior colliculus (SC) of adult cats. Is this target specificity present initially during development? RGCs that project to the SC at different times were identified by injecting rhodamine labeled microspheres into the SC at E38, E43 or postnatal day 4. Between E56 and P32, when alpha and beta cells are clearly identifiable if filled intracellularly with Lucifer Yellow (LY), retinae were removed and placed in a slice chamber. Microsphere-labeled RGCs from 8 cats were then filled with LY. As expected, beta cells were not retrogradely labeled by microspheres injected in the SC of postnatal cats (0 beta cells out of 47 RGCs filled that also included alpha and gamma cells). In contrast, microspheres injected prenatally in the SC of 3 cats always labeled beta cells morphologically similar to those projecting to the LGN (26 beta cells/ 86 RGCs examined at E56, P8 or P32). This was true even when microspheres were injected only into the caudal pole of the SC. These observations indicate that many beta cells initially send an axon to the SC during fetal life. Then, sometime between E43 and birth they must withdraw this collateral, possibly due to competition with alpha and gamma cells for territory within the SC.(NSF BNS 8616798 and March of Dimes).

188.4

IN VITRO DEVELOPMENT OF RETINAL GANGLION CELL STRUCTURE. P.R. Montague* and M.J. Friedlander (SPON: J.S. Tootle). Neurobiology Research Center, U.A.B. Sch. of Med., Birmingham, AL 35294.

Retinal ganglion cells from kittens postnatal age 2-42 weeks were retrogradely labeled with fluorescent markers and cultured. The development of their structure was followed with a time lapse video system (Soc. Neurosci. Abstr. 13:1299). The growth of the neurites of the cultured RGCs occurs primarily at the growing tips. The occurrence of a branch sprouting proximal to an already established branch is rare, and is independent of the final pattern that the neurites form. No significant overlap of neurites is observed for individual RGCs or between adjacent cells' neuritic arbors. (n = 17 identified RGCs).

We have used an estimate of the Hausdorff dimension of the images to generate a metric for the complexity of the neuritic patterns and to relate these patterns to models of process growth. After the neurons are plated, the Hausdorff dimension of the neuritic patterns increases with time (average **total** increase of 41%), and stabilizes after 10 days in culture. For a local region of an individual neuritic pattern, the Hausdorff dimension is dynamic and can change as much as 30% per hour, thus indicating a major remodeling of the neuritic pattern with time.

This work was supported by NSF grant BNS-8720069.

188.5

POSTNATAL DEVELOPMENT OF CAT RETINAL GANGLION CELLS. S.J. Ault and A.G. Leventhal. U. Utah Sch. Med., Anat. Dept., Salt Lake City, UT 84132.

We injected HRP into the LGNd and superior colliculi of 1 day old kittens and adult cats. Morphologies of labeled alpha (Y), beta (X) and all other cells (gamma (W) cells) were compared in kitten and adult retinae. In the neonate alpha, beta and gamma cells are recognizable (Ramoa et al., Science 237:522, 1987; Dann et al., Neurosci. Lett. 80:21, 1987). However, kitten alpha and beta cell bodies are significantly smaller than in the adult while remaining cell types are nearly adult sized. In fact, at birth beta cell bodies throughout the retina are significantly smaller than those of gamma cells. During the first 12 weeks of life, alpha and beta cell bodies increase in volume from 5 to 25 times depending upon eccentricity. Gamma (other) cells hardly increase in size at all. Also, the normal adult centro-peripheral cell size gradient for alpha and beta cells is not seen in the neonate. Gamma cells show no such gradient in the neonate or adult.

Thus, cat retinal ganglion cell classes seem to mature at different times. This observation may explain why some cell classes (e.g., alpha and beta) grow to be abnormally large in cell-poor regions while others (e.g., gamma) seem not to be affected when ganglion cell density is reduced by cutting one optic tract at birth (Leventhal et al., J. Neurosci. In press, 1988). These results may also explain why the sizes of LGNd W-cells are affected less by visual deprivation in early life than are those of X- and Y-cells (Hickey, JCN 189:467, 1980; Leventhal and Hirsch, JCN 214:59, 1983). Gamma (W) cells but not alpha (Y) and beta (X) cells normally stop growing soon after birth, prior to the time the foregoing surgical and environmental manipulations are carried out. Our results add to the growing body of evidence that different mechanisms mediate the morphological development of different classes of cells in cat retina.

188.6

RETINAL GANGLION CELL DEVELOPMENT IN PRIMATES. A.G. Leventhal, S.J. Ault and D.J. Vitek. Departments of Anatomy and Radiology, University of Utah School of Medicine, Salt Lake City, UT 84132

We studied the morphologies and retinal distributions of the different classes of ganglion cells in normal infant monkeys and in adult monkeys in which small strips of retina were depleted of ganglion cells at birth as a result of lesions made around the perimeter of the optic disc. New and old world monkeys were studied.

We find that the fovea is immature in neonates (see also Hendrickson and Kupfer, Invest. Ophthal. 15:746-756) as well as in adult monkeys in which the density of ganglion cells in central retina is reduced at birth. In both groups the foveal pit is small and contains many B(midgit) ganglion cells. B cells within the foveal pit project to the lateral geniculate nucleus and are larger than other foveal B cells. All of the morphological classes of retinal ganglion cells are recognizable in infant monkeys; cells in central and paracentral but not peripheral regions are close to adult size. Also, at birth an adult-like 'nasotemporal division' is evident.

In central retina of lesioned monkeys the cell bodies and dendritic fields of ganglion cells in depleted regions are up to ten times larger than normal. Unlike in cat central retina (Leventhal et al., 1988, J. Neurosci., in press) the dendrites of the abnormally large cells on the borders of cell poor zones are directed normally (toward the foveal pit) and are not directed away from neighboring cells.

We suggest that competition for afferents as well as direct interactions among neighboring ganglion cells in the immature retina contribute to the development of the structure and distribution of primate retinal ganglion cells.

188.7

SPONTANEOUS ACTIVITY OF RAT RETINAL GANGLION CELLS IN PRENATAL LIFE. L. Galli* and L. Maffei* (SPON: V. KAMP NIELSEN). Istituto di Neurofisiologia CNR 56100 Pisa, Italy.

The existence of spontaneous spike activity in retinal ganglion cells during early prenatal development has long been postulated. Such activity would play a key role in the formation of retinal connections. We have recorded from the retina of rat fetuses aged from 17 to 20 days post-conception.

Pregnant Long-Evans rats were anesthetized with urethane. Fetuses were taken from the mother uterus and placed in a low melting point wax bath, keeping the umbilical cord intact. Fetuses were then given a supplementary dose of urethane and paralyzed. Fetal body temperature and ECG were monitored continuously. Recordings were made with micropipettes inserted through a hole pierced into the sclera.

Spontaneously active cells were found in the retina at all ages studied. These cells showed irregular firing patterns, with average frequencies 0.5 - 1 spike/s. Relative long periods of silence altered to discharge periods of 4 - 5 spike/s.

These findings, which show for the first time the presence of spontaneous activity in mammalian retinal ganglion cells during prenatal life, strengthen the hypothesis of a possible role of electrical activity in the development of retinal connections.

188.8

TETRODOTOXIN (TTX) INFUSION DOES NOT PREVENT NATURALLY OCCURRING RETINAL GANGLION CELL DEATH. S. Friedman* and C.J. Shatz. (SPON: M. Siegel). Dept. of Neurobiology, Stanford University School of Medicine, Stanford CA 94305.

In the cat, only 20% of the retinal ganglion cells in the fetus will survive to adulthood. To determine whether action potential activity affects nerve cell death, TTX, a voltage-sensitive Na^+ channel blocker, was infused intracranially via osmotic minipumps into cat fetuses starting at E42, before the period of major ganglion cell loss. At E49 and E57, optic nerves were processed for EM to assess the effects of TTX treatment on axon number and ultrastructural organization.

The numbers of axons counted using the procedures of Williams et al. (J. Comp. Neurol. 246:32, '86) in TTX-treated animals examined at E49 or E57 were not significantly different from the counts of normal animals at comparable ages (Sretavan & Shatz, J. Neurosci. 6:990, '86): E48 normal- $3.3x10^5$; E49 TTX- $3.2x10^5$; E59 normal- $2.5x10^5$; E57 TTX- $2.1x10^5$. However, while E49 TTX-treated nerves were indistinguishable from normal nerves at the same age, E57 TTX-treated nerves were quite abnormal. Fasciculation of axons was disrupted by many pale, organelle-poor processes that had large cross-sectional areas. These processes were not axonal: they stemmed from glial cell bodies in longitudinal EM sections and acquired organelles typical of glial cells in serial EM sections. They were therefore not included in our axon counts.

These results indicate that intracranial infusion of TTX for the two week period during naturally occurring cell death does not rescue ganglion cells; they suggest that action potential activity does not control ganglion cell number. However, in view of the observed glial cell abnormalities, we suggest that action potential activity is important for the normal maturation of optic nerve glia. (Supported by NSF Grant BNS 8616798 and the March of Dimes)

188.9

DEVELOPMENT OF CHEMOSENSITIVITY AND ACTION POTENTIAL (AP) ACTIVITY IN RETINAL GANGLION CELLS (RGCs) OF THE RAT. A.S. Ramoa, S.S. Deshpande* and E.X. Albuquerque Lab. Mol. Pharm. II, Inst. Biophys. CCF, Fed. Univ. Rio de Janeiro, Brazil 20540 & Dept. Pharm. Exp. Ther., Univ. MD Sch. Med., Baltimore, MD 21201.

Although a good deal is known about morphology of developing RGCs, surprisingly little is known concerning development of their membrane properties. To address this issue, we labeled RGCs in vivo by retrograde transport with rhodamine-labeled latex microspheres. Next, RGCs obtained from rats at different stages of development were enzymatically dissociated and maintained in culture for up to 8 hours. Whole-cell patch-clamp recordings revealed that the number of RGCs firing APs increased from 40% of 28 cells at P3-5 to 92% of 36 cells at P20-23. At every age tested, tetrodotoxin (1 μM) blocked AP activity. Outside-out patch-clamp recording (n=28) revealed that, as early as P3 RGCs responded to N-methyl-D-aspartate (NMDA-10-50 μM), quisqualate (10-50 μM), and the agonist of the nicotinic-Acetylcholine receptor, (+)anatoxin (1-10 μM). The conductance values and the mean burst duration of the single channel currents elicited by NMDA (about 40 pS and 10 msec) and anatoxin (42 pS and 21 msec) remained unaltered during development. Application of phencyclidine, which is known to block the excitatory effect of NMDA, reduced the frequency and duration of ion channel openings elicited by both NMDA and anatoxin in the retina. In conclusion, concurrent with morphological changes that occur in neonatal retina, an increasing number of RGCs fire APs. In contrast, properties of post-synaptic receptors found in RGC membrane appear to remain unaltered during development. (CNPq-Brazil, U.S. Army Med. Res. Devel. Comm. DAMD17-84-C-4219, NIDA Grant DA02804)

188.10

THE DEVELOPMENT OF INDIVIDUAL RETINOGENICULATE AXONS DURING LAMINAR AND SUBLAMINAR SEGREGATION IN THE FERRET LGN. J. Hahm* and M. Sur. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

We have used an in vitro HRP labeling method (Sretavan & Shatz, J. Neurosci. 6:234, 1986) to study the morphology of individual retinal axons during the (postnatal) development of eye-specific laminae and On/Off sublaminae in the ferret lateral geniculate nucleus (LGN).

In the first 24 hours after birth, the LGN is not yet laminated, and retinal axons are relatively primitive in structure with a single main trunk extending through the nucleus. However, some axons have long, widely diverging branches covering a large portion of the nucleus. At this age the fibers are studded with extremely fine fibrils throughout their extent and have occasional side branches.

At P7-P8 the LGN shows contralateral and ipsilateral laminar zones (Linden *et. al.*, J. Comp. Neurol., 203:189, 1981). Retinal axons have begun to develop arbors which are confined to and span the width of one eye-specific layer. The main axon trunks are smooth, the fine fibrils no longer present. By P15, some retinogeniculate terminal arbors have taken on a distinct, narrow morphology. Arbors are more heavily branched and clearly confined to one eye-specific lamina, although there is no obvious indication of sublaminar segregation.

At P19 the axonal arbors appear to be segregating into sublaminae within the A and A1 layers. Cellular sublamination is definitive at P21. By P28-P35 the retinal axons have taken on an adult-like framework (Roe *et. al.*, Soc. Neurosci. Abs., 12:9, 1986) and well-formed boutons have replaced growth cones on many endings.

We propose the following sequence of events in the development of retinogeniculate axons: 1) fibers grow into the LGN and extend through the entire nucleus; 2) small branches and fibrils in inappropriate zones are lost as axons elaborate arbors in their eye-specific layer; 3) axons further refine their terminal arbors and consolidate their terminations in On and Off sublaminae.

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188.11

RETINAL PROJECTIONS INDUCED INTO AUDITORY THALAMUS IN FERRETS: VISUAL TOPOGRAPHY IN PRIMARY AUDITORY CORTEX. A. W. Roe, S. L. Pallas, J. Hahm*, Y. H. Kwon*, and M. Sur. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Removing normal retinal targets in neonatal ferrets and deafferenting the medial geniculate nucleus (MGN) induces an aberrant visual pathway from the retina to auditory thalamus and, in turn, to auditory cortex (Sur and Garraghty, Soc. Neurosci. Abstr. 12:592, '86). In these animals, we have studied the spatial pattern of thalamocortical projections to primary auditory cortex (AI) and the physiological map of visual space in AI. In normal adult ferrets and in operated ferrets reared to adulthood, we injected multiple tracers (WGA-HRP, Rhodamine, Fluoro-Gold) in AI, located in the medial ectosylvian gyrus (Kelly *et al.*, Hearing Res. 24:111, '86). AI in operated ferrets receives projections from regions of the lateral posterior/pulvinar complex. Otherwise, connections between the MGN and AI in operated animals closely resemble those in normal animals: single loci in AI receive projections from rostrocaudally oriented slabs of cells in deep dorsal, ventral, and medial divisions of MGN (Pallas et al., *this volume*).

In normal ferrets, the cochlea maps in a tonotopic fashion along roughly the mediolateral dimension of AI, while the anteroposterior dimension contains isofrequency lines (Kelly *et al., ibid.*). We find that the map of the contralateral visual field in AI of operated animals, while not as refined as in normal striate cortex, is two-dimensional and topographic. Azimuths increase in a mediolateral direction, and elevations increase roughly in a posterior-anterior direction in AI, though considerable variability exists along this axis. Since the axis of variable elevation is also the isofrequency axis in normal AI, one interpretation of our results is that this dimension of the visual field map is created physiologically in cortex from a highly overlapped thalamocortical projection system. Alternatively, the two-dimensional topography might arise from visual projections to AI through the LP/pulvinar complex.

Supported by EY 07023, the March of Dimes, and the McKnight Foundation.

188.12

RETINAL PROJECTIONS INDUCED INTO AUDITORY THALAMUS IN FERRETS: CHANGES IN INPUTS AND OUTPUTS OF PRIMARY AUDITORY CORTEX. S.L. Pallas, A.W. Roe, and M. Sur. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Retinal projections can be routed into the auditory thalamus (MGN) as a result of appropriate surgery in neonatal ferrets, conferring visual responsiveness onto primary auditory cortex (AI) (Sur & Garraghty, Soc. Neurosci. Abstr. 12:592, '86; Roe *et al.*, this volume). We have studied the thalamocortical, corticothalamic, and corticocortical connectivity in these animals to see whether the early lesions can influence the connectivity patterns of auditory cortex.

In normal adult ferrets and adult ferrets operated on as neonates, injections of tracers (WGA-HRP, Rhodamine, and Fluoro-Gold) were made into AI, located in the medial ectosylvian gyrus (Kelly *et al.*, Hearing Res. 24:111, '86). In normal ferrets, retrograde and anterograde label was found in the deep dorsal, ventral, and medial divisions of the MGN, and in the lateral division of the posterior thalamic nucleus, as in cats. Retrograde cortical label was found bilaterally in AI, areas anterior to AI, lateral to AI, and far lateral in the ventral convexity of the cortex. In neonatally operated ferrets, thalamic label was found in the same nuclei as in normal ferrets, and in new zones: the dorsal caudal division of the MGN and the LP/Pulvinar complex of the thalamus. Patterns of retrograde corticocortical labelling in operated animals included the same areas as in normal animals, and new zones in the ipsilateral and contralateral anterior lateral gyrus.

These results indicate that novel thalamocortical pathways as well as novel cortical connections can be induced into AI by the early lesions. The neonatal lesions either cause sprouting of these new projections, or stabilization of early exuberant projections that are normally eliminated.

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RESPIRATORY REGULATION I

189.1

EFFECTS OF EXPIRATORY MUSCLE AFFERENTS ON INSPIRATORY NEURONS OF THE DORSAL RESPIRATORY GROUP OF CAT. S. Iscoe*, L. Grelot* and A.L. Bianchi* (SPON: P. Zarzecki). Département de Physiologie et Neurophysiologie, Faculté des Sciences et Techniques St.-Jérôme, 13397 Marseille Cedex 13, France.

At elevated end-expiratory lung volumes, inspiratory duration (Ti) does not decrease despite increases in both pulmonary stretch receptor (PSR) feedback and chemical drive. We hypothesized that afferent activity from expiratory muscles inhibits the medullary neurons to which PSR project, blocking their excitation by PSR and, therefore, a decrease in Ti. We recorded intracellularly from 25 bulbospinal inspiratory neurons of the dorsal respiratory group (DRG) of decerebrate, paralyzed and ventilated cats. Sixteen neurons had excitatory monosynaptic projections from PSR. However, in only 2 neurons did the inhibition evoked by conditioning stimuli to afferents of expiratory muscles (internal intercostal or external oblique) block the PSR-mediated excitation. Instead, most neurons were either not affected (17) or excited (6) by stimulation of excitatory muscle afferents. Therefore, fixation of Ti at control values during respiration at elevated lung volumes is not due to inhibition of inspiratory neurons of the DRG by afferent activity from caudal expiratory muscles. Supported by the C.N.R.S. (France) and M.R.C. (Canada).

189.2

INTERRUPTION OF PULMONARY STRETCH RECEPTOR- (PSR) RELATED RESPIRATORY REFLEXES BY A DISCRETE COBALT INJECTION IN THE NUCLEUS TRACTUS SOLITARIUS (NTS) IN RATS. A.C. Bonham and D.R. McCrimmon, Dept. Physiology, Northwestern U., Chicago, IL. 60611.

We previously reported that (1) microinjecting DL-homocysteic acid (DLH, 1-6 nl, 20 mM) into a discrete site in the medial NTS produced an apnea similar to that elicited by activating PSR, and (2) pump cells (single units which discharged with lung inflation and were silent when lung inflation was withheld) were recorded in the DLH-sensitive site (Fed. Proc. 46:6442, 1987; Soc. Neurosci. Abs. 13:1638, 1987). To determine if neurons in this NTS site are important in the Breuer-Hering inspiratory shortening reflex, we interrupted neuronal transmission by microinjecting cobalt chloride (Co^{2+}) in the DLH-sensitive site and studied the effects on baseline respiratory rhythm and the reflex response to maintained lung inflation. We measured phrenic nerve discharge, expired CO_2 and arterial pressure in urethane-anesthetized, spontaneously-breathing or ventilated rats. A double-barrel pipette was used for microinjecting; one barrel contained DLH (20 mM)and the second contained Co^{2+} (20 - 100 mM). Once the site was established where microinjecting DLH produced an apnea (0.5 mm rostral to calamus and 0.7 mm lateral to midline), we maintained lung inflation at pressures from 2.5 cm to 20 cm H_20 and determined the effect on phrenic nerve discharge. Co^{2+} was then microinjected in the DLH-sensitive site and the lung inflation maneuver and DLH injection repeated. Co^{2+} microinjected within the DLH-sensitive site (1) prolonged the inspiratory and expiratory periods and (2) markedly diminished the inhibitory effects on phrenic nerve discharge produced by maintaining lung inflation. Co^{2+} did not prevent the DLH-induced apnea, indicating that Co^{2+} impaired synaptic input but did not directly depress postsynaptic neuronal excitability. The Breuer-Hering reflexes recovered within 1 hour following Co^{2+}. These results suggest that neurons in a discrete site in the medial NTS are important in the Breuer-Hering inspiratory shortening reflex. Supported by NIH grant RR-05370 and NRSA, HL07717-01, to Dr. Bonham.

189.3

LOCALIZATION OF THYROTROPIN-RELEASING HORMONE (TRH) RECEPTORS IN SUBNUCLEI OF THE NUCLEUS TRACTUS SOLITARIUS (NTS). S. Manaker and G. Rizio*, Cardiovascular-Pulmonary Division, Dept. of Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA 19104-4283

The nucleus tractus solitarius (NTS) is an important site for the integration of pulmonary vagal afferents with the central nervous system. The NTS is divided cytoarchitectonically into different subnuclei, each of which may have different physiological functions. The neuropeptide thyrotropin-releasing hormone (TRH) is localized within the NTS, and central administration of TRH produces tachypnea. In addition, specific high-affinity receptors that may mediate the respiratory actions of TRH are present within the NTS. In the present study, we determined the concentration of TRH receptors within individual NTS subnuclei using quantitative autoradiography and our previously published technique (Manaker et al, J Neurosci 5:167-174, 1985).

The highest concentration of TRH receptors (200 fmol/mg P) was observed in the gelatinosus subnucleus of the NTS. High concentrations (100-150 fmol/mg P) were also observed in the medial and intermediate NTS subnuclei. Moderate concentrations of TRH receptors (40-80 fmol/mg P) were observed in the remaining NTS subnuclei (commissural, ventrolateral, dorsal) as well as the solitary tract. These results suggest that TRH and TRH receptors may play a role in the integration of pulmonary vagal afferents in the gelatinosus, medial and intermediate NTS subnuclei.

189.4

CAROTID BODY CHEMORECEPTOR (CR) INPUT TO INSPIRATORY HYPOGLOSSAL MOTONEURONS (IHMs). S.W. Mifflin Department of Pharmacology, The University of Texas Health Science Center, San Antonio, Texas 78284-7764.

The phasic discharge of IHMs aids in maintaining upper airway patency. To better understand the behavior of IHMs in normal and pathological states IHM responses to CR inputs were examined in pentobarbital anesthetized, vagotomized, mechanically ventilated and paralyzed cats. Intracellular recordings were obtained from 12 IHMs. Injections through an intra-carotid catheter of .1-.5 ml of .15M $NaHCO_3$ gassed with 100% CO_2 increased phrenic nerve discharge. During CR evoked changes in phrenic nerve discharge the inspiratory depolarization and discharge frequency of the 12 IHMs increased. Injection of non-CO_2 gassed saline had no effect. In 5 IHMs during CR activation the depolarization at the beginning of inspiration was 3-8mV greater than under control conditions so that discharge began virtually at the onset of inspiration. The time difference between the onset of inspiratory depolarization and the beginning of IHM discharge ranged from 280-600msec under control conditions compared to 86-320msec during CR activation (paired t-test, $p<.05$).

In conclusion, during a CR evoked increase in inspiratory drive to phrenic motoneurons, the parallel increase in IHM discharge should aid in the maintenance of upper airway patency. (Supported by NIH Grant HL41894).

189.5

AGE DIFFERENCES IN CAT RESPIRATORY RESPONSE TO INCREASED INSPIRED CO_2. Bada, F., Archer, P. Jackson, E., Whittaker, J., Bernard, D.G., Millis, R.M., Trouth, C.O. Dept. Physiol., Coll. Med. Howard University, Washington, D.C. 20059.

This study examines the hypothesis that the brainstem respiratory chemosensory system might not be functionally mature at birth. In 3 age groups (newborns 1-6 days; young pups 4-6 weeks, and adult >7 months) of spontaneously breathing cats anesthetized with chloralose urethane, respiratory response to increased inspired CO_2 ($PICO_2$) were examined. In the ADULT and Young minute ventilation ($\dot{V}_E$) increased in response to 7% inhaled CO_2 and arterial pH 7.2 by approx. 138% and 106% respectively whereas in the NEWBORN only a 23% increase in $\dot{V}_E$ was observed. Neurons in the caudal chemosensitive area were tested for their responsiveness to increased inspired CO_2 ($PICO_2$) and CSF-pH. In the ADULT and Young animal, non-phasic pH sensitive units increased their firing rate by approx. 200% and 110% respectively in response to increased $PICO_2$ and acid CSF, whereas in the NEWBORN, increased $PICO_2$ caused only slight increases to no changes in neuronal activity. Electron microscopic examination at the caudal chemosensitive area (Area "L") revealed that evaginations of the ventral surface abutting the overlying pia mater which delimit discrete compartments were more pronounced in the adult animal and least marked in the newborn. Neuronal elements in vascular walls, reminescent of neurovascular junctions in cerebral and systemic arteries and arterioles, were most pronounced in the young. Support: MBRS-2-S06-RR-08016-16.

189.6

PHRENIC MOTONEURON PROPERTIES IN THE PERFUSED GUINEA PIG. Corey Cleland and Peter Getting, Department of Physiology and Biophysics, Univ. of Iowa, Iowa City, Iowa 52242

Mammalian respiration arises from neurons in the brainstem and spinal cord, however, the neural mechanisms underlying formation of the respiratory motor pattern remain unclear. Our goal is to characterize the cellular, synaptic and network features of phrenic motoneurons using a brain-spinal cord preparation perfused with artificial blood, which permits stable recordings and control over blood composition [Richerson and Getting, *Brain Res.* 409(1987)128].

Anesthetized guinea pigs (350 gm) were instrumented with EEG, phrenic and vagal electrodes. The C3-C6 segments were exposed and the descending aorta ligated. The rostral half of the guinea pig, including the brain and spinal cord, was kept alive by non-pulsatile perfusion with a fluorocarbon-based artificial blood containing pentobarbital and Flaxedil. Fictive respiration remained normal for up to 9 hours at 37°C. Phrenic motoneurons were identified by antidromic activation.

We have recorded intracellularly from 24 phrenic motoneurons during fictive respiration. Their firing patterns and underlying membrane potential (Vm) oscillations resemble those observed in cat phrenic motoneurons. Fictive respiration, although essential for identifying and characterizing respiratory-related neurons, complicates the determination of cellular properties. Therefore, we reversibly eliminated respiratory modulation by repetitive stimulation of the vagus nerve to evoke the inspiration-supressing Breuer-Hering reflex. Compared to hypercapnea or spinal block, vagal stimulation can be rapidly applied, is easily controlled and does not alter pH or pCO_2, parameters that affect cellular properties and cerebral blood flow. Our preliminary results indicate that Vm during vagal stimulation is constant and similar to that during expiration. Furthermore, stimulus-triggered averaging did not reveal any detectable PSPs. Thus, vagal stimulation appears to produce a stable, reproducible Vm during which we can evaluate the cellular properties of phrenic motoneurons.

Funded by NIH grant NS15350 to PG.

189.7

BRAINSTEM PROJECTIONS TO THE VENTRAL RESPIRATORY GROUP (VRG) IN THE RAT: COMPARISON WITH CATECHOLAMINE CELL GROUPS. H.H. Ellenberger, C.A. Connelly & J.L. Feldman, Systems Neurobiology Lab., Dept. of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

Rhodamine impregnated latex beads (40 nl; 20% in saline) were injected into physiologically identified regions of the inspiratory (rostral) (r)VRG to retrogradely label caudal brainstem neurons projecting to rVRG. Norepinephrine and epinephrine containing neurons were labeled simultaneously by immunoreaction of dopamine β-hydroxylase (DBH) in the same tissue sections. Populations of rhodamine (Rh)-labeled and DBH-labeled neurons formed partially overlapping, yet distinct cell groups in 3 regions of the caudal brainstem: 1) The nucleus of the solitary tract, where Rh-labeled neurons were found lateral, ventrolateral, ventral, medial and dorsomedial to tractus solitarius (TS) and in area postrema. Rh-labeled neurons medial to TS were intermingled with DBH-labeled neurons of the A2 and C2 groups. 2) The ventrolateral medullary reticular formation; where Rh-labeled neurons of the VRG were dorsomedial to, but partially intermingled with the A1 and C1 cell groups. In the rostral medulla, Rh-labeled neurons were clustered dorsal and ventral to the C1 group forming 2 distinct groups in the lateral paragigantocellular nucleus. 3) The Kölliker-Fuse nucleus; where Rh-labeled neurons were dorsal to, but partially intermingled with DBH-labeled neurons of the A7 cell group.

The proximal relationship between presumptive respiratory (rhodamine-labeled) and DBH-labeled neurons in these regions suggests a parallel organization of respiratory and other autonomic pathways in the brainstem. Furthermore, local processing within these regions may be important in the central coordination of respiratory and cardiovascular function. Supported by NIH grants HL-37941 and HL- 07363.

189.8

RAPHE PROJECTIONS TO THE VENTRAL RESPIRATORY GROUP (VRG) IN THE RAT: COMPARISON WITH SEROTONIN (5-HT) IMMUNOREACTIVE CELLS. C.A. Connelly, H.H. Ellenberger & J.L. Feldman, Systems Neurobiology Lab., Dept. of Kinesiology UCLA, Los Angeles, CA 90024-1568.

Rhodamine impregnated latex beads (40 nl; 20% in saline) were injected into physiologically-identified regions of the inspiratory (rostral) (r)VRG to retrogradely label caudal brainstem neurons projecting to rVRG. Immunohistochemical methods were then used to identify 5-HT containing neurons in tissue sections containing rhodamine-labeled neurons.

5-HT neurons were found throughout the raphe nuclei from the level of the pyramidal decussation to the inferior colliculus. Rhodamine labeled neurons were located bilaterally, intermingled with 5-HT immunoreactive cells in raphe obscurus, raphe pallidus, and raphe magnus. Rhodamine-labeled neurons projecting to rVRG were similar in morphology (fusiform or multipolar) and size (5-20 μm somal diameter) to the 5-HT neurons; some cells contained both labels. In addition to the raphe nuclei, 5-HT containing neurons in the B3 serotonin cell group were juxtaposed with rhodamine-labeled cell bodies. No rhodamine-labeled neurons were found in raphe dorsalis or pontis. Other rhodamine-labeled neurons were located predominantly within respiratory-related nuclei such as the nucleus of the solitary tract, VRG and Kölliker-Fuse nucleus, substantiating the relative specificity of the injection site within rVRG.

These data suggest that non-serotonergic as well as serotonergic neurons projecting from raphe nuclei may influence activity of rVRG neurons. Supported by NIH grants HL-37941 and HL-07363

189.9

AFFERENT-EFFERENT ORGANIZATION AND CYTOARCHITECTURE OF PHRENIC SENSORIMOTOR CORTEX IN THE CAT. J.J. Warner, J.P. Coffey, F.J. Thompson, P. Davenport, M. Davda, G. Schrimsher. Depts. of Neurology and Neuroscience, Colleges of Medicine, Dentistry and Veterinary Medicine, University of Florida, Gainesville,FL 32610.

Phrenic nerve projections to sensorimotor cortex have been proposed to participate in respiratory kinesthesia (Davenport, 1985). Sensorimotor cortical stimulation has been shown to modulate medullary respiratory output (Bassal, 1982) and phrenic activity (Lipski, 1986), (Thompson, Davenport, Warner, 1987). The present study was undertaken to investigate the localization, organization, and cytoarchitectonics of phrenic sensorimotor cortex.

Dorsolateral frontoparietal craniotomy and contralateral C5 phrenic nerve exposure were performed on vagotomized adult cats under chloralose anesthesia. C5 phrenic nerve stimulation was utilized for mapping of cortical evoked potentials. Low-threshold cortical sites for elicitation of contralateral short-latency phrenic nerve action potentials were mapped, and depth penetration laminar analysis performed. Regions producing inhibition of phrenic inspiratory activity were also identified. Following perfusion fixation, Klüver-Barrera and Nissl stained serial coronal or sagittal sections were used for cytoarchitectonic analysis and laminar data correlation.

Phrenic nerve stimulation produced evoked potentials over the posterior sigmoid gyrus, and lateral to the coronal sulcus. Low-threshold efferent sites were identified on the banks of the coronal sulcus and rostral to the ansate sulcus, however their location did not necessarily correspond to maximum evoked potential sites for each experiment. Localization of maximal afferent and efferent sites corresponded to cytoarchitectonic areas 3, 1, and 2 of Hassler and Muhs-Clement (1966).

189.10

HIPPOCAMPAL SLOW WAVE CORRELATES OF RESPIRATORY PATTERNING. R.M. Harper, C.A. Richard, H. Ni*, and R.C. Frysinger. Department of Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA, 90024-1763.

Attributes of hippocampal slow wave EEG activity have been associated with particular locomotor patterns in freely moving animals; higher frequencies of rhythmical slow wave activity, for example, have been correlated with enhanced whole body movement, while lower frequencies have been associated with localized movements. Respiratory movements are a form of locomotive patterning that continues during the motoric paralysis of rapid eye movement sleep, a state accompanied by diverse respiratory patterns as well as marked hippocampal EEG variation, including pronounced rhythmical slow wave activity. Since electrical stimulation of the hippocampus elicits profound changes in respiratory patterning, we examined momentary changes in hippocampal EEG patterns during different sleep-waking states along with breath-by-breath attributes of the respiratory cycle. Bipolar macroelectrodes were placed in the hippocampus of cats, together with diaphragmatic EMG leads and electrodes to record eye movements, nuchal EMG, and cortical EEG. Scattergrams were calculated between interpeak intervals of synchronous slow wave hippocampal activity, total respiratory cycle duration, and inspiratory area. Among a number of correlations, higher frequency rhythmical slow activity was frequently associated with diminished respiratory period. Supported by HL 22418-11.

BIOLOGICAL RHYTHMS: MECHANISMS

190.1

SUPRACHIASMATIC NUCLEUS TRANSPLANTATION RESTORES DONOR SPECIFIC CIRCADIAN RHYTHMS TO ARRHYTHMIC HOSTS M.R. Ralph, F.C. Davis and M. Menaker. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

The suprachiasmatic nucleus (SCN) contains circadian pacemaker cells that oscillate in vitro. Although ablation of the nucleus or its isolation from the rest of the brain results in behavioral arrhythmicity, there is still some question about the role played by the SCN within the circadian system of mammals especially in view of the finding that methamphetamine can restore rhythmicity to SCN lesioned rats (Honma et al., Physiol. & Behav. 40: 767 (1987). Experiments in which rhythms have been restored to SCN lesioned hamsters with SCN tissue from fetal donors have approached this question; however, as yet, it has not been possible to identify donor derived properties in the restored rhythms.

We have performed reciprocal SCN transplant experiments between wild-type hamsters and a recently discovered period mutant (tau) that shortens the period from 24 hr. to 22 hr. in the heterozygote and to 20 hr. in the homozygote. We have found that implants of fetal (E13.5) tissue containing SCN restore rhythmicity to SCN lesioned, arrhythmic hosts. The period of the restored rhythm always matches that of the donor regardless of the direction of the transplant. These data indicate that a small brain region containing SCN determines the period of the overt locomotor rhythm, and greatly strengthens the currently held view that the SCN occupies a position at the top of the circadian hierarchy in mammals.

Supported by NIMH grants MH09483 to MRR and HD13162 to MM.

190.2

FETAL HAMSTER SUPRACHIASMATIC NEURONS EXPRESS VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN DISSOCIATED CELL CULTURES. M.N. Lehman, J.L. Vest* S. Warren* and R.A. Akeson. Dept. Anat.& Cell Biol., Univ. Cinti. Coll. Med., and Inst. Develop Res., Children's Hosp. Res. Found., Cinti, OH 45267.

We recently demonstrated that dissociated cell grafts of the fetal suprachiasmatic nucleus (SCN) restore circadian locomotor rhythms to arrhythmic SCN-lesioned hamsters (Neurosci. Abst., 13:212). To explore the potential for *in vitro* manipulation of SCN cells prior to grafting, their survival and expression of vasoactive intestinal polypeptide (VIP) were examined in primary culture. Tissue chunks of either anterior hypothalamus (containing the SCN) or cortex were microdissected from E13 hamster fetuses, trypsinized and mechanically dissociated; approx. 5 X 10^5 cells were plated upon glass coverslips coated with either poly-L-lysine; poly-L-lysine and laminin; or a primary monolayer of either rat or hamster astrocytes. Cells were grown for 1-4 wks. in Bottenstein/Sato media with 5% horse serum, with or without added glial conditioned media. They were fixed in 4% paraformaldehyde and immunostained for VIP. After 2 wks., SCN cultures plated on either poly-L-lysine alone, or poly-L-lysine in combination with laminin or glial conditioned media, contained 5-10 immunoreactive VIP cells which possessed few if any immunodetectable processes. In contrast, SCN cultures grown on either rat or hamster astrocytes contained 20-30 isolated VIP neurons, most of which had multiple, extended neurites with beaded VIP-containing varicosities. Suprisingly, we saw no immunoreactive VIP cells in any cortical cultures. In preliminary experiments we have found that VIP cells of the SCN grown on astrocytes for 1-2 wks. survive subsequent grafting and ramify within the brains of intact adult hamsters. [Supported by NIH NS24292 (MNL) and NS23348 (RAA).]

190.3

FURTHER EVALUATION OF THE TETRODOTOXIN-RESISTANT CIRCADIAN PACEMAKER IN THE SUPRACHIASMATIC NUCLEI USING 50% PROCAINE & 20mM KCl INFUSIONS. W.J. Schwartz & P. Zimmerman*. Dept. Neurol., U. Massachusetts Med. Sch., Worcester, MA 01655.

Previous work (PNAS 84:1694,'87) has shown that chronic TTX infusion into the rat SCN blocks rhythm entrainment & expression without affecting the oscillatory mechanism of the circadian pacemaker in the nuclei. Further experiments now help to validate and extend these findings.

After free-running circadian drinking rhythms were recorded for 2 wks, rats were anesthetized and cannula assemblies stereotaxically inserted into the SCN & connected to subcutaneously implanted mini-osmotic pumps. Free-running rhythms were then recorded during the 14-d infusions (@ 0.5 µl/hr) and for an additional 4 wks.

Like TTX, chronic infusion of 50% procaine (n=5) produced behavioral arrhythmicity, & drinking rhythms were restored without phase shift after infusions ended. Like TTX, procaine suppresses Na^+-dependent spikes; unlike TTX, the mechanism involves binding to an intracellular receptor. Chronic infusion of 20 mM KCl (287 mosm/l)(n=7) also resulted in behavioral arrhythmicity, but these rhythms returned with anomalous phases (advanced by 4.4 $\pm$ 0.5 hr from expected). Thus, the infusion pump paradigm can detect alterations of the pacemaker's endogenous oscillation; the TTX & procaine results are not merely artifacts of an insensitive method. Infusions of 20 mM KCl + 10^{-6}M TTX are currently underway.

190.4

SUPRACHIASMATIC (SCN) NEURON ACTIVITY IN VITRO FROM HAMSTERS WITH SPLIT ACTIVITY RHYTHMS. R.Y. MOORE, L.P. MORIN and S.SHIBATA*, Depts. of Neurology and Psychiatry, SUNY, Stony Brook, N.Y. 11794 and Dept. of Pharmacology, Kyushu University, Fukuoka, Japan.

The splitting of activity rhythms in the hamster into two free-running components in constant light is one of the bases for the view that the circadian system contains multiple oscillators. To determine the localization of those oscillators, adult male hamsters were maintained in constant light and a group of 9 animals with clear, split rhythms was studied and compared with 12 control, free-running animals with intact activity rhythms. Hypothalamic slices including the SCN were prepared and single unit recordings made. Slices from controls exhibit the typical pattern of high firing rates of SCN neurons during subjective day and low firing rates during subjective night. The slices from split animals were analyzed to test four hypotheses concerning splitting: 1) dissociation of the two SCN's; 2) dissociation of two anatomically identifiable components of the SCN; 3) dissociation of the SCN from another oscillator outside the SCN; 4) dissociation of components within the SCN on a non-anatomic basis. The data obtained are explicable only by the last hypothesis. Units in all areas of the SCN on each side exhibit a pattern of firing rates which has the appearance of two peaks of lower amplitude than controls but corresponding to the appropriate components of the activity rhythm. Supported by NIH grants NS-16304 and NS-22168.

190.5

OUBAIN MIMICS THE EFFECTS OF DARKNESS ON THE CIRCADIAN PACEMAKER IN CHICK PINEAL CELLS. M. Zatz and D.A. Mullen* Lab. of Cell Biology, N.I.M.H., Bethesda, MD 20892

Chick pineal cells in primary culture display a persistent circadian rhythm of melatonin output in constant red light. Four hour pulses of "unexpected" white light (L), or darkness (D), induce phase-dependent phase shifts in subsequent cycles of the melatonin rhythm, indicating an action on the underlying pacemaker. L and D pulses each cause both phase delays and phase advances, but their phase response curves (PRC's) differ.

Four hour pulses of oubain, a specific inhibitor of the Na,K-ATPase, also induced both phase delays and phase advances, with a PRC similar to that for dark pulses. Eight hours exposure to low external potassium (estimated below 0.5 mM) also caused dark-like phase delays and advances. Exposure of cells to 8 hours oubain before and during exposure to 4 hours L had assymetric effects: the phase advancing effect of L was counteracted by oubain but the phase delaying effect of L was converted to an enhanced phase advance. Such results support and are explained by "instantaneous" and additive shifts of the pacemaker and of its PRC in response to oubain and light. Taken together, these results suggest that inhibition of the Na,K-pump has consequences similar to those of darkness (and thus opposite to those of L) on the circadian pacemaker in chick pineal cells.

190.6

[14C] 2-DEOXYGLUCOSE UPTAKE IN SQUIRREL BRAIN ACROSS THE HIBERNATION CYCLE. T. S. Kilduff, C. M. Radeke*, J.D. Miller, and H. C. Heller*. Depts. of Psychiatry and Biol. Sci., Stanford Univ., Stanford, CA 94305.

To provide a comprehensive view of brain activity during hibernation, we used the autoradiographic [^{14}C]2-deoxyglucose (2DG) method to compare the relative metabolic activity of 96 brain regions across 7 phases of the hibernation cycle: euthermia, 3 body temperature (T_b) intervals during entrance (30°C to 25°C; 25°C to 20°C; and 20°C to 15°C), deep hibernation (T_b < 8°C), and both early (T_b < 8°C) and late (T_b = 25°C) arousal. Cluster analysis of relative 2DG uptake (R2DGU) values across these phases resulted in grouping the 96 neural structures into 5 categories. In cluster 1 (N=18 brain regions), R2DGU paralleled the change of T_b (e.g. the cerebellar nuclei). Cluster 2 (N=5), exemplified by VIII nerve efferent nuclei, was similar to cluster 1 in that R2DGU paralleled the change of T_b, but the euthermic R2DGU was lower than the entrance R2DGU. Cluster 3 (N=39) was comprised of gray matter regions whose R2DGU was inversely related to the change in T_b and included reticular formation and hypothalamic nuclei, limbic regions and somatosensory structures. Cluster 4 (N=5) constituted white matter structures in which the R2DGU was also inversely related to T_b. Cluster 5 (N=29) was a heterogeneous group whose R2DGU values could not be readily classified in any particular pattern, but included all 6 cortical regions examined.

190.7

DIURNAL RHYTHM OF CORTICOTROPIN-RELEASING HORMONE (CRH) IN RAT CEREBROSPINAL FLUID (CSF). C.M. Barksdale,* L.K. Takahashi, and N.H. Kalin (SPON: L. Hegstrand). Dept. of Psychiatry, Univ. Wisconsin-Madison, and Psychiatry Service, Middleton Veterans Hospital, Madison, WI 53705.

CSF levels of peptide hormones are thought to reflect function of brain peptidergic systems and have been used to investigate the involvement of these systems in the pathophysiology of neuropsychiatric illnesses. Data suggest that CRH systems modulate the endocrine, autonomic, and behavioral consequences of stress.

We have studied the parameters that affect concentrations of CRH in CSF. Cisternal CSF was obtained from adult male rats at 0300, 0700, 1100, 1500, 1900, and 2300 hr. Eight rats were used for each time point. Rats were maintained on a light-dark cycle with lights on from 0600 to 1800 hr. CSF was aliquotted for each rat, and all CSF CRH-IR samples were measured in one assay.

CSF concentrations of CRH-IR varied rhythmically over time ($F=2.97$; $df=5,43$; $P<.02$). The peak concentration of 9.7±1.3 pg/ml (mean±SEM) occurred at 0300 hr. The nadir, 5.1±0.6 pg/ml, occurred at 1100 hr.

These findings demonstrate a diurnal rhythm in rat CSF CRH-IR similar to reported changes in rat hypothalamic CRH content and plasma concentrations of ACTH and corticosterone. Interestingly, our studies in rhesus monkeys show a diurnal rhythm of CSF CRH-IR opposite to that of peripheral pituitary-adrenal activity.

190.8

ANALYSIS OF THE *Clock* CIRCADIAN RHYTHM MUTATION IN *DROSOPHILA MELANOGASTER*.
John R. Nambu, Mitchell S. Dushay*, Michael Rosbash*, and Jeffrey C. Hall. Department of Biology, Brandeis University, Waltham, MA 02254.

In *Drosophila*, several loci have been identified which when mutated, lead to aberrant circadian and/or ultradian rhythm properties. We are employing genetic and molecular techniques to characterize one such locus, defined by the $Clock^{KO6}$ mutation. *Clock* was originally isolated by D. Orr and R. Konopka (D. Orr PhD thesis, 1982) based on ca. 22.5 hour periods for locomotor activity rhythms (1.5 hours shorter than normal). We have shown that in addition, cultures of $Clock^{KO6}$ exhibit 22.5 hour eclosion rhythms. In contrast, the ultradian rhythm of male courtship song is apparently normal (M. Greenacre and C. P. Kyriacou, unpublished results). Similarly to other rhythm mutations in *Drosophila* and other organisms, the *Clock* mutation is semi-dominant.

The *Clock* gene is X-linked; we have mapped the mutation via recombination analysis to the distal portion of the X chromosome in the region between the *yellow* and *white* genes. Preliminary deficiency mapping data suggest that *Clock* is uncovered by the small *white* deficiency, $Df(1)w^{258-45}$. *Clock* is thus quite near to *white* (3C1-2) yet appears to be distinct from and proximal to another nearby *Drosophila* rhythm gene, *period* (3B1-2), in that we have shown the w^{258-45} deletion to be $period^{+}$. Our cytogenetic data, and extant molecular clones isolated from the 3B-3C X-chromosomal interval, should facilitate the molecular isolation and characterization of this gene and its product(s).

190.9

DROSOPHILA CIRCADIAN RHYTHMS ARE DRASTICALLY DISRUPTED BY *disconnected* VISUAL SYSTEM MUTATIONS. Mitchell S. Dushay*, Michael Rosbash*, and Jeffrey C. Hall (SPON: H. T. Epstein) Dept. of Biology, Brandeis University, Waltham, MA 02254.

Wild-type *D. melanogaster* that are never exposed to light do not express circadian rhythms (Dowse, H. et al., Behav. Genet., 17: 19, 1987). Functional eyes are not necessary for flies to be rhythmic (Helfrich, C., J. Neurogenet., 3: 321, 1986), so flies must have extra-retinal 'circadian' photoreceptors. Blind flies that have defects in their 'circadian' photoreceptors should be insensitive to light and therefore arrhythmic. 96% of *disco* flies were arrhythmic in locomotor activity, and mutants showed weak and variable eclosion rhythms. Since 5-10% of *disco* flies have eyes connected to their brains (Steller et al., Cell, 50: 1139, 1987), we tested whether eye connection is sufficient for rhythmicity. Using both the deep pseudopupil and histological sectioning, we found no correlation between connected eyes and rhythmicity. However, this did not test if eye input is sufficient for rhythmicity, since *disco* phototaxis and optomotor responses were similar to those of blind flies whether the *disco* eyes were connected or not. *disco* flies are able to perceive and to respond to light, however. When tested in an LD cycle, mutant flies showed cyclic activity similar to the behavior of the other arrhythmic mutants; per^{0}. Therefore the *disco* mutations do not entirely abolish light perception, but must disrupt connections between photoreceptors and the circadian pacemaker, and/or disrupt the central pacemaker itself.

190.10

GASTROPOD CIRCADIAN PACEMAKER NEURONS STAINED BY ANTIBODY TO *DROSOPHILA PERIOD* PROTEIN. K.K. Siwicki*, J.W. Jacklet, and J.C. Hall. Biology Dept., Brandeis University, Waltham, MA 02254, and Dept. Biology, Neurobiology Research Center, SUNY Albany, Albany, NY 12222

The *period* (*per*) gene of *Drosophila melanogaster* regulates the period of circadian rhythms of eclosion and locomotor activity, as well as a short-period rhythm in the male fly's courtship song. A polyclonal antibody specific for a small domain (14 amino acids) of the *per* protein has been used immunocytochemically to label the cells and tissues in the fly that express the gene (Neuron *1:* 141-150, 1988). The affinity purified anti-*per* antibody was used to stain the eyes of the marine gastropods, *Bulla* and *Aplysia*, which contain endogenous circadian pacemakers. In the *Bulla* eye, a *per*- like antigen was detected in the cytoplasm of cell bodies of the basal retinal pacemaker neurons, and in the adjacent neuropil. No staining was present in the *Bulla* optic nerve, or in the photoreceptors of the retina. In the *Aplysia* eye, stained pacemaker neurons and varicosities were distributed around the eye, and stained axons were present in the optic nerve. Similar results were obtained in eyes examined during both the light and dark phases of the animals' daily entrainment cycles (12 hr L: 12 hr D). These results suggest that a *per*- like molecule may function in the circadian clocks of diverse species.

(Supported by NIH GM-33205 and NSF BNS 8510626.)

190.11

MECHANISM FOR REGULATION OF CIRCADIAN AND PINEAL PHYSIOLOGY BY ULTRAVIOLET RADIATION IN RODENTS
G.C. Brainard, R.A. Hoffman,* M.H. Stetson,* F.M. Barker* and M.D. Rollag,* Jefferson Med. Coll. Phila., PA 19107; Colgate Univ., Hamilton, NY 13346; Univ. Delaware, Newark, DEL 19711; Penn. Coll. Optometry, Phila., PA 19141; USUHS Bethesda, MD 20814.

Visible light (400-760 nm) is considered the primary environmental stimulus which regulates circadian and pineal physiology of mammals. Recently, near-ultraviolet radiation (UV-A, 320 -400 nm) has been shown to influence selected circadian and pineal functions in rodents. A set of experiments have been tested for the mechanism by which UV-A exerts these biological effects. These studies indicate: 1) UV-A exerts its CNS effects via the eyes; 2) ultraviolet radiation down to 300 nm is transmitted through the cornea, aqueous humor, lens and vitreous humor to the retina; 3) pigment of the iris and retinal pigment epithelium does not mediate the effects of UV-A on the circadian system; and 4) the harderian gland, anatomically close to the eye, does not mediate the responses to UV-A. These results support the hypothesis that elements in the rodent retina directly transduce UV-A photic information to the circadian and neuroendocrine system in rodents. Supported by Nat. Elec. Manuf. Assoc. (LRI 87:DR:2).

190.12

RUNNING ACTIVITY ENHANCES THE PHASE-SHIFTING EFFECT OF DARK PULSES ON HAMSTER RHYTHMS.
S.G. Reebs*, R.A. Lavery* and N. Mrosovsky. Dept. of Zoology, University of Toronto, Toronto, M5S 1A1, Canada.

Dark pulses can phase-shift the activity rhythms of hamsters kept in constant light (Boulos, Z., and B. Rusak, *J. Comp. Physiol.*, 146: 411, 1982; Ellis, G.B., *et al., Am. J. Physiol.*, 242: R44, 1982). Dark pulses under those conditions alter photic input to the circadian system, but they also commonly trigger running activity. Running activity is now known to affect the circadian system of hamsters (Mrosovsky, N., and P.A. Salmon, *Nature*, 330: 372, 1987). Therefore we asked: is the phase-shifting effect of dark pulses caused, at least in part, by running activity?

Male Syrian hamsters in constant light were given 3-h dark pulses at circadian time (CT) 9. Half of the animals did not have normal access to their running wheels. Their phase shifts averaged 0.56 h (S.D. = 0.32, n = 9). The other hamsters had access to their wheels and ran for more than half of the pulse duration; their phase shifts averaged 1.07 h (S.D. = 0.53, n = 8). The difference is significant ($p < 0.05$, t-test). Preliminary results suggest a similar difference for 6-h pulses given at CT 6. This indicates that running activity accounts for at least part of the phase-shifting effect of dark pulses, and warns about the confounding effects of behavioural states in photic manipulations.

Supported by the Natural Sciences and Engineering Research Council of Canada.

190.13

FEEDING AND LOCOMOTOR ACTIVITIES OF THE HOMING PIGEON: MELATONIN AND ITS EFFECTS ON RHYTHMICITY.
C.C. Chabot* and M. Menaker. Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901.

Measurement of general locomotor activity in homing pigeons often does not allow for accurate interpretation of period or phase of the circadian rhythm. Feeding activity, a more specific behavior, often provides a better assay. Feeding activity of both intact and pinealectomized (P-X) pigeons is rhythmic in L:D (12:12) cycles and in constant darkness (DD). Pigeons exposed to several intensities of constant light (LL) exhibit clear changes in both feeding and locomotor rhythms. In general, rhythms persist at low intensities of LL, while at higher intensities the birds become arrhythmic. The implantation of melatonin filled silastic capsules into intact pigeons abolishes rhythmicity in both behaviors; their removal immediately restores it.

Intra-cardial and sub-cutaneous cannulae were implanted into intact pigeons enabling the simultaneous infusion of melatonin and withdrawal of blood samples in freely moving birds. Melatonin was delivered via the sub-cutaneous cannula for six hours (1.3 ug/hr) each day in the middle of the subjective day to five P-X pigeons in DD. One of the five became arrhythmic while the other four entrained to the exogenous melatonin cycle by shifting their subjective day by about 12 hours. They remained entrained until the timing of the melatonin infusion was changed. The changes in behavior produced by melatonin infusion will be correlated with changes in blood levels of the hormone.

OPIATES, ENDORPHINS AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS III

191.1

OPIOIDS RECIPROCALLY REGULATE THE M-CURRENT IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS IN VITRO.
S.D. Moore, S.G. Madamba and G.R. Siggins. Research Inst. Scripps Clinic, 10666 N. Torrey Pines, San Diego, CA, 92037.

Two opioid systems, one containing enkephalin and one dynorphin, exist in the hippocampal CA3 field. Previous studies in our lab revealed multiple effects of these opioids on CA3 neuronal discharge and membrane potential. To determine if such diverse responses arise from voltage-dependent mechanisms, we voltage-clamped CA3 neurons in submerged, superfused hippocampal slices. At holding potentials of -40 to -45 mV, hyperpolarizing commands elicited ohmic current drops followed by slow carbachol-sensitive current relaxations likely to represent the voltage-dependent K^+ current, I_M. Superfusion of the relatively receptor-unselective opioid D-ala^2, D-leu^5 enkephalin decreased I_M, measured from the instantaneous ohmic step to steady state. High dynorphin concentrations (1 μM) either had no effect or decreased I_M. However, low (0.1 μM) dynorphin (likely to be selective for κ receptors) significantly augmented I_M by 40-70% (n=7). The κ-selective agonist, U-50,488H (5-10 μM) also significantly increased I_M by 66-74% (n=8). This effect is similar to that recently described (Moore et al. Science 239:278,1988) for somatostatin. We propose that μ and κ receptor activation may, depending on membrane potential, reciprocally decrease and increase I_M, respectively, causing the divergent dynorphin effects seen previously. Supported by NIDA (DA-03665).

191.2

POSSIBLE INVOLVEMENT OF LOCUS COERULEUS IN FENTANYL-INDUCED MUSCULAR RIGIDITY IN THE RAT.
P.W. Lui*, T.Y. Lee* and S.H.H. Chan (SPON: T.C. Fu). Department of Anesthesiology and Institute of Pharmacology, National Yang-Ming Medical College and Veterans General Hospital, Taipei 11217, Taiwan, Republic of China.

Whereas muscular rigidity is a well-known side effect that is associated with high-dose fentanyl anesthesia, a paucity of information exists with regard to its underlying mechanism(s). We investigated in this study the possible engagement of locus coeruleus in the pons in this phenomenon, using male Sprague-Dawley rats. Under proper control of respiration, body temperature and end-tidal CO_2, intravenous administration of fentanyl (50 and 100 µg /kg) consistently promoted an increase in electromyographic (EMG) activities recorded from the gastrocnemius and abdominal rectus muscles. Such an induced muscular rigidity by the narcotic agent was significantly antagonized or even reduced by prior bilateral electrolytic lesions of the locus coeruleus or pretreatment with the α-adrenoceptor blocker, prazosin (200 µg/kg, i.v.), signifying the involvement of this norepinephrine-containing pontine nucleus in that process. These data suggest that our hypothesis that locus coeruleus may participate in fentanyl-induced muscular rigidity is valid, although the functional role of this nucleus awaits further investigation.
(supported by NSC 77-0412-b075-18 and CRC, VGH 46500-005)

191.3

DIFFERENT MU OPIOID RECEPTOR SUBTYPES MEDIATE SPINAL AND SUPRASPINAL ANALGESIA. G.W. Pasternak, D. Paul,* and R.J. Bodnar. The Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Cornell U. Medical College, NY, NY 10021.

Previous studies have implicated mu_1 receptors in the analgesia produced by microinjection of the opioid peptides DAGO (mu_1 + mu_2) and DSLET (mu_1 + delta) into the PAG, n. raphe magnus, and locus coeruleus, based in part on their sensitivity towards the mu_1-selecitive antagonist naloxonazine. We have examined the relative roles of mu_1 and mu_2 receptors in spinal and supraspinal opioid analgesia in mice following intrathecal (IT) or intracerebroventricular (ICV) injections of the selective agonists DAGO, DSLET and DPDPE. Naloxonazine shifted the analgesic dose-response curves of ICV DAGO and DSLET 7-fold and 10-fold, respectively. In contrast, naloxonazine did not affect the potency of either compound following IT adminstration. Although naloxone reversed IT DAGO and DPDPE analgesia, DAGO was ~10-fold more sensitive, implying that IT DAGO was not acting through delta receptors. The high selectivity of DAGO for mu receptors and its potency both ICV and IT implies mu mechanisms at both levels of the neuroaxis. Yet, naloxonazine dramatically reduces the analgesic potency of DAGO ICV but not IT. These findings suggest that DAGO produces analgesia supraspinally through mu_1 receptors and spinally through mu_2 receptors.

191.4

AREA TEMPESTAS MODULATES THE ANALGESIC RESPONSES OF SYSTEMIC INJECTION OF MORPHINE IN THE RAT. M. Massotti, P.Lorenzini*and A.D'Amore*. Lab. di Farmacologia, Istituto Superiore di Sanità, Roma, Italy, 00161.

Various solutions of naltrexone-HCl [NLT] were microinfused into the Area Tempestas [AT] in a volume of 120 nl/rat 20 min after injection of morphine sulfate [MS] (5 mg/kg sc).

Twenty min after systemic MS, an increase in the reaction latencies can be observed in the hot-plate test (from 1.9±0.4 to 19.1±1.1 sec). This effect lasts 100-140 min, returns to the basal values at the 5th h and is not affected by local application of 120 nl of saline. Local application of NLT (8-60 ng/rat) dose-dependently reduces the values of tail-withdrawal reaction between 20-30 min after the end of the infusion.

In the hot-plate test, twenty min after systemic MS the latencies in licking reaction time increases from 6.4±0.8 sec to 35.4±4 sec. Five-ten min after unilateral application of NLT,associated with contralateral application of saline, a dose-dependent reduction in the delay of the response in both the contralateral (8-30 ng/rat) and ipsilateral (15-30 ng/rat) paws is noticed.

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191.5

EFFECTS OF ACUTE ADMINISTRATION OF NALTREXONE ON PLASMA CONCENTRATIONS OF NALTREXONE, 6-β-NALTREXOL, β-ENDORPHIN AND CORTISOL IN CHILDREN. B.H. Herman, A. Arthur-Smith*, K. Verebey*, J. Alrazi* and M.K. Hammock*. Brain Res Cen & Dept Psychiatry of CHNMC & George Washington Univ Sch Med, Washington, DC and PDLA, South Plainfield, NJ.

The opiate antagonist, naltrexone (NTRX), may be useful in treating self-injurious behavior (SIB) and autism in children (Herman et al. Ann Neurol 22:550, 1987). Here we measured the concentrations of NTRX, 6-β-naltrexol (6βNLT), major metabolite), β-endorphin (β-E) and cortisol (COR) after administration of NTRX in children. Groups studied: autistics (N=5, 4-12 yrs) without SIB and SIB subjects (SIBS) (N=3, 10-17 yrs). Blood was drawn 1h after oral NTRX or placebo (P). NTRX(ug/L) and 6βNLT(ug/L) were analyzed on a GC/MS; β-E(pmol/L) and COR(nmol/L) by RIA. Table presents plasma values for autistics.

		Naltrexone (mg/kg)				
	P1	0.5	1.0	1.5	2.0	P2
NTRX	0±0	13±1	21±2	32±5	42±7	0±0
6βNLT	0±0	50±6	60±12	107±20	159±46	0±0
β-E	8±1	9±2	7±3	8±1	8±2	8±1
COR	357±31	294±88	399±159	415±124	404±132	383±87

Significant concentrations of NTRX and 6βNLT were detected at every dose. There were no significant differences between autistics and SIBS in plasma NTRX and 6βNLT levels. In both groups, there were no significant effects of NTRX on plasma concentrations of β-E or COR.

191.6

β-ENDORPHIN-(1-27) IS A POTENT ENDOGENOUS HYPOTENSIVE AGENT. M.D. Hirsch, W.R. Millington, J.E. McKenzie* and G.P. Mueller. Dept. of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Like morphine, β-endorphin(βE)-1-31 has a pronounced influence on peripheral hemodynamics by acting upon putative opioid binding sites in the dorsal medulla. The present study utilized this central regulatory system to elucidate how the post-translational processing of βE-1-31 may contribute to diverse processes of bioregulation. Direct measures of mean arterial pressure (MAP) and heart rate (HR) were made in anesthetized rats. Unexpectedly, βE-1-27 displayed a 10-fold greater central hemodynamic potency as compared to βE-1-31: By 60 min post injection, 0.15 nmol βE-1-27 (i.c.) produced a reduction in MAP (25.9 ± 4.7 mm Hg below control (94.7 ± 1.9 mm Hg)) which equalled that of 1.5 nmol βE-1-31 (29.7 ± 3.9 mm Hg). A higher βE-1-27 dose (1.5 nmol) further reduced MAP (47.3 ± 5.3) to 50% of control values. Thus in contrast to analgesia, in which C-terminal proteolysis converts βE-1-31 to a potent opioid receptor antagonist, βE-1-27, this processing step enhances the hemodynamic potency of the peptide. However, as shown previously for analgesia, C-terminal cleavage of βE-1-27 to βE-1-26 and N-acetylation of both βE-1-31 and βE-1-27 eliminated hemodynamic activity. These results suggest that the C-terminal proteolysis of βE-1-31 subserves quite different roles in defining the cardioregulatory and analgetic activities of the peptide.

191.7

HAMSTER ENKEPHALIN-PEPTIDES AND mRNA ARE ALTERED BY RESERPINE. S.O. Franklin*, A.D. Branch*, H.D. Robertson*, B.C. Yoburn and C.E. Inturrisi. Pharmacology, Cornell Univ. Med. Coll. and Rockefeller Univ., New York, NY 10021.

Hamster adrenal, in contrast to the rat, contains high levels (200 pmole/gland) of enkephalin-containing peptides (ECPs) so that this species is particularly useful for the study of the modulation of ECPs. The adrenal ECPs appear to be proenkephalin while striatal ECPs are low molecular weight (free enkephalins). Two consecutive daily treatments with reserpine produce a dose (1.25 to 10 mg/kg) dependent depletion of adrenal ECPs at day 1 (max. 75% decrease with 10 mg/kg reserpine) and a 60-100% increase in preproenkephalin mRNA at day 1 by Northern analysis. Known amounts of unlabeled proenkephalin RNA transcripts were used for quantitation. Thereafter, ECPs increase above control at days 4 and 12. Adrenal preproenkephalin mRNA is still elevated (200%) at 12 days after reserpine treatment. At day 1 striatal ECP levels show a small (40%) but significant increase which disappears by days 4 and 12 post reserpine. However, at day 1 there is a dose dependent increase in striatal preproenkephalin mRNA of 75 to 200%, which has returned to control values by day 12. Thus, reserpine induced changes in ECPs are associated with changes in proenkephalin gene expression in both adrenal and striatal tissues. Transcriptional regulation after reserpine treatment has a different time-course for adrenal proenkephalin (prohormone) than for striatal enkephalins (neurotransmitter). Supported by DA-01457 and DA-05130.

191.8

IN VIVO DIALYSIS MEASURES OPIOID PEPTIDE RELEASE IN THE RAT GLOBUS PALLIDUS. N.T. Maidment*, D.R. Brumbaugh*, V.D. Rudolph*, E.Erdelyi*, K.F. Faull, M.C. Dement, J.D. Barchas and C.J. Evans. Pritzker Laboratory and Sleep Research Center, Dept. of Psychiatry, Stanford University Medical Center, Stanford, CA 94305

We report the measurement of opioid peptides in vivo using both commercially available dialysis probes and smaller custom-made units. The recovery values obtained in 1nM solutions of met-enk were 15-20% and 10% respectively. A "universal" opioid RIA with an IC_{50} of 1 fmole was developed with Ab's directed towards the N-terminal Try-Gly-Gly-Phe sequence. Basal release produced approx. 3fmoles of opioid peptide-like immunoreactivity per 30min sample in the absence of peptidase inhibitors. Incorporation of bestatin (1μM) and thiorphan (200μM) elevated the recovered immunoreactivity approx. 3-fold. The release was increased by a factor of 10-15 by 100mM KCl. Samples were subjected to reverse-phase HPLC and the immunoreactivity tentatively identified as Met-enk, Leu-enk and Met-enk-Arg-Phe. We are investigating changes in this profile in the presence of peptidase inhibitors and the regulation of opioid peptide release by dopamine using the dialysis probes for simultaneous drug administration and peptide measurement.

191.9

THE EFFECTS OF DOPAMINE AGONISTS AND ANTAGONISTS ON PROENKEPHALIN mRNA AND OPIOID PEPTIDES MEASURED IN THE SAME TISSUE EXTRACTS. M.E. Abood*, J.H. Eberwine*, E. Erdelyi*, J.D. Barchas and C.J. Evans, (SPON: Stuart Leff). Nancy Pritzker Laboratory, Dept. of Psychiatry, Stanford University, Stanford, CA 94305.

In order to reduce intraspecies variation, differences in dissection, and the number of animals required for statistical significance, we have developed a technique for analysis of mRNA and opioid peptides in the same tissue extracts. This method involves the extraction of tissue in 5M guanidine thiocyanate, 10 mM EDTA, 50 mM Tris, pH 7.5, 8% ß-mercaptoethanol, and subsequent precipitation of the RNA with 4M LiCl. The RNA is isolated by a second precipitation in 3M LiCl, followed by solubilization in 10 mM Tris, 1 mM EDTA, 0.1% SDS, phenol/chloroform extraction, and ethanol precipitation. After this procedure, the RNA can be quantitated using standard methods. The peptides, which are in the supernatant of the 4M LiCl precipitation, are adsorbed onto Sep Pak (C18 resin) and eluted by acid acetone. The total opioid peptides are measured by chemical acetylation of the eluted peptides, followed by RIA using an assay specific for α-N-acetyl-Tyr-Gly-Gly-Phe-X. We have used this technique to measure the effects of haloperidol and bromocryptine on proenkephalin mRNA and total opioid peptides contained in the tissue samples from various discrete regions of the rat brain.

191.10

EVIDENCE FOR THE ACTIVATION OF SPINAL KAPPA OPIATE RECEPTORS DURING PREGNANCY. H.W. Sander*, P.S. Portoghese*+ and A.R. Gintzler. (Spon: H. Begleiter). Depts. of Biochemistry and *Medicinal Chemistry, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203 and +College of Pharmacy, Univ. of Minn., Minneapolis, MN 55455.

In a variety of laboratory animals as well as humans, pregnancy has been associated with an activation of a maternal opioid system(s) and with a concomitant elevation in the threshold for maternal responsiveness to aversive stimuli. This analgesia is mediated via the activation of spinal opiate receptors and does not require an intact peripheral opioid system(s). The recently developed kappa-selective opioid antagonist, nor-binaltorphimine (nor-BNI), significantly reduces the threshold for reflexive jumping in response to electric foot shock when administered to the lumbar intrathecal (i.t.) space of pregnant rats (day 20 of gestation). In contrast, i.t. nor-BNI when administered to nonpregnant rats as well as systemic (intraperitoneal) administration of an intrathecally effective dose of nor-BNI to pregnant rats is without effect on the jump thresholds. These data indicate that the kappa type of opiate receptor mediates, at least in part, the analgesia observed during gestation thus providing an important physiological function for this receptor type.

191.11

KAPPA OPIOID RECEPTORS ACTIVATION COUNTERACTS URINE RETENTION DUE TO INTRA SPINALCORD INJECTION OF COLCHICINE IN RATS. M. Baraldi and P. Zanoli*. Chair of Pharmacology and Pharmacognosy, Modena University, School of Pharmacy, 41100 Modena, Italy.

Continence and urination require a good coordination between the inhibitory sympathetic influence and the parasympathetic bladder excitatory activity. This coordination is controlled by intra and supraspinal impulses. We have documented that the intraspinalcord (i.s.c.) injection of low doses (2.5-10 ug/rat) of colchicine (C) in rats induces in a dose related fashion an irreversible urine retention due to inability of bladder to void. Supraspinal influence activated by i.s.c. injected C was excluded by the demonstration that the toxin remains at the site of injection. The notion that μ and δ opioid receptor agonists inhibit bladder motility prompted us to study the levels of endogenous opioids and the characteristics of opioid receptors in the spinal cord of normal rats and rats with C-induced urine retention. Here we report that 72 hs after the i.s.c. injection of C we found a significant increase (0.15 vs 0.08 ng/mg prot.) of Met-Enkephalin (M-Enk) in the lumbar region of the spinal cord which was parallelled by a decreased affinity of δ receptors labelled by ^{3}H-DADLE and which was thought to be responsible of urine retention throught a spinal action. It is noteworthy however that the C-induced urinary retention was minimized by the systemic chronic injection of the kappa receptor agonist bremazocine. In conclusion our observation seems to suggest that the spinal mechanism(s) which regulates bladder motility might be modulated in an opposite direction by M-Enk and dynorphin.

191.12

MULTIPLE OPIOID MODULATION OF PROLACTIN RELEASE IN THE RAT: SUBCHRONIC TOLERANCE STUDIES. S. Iyengar, H. S. Kim, P. Callahan and P. K. Wood. Ciba Geigy Corp., Summit, NJ 07901 and R. W. Johnson Medical School, Piscataway, NJ 08854.

Opioid modulation of prolactin release was evaluated using opiate drugs and opioid peptides in subchronic tolerance studies. The mu opiate agonist morphine (MS) and several kappa opiate agonists including U50488H (U50), potently increased prolactin release in a dose-dependent, antagonist reversible manner. Opiate antagonists naloxone and WIN 4441-3 potently inhibited prolactin release in opiate-naive rats, implicating endogenous opioid peptides in modulating prolactin release. B-endorphin (END), dynorphin (DYN) and the specific enkephalin analogue DPDPE also caused prolactin release with END being the most potent of all compounds tested. MS, U50, END and DYN were then injected into animals made subchronically tolerant to either MS, U50 or END, (Iyengar et al, Brain. Res., 1987, 435: 220). Animals exhibited no cross tolerance with respect to the effects of MS and U50, implying action at independent receptors. The effect of DYN was supressed in U50-tolerant rats, indicating action at kappa opioid sites. The effect of END did not show cross-tolerance to either U50 or MS while the effect of MS was completely inhibited in END-tolerant rats. Thus, while some of the effects of END may be mediated through mu opioid sites, they may also be mediated via another site, possibly the putative "epsilon" opioid receptor site. These studies also implicate independent kappa opioid receptor sites in modulating prolactin release.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: POSTURE AND MOVEMENT V

192.1

MOTOR STRATEGIES FOR THROWING A HEAVY WEIGHT. P.R. Burgess and J.W. Woodbury, Dept. of Physiol., Univ of Utah School of Medicine, Salt Lake City, UT 84108.

A volunteer faced a force transducer mounted waist high on a horizontal track and placed his right hand on the transducer. The transducer was attached via a cable and a pulley to a load weight (LW) exerting 155 Newtons (35 lb). The task was to lift and accelerate the LW by "throwing" the carriage from right to left. Force (F), position (x) and velocity (v) were measured. During hard throws, F rose rapidly until F exceeded LW, then rose more slowly with an upward concavity to a 50% higher peak, and then fell sharply. Peak F's ranged from 225-275 Newtons; peak v's from 160-190 cm/s; x's at release from 23-33 cm; and duration from 0.65-0.95 sec. Peak v varied less than duration; shorter durations (correlated with smaller x's at release) were compensated by faster F buildup. For constant muscle activation, the F-v relation of muscle requires that F decreases as v increases. Conclusions: (1) F-v effects within the muscle appear to be compensated by muscle activation that increases faster than v increases. (2) The throw is terminated when velocity reaches a predetermined value. (3) The concave upward F vs. t curve reduces the amount of energy "wasted" in shortening the muscle against its internal viscosity.

192.2

TWO STRATEGIES OF SINGLE JOINT HUMAN ELBOW MOVEMENT: THE SPEED-INSENSITIVE STRATEGY. G.L.Gottlieb, D.M.Corcos & G.C.Agarwal. Rush-Presbyterian St. Luke's Medical Center, Chicago, IL, 60612.

We propose organizing principles for the control of single joint human movements in which tasks are performed by one or a combination of two strategies. These are Speed-Insensitive and Speed-Sensitive strategies.

The tasks were to make elbow flexions with different inertial loads or to targets at different distances. Movement speed was altered by both variations of the task. In this experimental paradigm, subjects used a Speed-Insensitive strategy.

Inertial torque, acceleration, velocity, and movement time all highly correlated with the task variable, load or distance. The significant variable with which EMG, integrated over the acceleration phase of the movement remained strongly and positively correlated, was inertial torque.

The rate at which torque was developed to accelerate the movements was invariant over changes in the value of the task variable, as was the agonist EMG, integrated for the first 30 ms of activity.

Two components can be distinguished in the antagonist EMG. The first, of constant latency and amplitude is terminated by the second and larger component at a latency proportional to movement time. The area of the second antagonist component is proportional to the decelerating force which is proportional to both task variables. When target distance varied, this could only be demonstrated by adjusting for the effects of muscle length on force and on the EMG.

A model is proposed in which movements made under a Speed-Insensitive strategy are executed by controlling only the timing of an invariant pattern of activation of the motor neuron pool.

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192.3

TWO STRATEGIES OF SINGLE JOINT HUMAN ELBOW MOVEMENT: THE SPEED-SENSITIVE STRATEGY. D.M.Corcos, G.L.Gottlieb, and G.C.Agarwal. Rush-Presbyterian St. Luke's Medical Center, Chicago, IL, 60612.

We propose organizing principles for the control of single joint movements in which tasks are performed by one or a combination of two strategies. These are Speed-Sensitive and Speed-Insensitive strategies.

Subjects made discrete flexions of the elbow from a stationary initial position to a visually defined target at a fixed distance. Movement speed was operationally controlled either by explicit instruction to the subject or by adjusting the size of the target to which the subject moved. Instructions always included the requirement to accurately hit the target. These experimental paradigms cause subjects to use a Speed-Sensitive strategy of movement control.

Inertial torques, accelerations and movement times correlated with speed. The agonist EMG, integrated over the acceleration phase of the movement, was strongly and positively correlated with inertial torque.

When subjects perform tasks which require control of movement speed, they accomplish this by adjusting the rate at which torque is developed by the muscles. This rate is modulated by the way in which the muscles are activated. The rate at which joint torque develops is correlated with the rate at which the agonist EMG rises.

The antagonist EMG shows two components which scale with torque. The first is a coactivation component which has a latency independent of movement dynamics. The second component is a deceleration burst which has a latency of onset proportional to movement time.

A model is proposed in which movements made under a Speed-Sensitive strategy are executed by controlling the intensity of activation delivered to the motor neuron pool. The kinematic effect is to regulate the rate at which torque, and consequently acceleration, increases.

Supported in part by NIH grants AR 33189 and NS 23593

192.4

UPDATING OF ISOMETRIC FORCE TRAJECTORIES: CORRECTIONS FOR UNEXPECTED CHANGES IN TARGET AMPLITUDE. J. Gordon, R. Bermejo, & C. Ghez. Ctr for Neurobiol & Behav, Columbia Univ and NYS Psych Inst, New York, NY 10032.

The purpose of this study was to determine whether human subjects can utilize new visual information to modify the dynamic phase of rapidly rising force impulses aimed to an earlier visual target. This question was addressed by comparing the trajectories of impulses of elbow flexion force to a single target occurring 450 ms before the response (controls) with ones in which a second target step occurred 80–140 ms before response initiation (double steps). Response timing was controlled by having the subjects initiate their responses in synchrony with the last of a series of four regularly spaced tones. In all trials, an initial target step of one of 3 possible amplitudes was presented 450 ms prior to the synchronizing tone, then, unpredictably in half the trials, the target was again stepped to a new randomized level 120 ms before the tone. Subjects were instructed to correct their responses when a second target step occurred. In all subjects, the peak forces of responses to the double steps were significantly influenced by the amplitude of the second target (r^2 = .51 to .65). This was achieved by adjustments in the rise time of the force. Mean time from response onset to peak force ranged from 104 to 127 ms in control trials, while in double step trials it ranged from 109 to 143 ms. The gain of these corrective adjustments (actual correction divided by required correction) varied between .5 and .6, indicating that subjects incompletely compensated for the new target. Corrections were more effective when the second target was larger than the first. As expected from our earlier studies of response scaling when specification is prematurely interrupted (Neurosci Abstr, 10:801, 1984), the correction for the second target step showed a prominent central tendency bias: the amplitudes of corrective adjustments, while appropriate in direction, were stereotyped in their amplitudes. These results contradict the view that rapid responses cannot be corrected during their course. New target information can be used to modify ongoing responses; however, the reduced gain and central tendency bias of corrective adjustments may have obscured their presence in other studies. (Supported by NS22715)

192.5

DISORDERS OF TRAJECTORY FORMATION IN EARLY PARKINSONISM. T. A. Zeffiro and D. L. Claman. Department of Neurology, University of Wisconsin School of Medicine, Madison, WI 53792.

Analysis of motor performance in patients with Parkinsonism (PD) has been utilized to study the role of the basal ganglia in the process of movement planning and execution. Nevertheless, interpretation of these studies has often been limited by difficulties inherent in analyzing movements of great kinematic complexity and the often uncontrolled and confounding effects of antiparkinsonian medications. To draw inferences concerning mechanism from such data, the observed trajectory abnormalities should not be too complex or variable. In an attempt to simplify the problem, we studied the kinematics of visually triggered arm movements in unmedicated patients with early PD.

Hand trajectories of patients with clinically mild to moderate PD were compared to those of an age-matched, healthy control group. All subjects were free of medication for two weeks prior to the experiment. Movements were recorded as the subject, while sitting in total darkness, tracked a visual stimulus on the surface of a 20" x 20" digitizing tablet. The only instructions were to move as quickly and accurately as possible to the target when it became visible. Vision of the target during trial was manipulated as follows: In one condition the target vanished as the movement began (Toff); and in the other the target remained visible throughout the trial (Ton). A minimum of 200 movements were recorded in each condition.

Quantitative trajectory analysis revealed consistent spatial and temporal abnormalities. In the Toff condition, the group with mild PD exhibited normal movement times and peak velocities. Both spatial and temporal properties scaled appropriately with movement size. Although vision of the target (Ton) resulted in normal improvements in accuracy, this increase was accompanied by abnormal scaling and inappropriately large deceleration times. This effect increased in relation to the clinical severity of the PD. In more severe cases, there appeared additional abnormal scaling during acceleration with inappropriately small peak velocities and hypometria (Ton>Toff).

These results suggest that one function of the basal ganglia may be to integrate visual information during the latter part of the trajectory formstion process.

192.6

REPRESENTATION OF SPEECH MOVEMENTS: EVIDENCE OF BASIC MOTOR PROCESSES. V. L. Gracco. Haskins Laboratories, New Haven, CT 06510.

Human motor actions involve the production of numerous movement patterns reflecting various goal-related activities. Speech, for example, involves the production of 44 basic sounds, each of which is unique in terms of acoustic characteristics. However, it is not clear whether the movements associated with each sound are singly represented and conversely whether their coordination is fundamentally different for the basic sound productions. The present study evaluated the similarity of the kinematics and coordination among the multiple movements associated with a class of speech sounds.

Subjects were asked to repeat six speech-like words constructed to allow manipulation of the extent of oral opening prior to the first closing movement. By using all possible combinations of sounds made with both lips and jaw (e.g., p,b,m) it was possible to evaluate the similarity of the geometric form of the velocity profiles and the coordinative timing for this class of speech sounds.

Inspection of the the geometric form of the velocity profiles from the upper and lower lips suggested a scalar relation underlying the different lip sounds regardless of the preceding vowel. Further, although the absolute timing of the coordinative adjustments were different for the different sounds, the relative timing relations were similar. Variation in the degree of oral opening due to different preceding vowels modified the relations among the lip and jaw movements.

The scaling of velocity profiles in the present and previous studies and the systematic timing relations among the multiple articulators suggest that speech movements may be organized around basic movement relations and control actions. Further, while certain aspects of the basic control actions are invariant, certain aspects are context dependent reflecting the multiplicity of processes underlying motor control. Supported by NIH (NS-13617).

192.7

TIMING AND MAGNITUDE CHANGES IN PRE-PULL ANKLE TORQUES AND EMG ACTIVITY FOR HANDLE PULLS OF VARIED MAGNITUDE. W.A. Lee, C.F. Michaels* and Y.C. Pai*. Physical Therapy, Northwestern Univ., Chicago IL 60611.

This study describes how pre-pull ankle torques and EMG activity in leg muscles of standing subjects change when the magnitude of the pulling torque is increased. Five well-practiced subjects made 10 abrupt pulls on a fixed handle attached to a load cell located at elbow height, at 5, 10, 20, 40, 60, 80 and 95% of maximum pulling force (MPF). Initial location of the center of pressure and handle force were controlled. Ankle torques associated with shear (anteroposterior: Tsh) and vertical (Tv) ground reaction forces were computed from force platform data. The torque about the ankle joint due to the pull, Tp, was computed from load cell force. EMG activity was recorded from gastrocnemius (GS), tibialis anterior (TA), biceps femoris (BF) and quadriceps (QD) muscles. All variables were sampled at 200Hz. We measured torque and EMG onset times, peak torques, pre-pull impulses due to Tsh and Tv (Ish; Iv) and the impulse due to Tp from the onset to the peak of the pull.

The onset times of Tsh and Tv were correlated with Tp ($r < -.94$); Tv onset always preceded Tsh, and led Tp onset by up to 700ms. Iv and Ish increased with peak Tp, due to both longer durations and higher peaks for Tsh and Tv. Ish had no abrupt changes in magnitude, although subjects appeared to switch to a "hip strategy" for larger pulls. GS onset was correlated with ($r > .96$) and preceded Tv, indicating a causal role in generating Tv and the pull. No other muscle was activated consistently before Tv by all subjects. All subjects activated fewer muscles at low than at high forces. TA, BF, and QD were recruited at various percentages of MPF. All muscles were recruited by 80% of MPF. Thus, continuous changes in pre-pull ankle torques and gradual recruitment of muscles were observed as the magnitude of the pull increased.

192.8

VESTIBULAR AND PROPRIOCEPTIVE INFLUENCES ON POSTURAL STRATEGIES. J.H.J. Allum, F. Honegger* and C.R. Pfaltz*. ORL Dept.,Basel Univ.,Basel,CH.

Human postural strategies responsible for the coordinated activation of leg and trunk muscles during equilibriating movements may be elicited by proprioceptive and/or vestibular inputs. To test the relative influences of these inputs on EMG response strategies support surface rotations and translations with equal (30 deg/s) ankle rotations, but oppositely directed head accelerations were imposed on normals and patients with absent vestibular function. The major response to toe-up rotation consisted of a coactivated stabilising action in tibialis anterior (TA) and quadricepts (QUAD) assisted by activity in paraspinal (PARAS) and trapezius (TRAP). Onset order was TRAP=PARAS, TA=QUAD. Rearward translations elicited the major reaction in soleus (SOL) and hamstring (HAM) muscles, and enhanced responses in PARAS. Onset order was SOL=ABDOM, HAM=TRAP, PARAS. Patient leg muscle responses to rotation were significantly less than normal, whereas those to translation were only altered in latency. These results in normals and patients indicate that vestibular and proprioceptive inputs, other than at the ankle, are responsible for eliciting the correct equilibriating strategy.

192.9

MUSCLE SPINDLES INFLUENCE NOT ONLY THE PLANNING BUT ALSO THE EXECUTION OF ANTAGONISTIC MUSCLE ACTIVITY IN HUMANS. Benoni B. Edin (SPON: J.H. Abbs). Dept of Physiology, University of Umea, S-901 87 Umea, Sweden (Present address: Speech Motor Control Lab, Waisman Center, Univ of Wisconsin, Madison WI 53706).

It has recently been shown that human muscle spindles (MS) become sensitized, i.e. show an enhanced response to subsequent slow stretch, when their parent muscle has been subjected to repeated rapid stretches and then kept at a short length (Edin, B.B. and Vallbo, A.B., J Physiol, 400:0-0, 1988). In contrast, MS become desensitized when kept at a long length after the rapid stretches. This property is conceivably exclusive to MS, and was in the present study exploited to assess the role of MS in planning and executing finger movements.

Passive, slow ramp-and-hold movements were imposed on a metacarpophalangeal joint and subjects reproduced these movements actively. The MS of the finger extensor muscles were either sensitized or desensitized before the imposed movement and either sensitized or desensitized before the active movement. In some experiments, microneurography recordings from muscle spindle primary afferents were obtained.

1. Afferent inputs from MS influence the planning of antagonistic muscle activity: The discharge of MS during muscle stretch is larger when MS are in the sensitized state compared to the desensitized state. As such, discharge of sensitized MS should give the impression of a larger movement then do desensitized MS. With all subjects, the reproduced movements showed significant differences in the predicted direction.

2. Afferent inputs from MS influence the ongoing execution of pre-planned antagonistic muscle activity: If MS of the finger extensor muscles access the motoneurone pool of the flexor muscles via the Ia inhibitory interneurones, then reproduced movement should be smaller when the MS of the extensor muscles are sensitized vs. desensitized. In fact, such differences were found with all subjects.

192.10

CONTRIBUTION OF PROPRIOCEPTION VS. EFFERENCE COPY TO THE CONTROL OF POINTING MOVEMENTS IN MAN. O.Bock*, R.Eckmiller, and K.Holthoff*. Div.of Biocybernetics, Univ.of Düsseldorf, 4000 Düsseldorf, FRG.

Ss. pointed with their right arm at visual targets, sequentially presented on a cylindric screen 45 cm in front of their eyes. The hand grasped a lever, restricting motion of index fingertip to an arc just in front of cylinder, and just below targets. A horizontal panel prevented vision of the arm, but a flag could pop up for visual feedback (VF). The arm was moved actively by Ss. (A), or passively by torque motor (P).

One set of expts. analyzed pointing accuracy when VF was available only at the starting position of each movement. The gain (right arm amplitude / target displacement) was in A around 0.90 for all movements, and averaged in P 0.84 for rightward, and 1.00 for leftward movements, independent of starting position; these direction differences were highly significant ($p<0.01$). Variability (s.d.) was 30% smaller in A vs. P. These findings suggest that efference copy is available to centers for pointing movement control, improving their performance, and that direction-specific control mechanisms are involved (as suggested before from other evidence by Bock et al, Soc Neurosci Abstr 13, 1987, p716).

Another set of expts. analyzed unidirectional pointing sequences with VF only at the first target of sequence. Both in A and P, pointing errors accumulated within sequences; successive errors were closely correlated ($r=0.74$ in A, $r=0.80$ in B). From this we conclude that error accumulation can not be attributed to the open-loop character of efference copy, but rather suggests that amplitude is a major controlled variable of active as well as passive pointing movements.

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192.11

CEREBRAL ACTIVITY ASSOCIATED WITH THUMB FLEXION IN MAN. D.H. York, D. Fousek* and S. Dulebohn*. Dept. of Physiology, Sch. of Med., Univ. of Missouri, Columbia, MO 65212.

The present study was undertaken to evaluate whether force is the principle variable which is defined by the motor output for flexion movement of the thumb in man. Studies were undertaken in normal volunteers who had scalp recording electrodes placed over the motor and sensory hand areas. Subjects performed a ballistic thumb flexion-extension movement over a fixed displacement against a known load. Each response was segregated into different files according to the velocity of the thumb flexion movement. In recordings from the sensory hand area, an inverse correlation was observed between the latency of an N3 component and the velocity of thumb flexion, which was consistent over four different loads. An inverse correlation was found between load and the latency of a P1 component recorded from the motor hand area. The latency of P1 varied from 8.8±sd8.3 msec to 44±sd25 msec for loads ranging from 792-300 gms. Increasing the displacement of the thumb flexion from 2-6 cm was observed to cause an increase in amplitude of the wave component immediately following the onset of movement. In summary, scalp recordings obtained from the sensory and motor hand areas of humans during ballistic thumb flexion-extension movements have revealed specific components of brain activity which are correlated with movement variables of load, velocity and displacement. (Supported by NIH NS24960).

192.12

CORTICAL TOPOGRAPHY OF THE PREMOTOR AND MOTOR POTENTIALS PRECEDING SELF-PACED VOLUNTARY MOVEMENT IN MAN. I.M. Tarkka* and M. Hallett. (SPON: J. Grafman). NINCDS, NIH, Bethesda, MD 20892.

A series of potentials precede the onset of unilateral voluntary finger movements. The bereitschaftspotential (BP) begins approximately 1000 ms before movement, NS' starts about 400 ms before movement, the premotor positivity (PMP) starts about 80 ms before movement, and the negative motor potential (MP) starts at the peak of the PMP. This study was designed to investigate the detailed topography of these potentials.

29 channels of EEG, one channel of EOG and the first dorsal interosseus EMG in right and left hands were recorded for a 499 ms window. The EEG montage consisted of the International 10-20 System with 10 additional electrodes between frontal and central, and central and parietal electrodes. Healthy subjects were asked to make self-paced, unilateral index finger abductions. The rectified EMG of the moving finger triggered the computer, the recordings were aligned according to the start of the EMG, and topographic maps were generated from averaged waveforms.

NS' peaked at 82.3 ms (S.E. ±9.9 ms). The peak of PMP occurred at 35.6 ms (S.E. ±5.4 ms). The peak of NS' and the peak of PMP were both closer to the onset of movement in the dominant hand. In the topographic maps NS' localized in central and parietal areas with maximal intensity in CZ and PZ with slight contralateral predominance. The PMP was localized in the central area ipsilateral to the finger moved. The MP appeared in a small, well-localized area in the contralateral central region, corresponding to the motor map obtained by Cohen & Hallett (Neurology, in press) using non-invasive electrical stimulation.

HORMONAL CONTROL OF BEHAVIOR IV

193.1

SEX DIFFERENCES IN LORDOSIS EFFECTS OF ESTROGEN METABOLITES: IMPLICATIONS FOR RAT BRAIN ESTROGEN RECEPTOR DYNAMICS. R.H. Lustig, C.V. Mobbs, D.W. Pfaff, and J. Fishman , The Rockefeller University, New York, NY 10021.

16α-hydroxyestrone (16OHE1), a metabolite of estradiol (E2) and precursor of estriol (E3), binds to the estrogen receptor (ER) with an affinity of 3% of E2, but is equipotent with E2 in *in vitro* systems, due to covalent binding to the ER via a Heyns rearrangement of an intermediate Schiff base. We used this novel property to assess the dynamics of estrogenic effects on rat reproductive behavior by measuring the lordosis score (LS) to manual stimulation in response to exposure and withdrawal of these steroids. Male and female castrate Fisher rats were implanted with Alset osmotic minipumps to deliver vehicle, or 150 nmol/kg/d E2, 16OHE1, or E3. In both sexes, LS increases with 16OHE1 were delayed versus E2 and E3 for 2-3 days ($p<0.05$), but ultimately reached similar maximal levels within a sex (males: max LS ~1.1; females: max LS ~2.3; range 0-3). Upon pump removal, E2- and E3-stimulated LS fell to control levels within 2-4 days in both sexes. 16OHE1-stimulated LS in females also fell as quickly, but in males, LS remained maximal for 6 days ($p<0.05$) before decreasing to control levels.

These results suggest that: 1) 16OHE1 and E3 are equipotent with E2 in promoting lordosis in both sexes; 2) the onset of LS with 16OHE1 is delayed, consistent with its poor ER affinity; 3) males demonstrate a prolonged action of 16OHE1 relative to E2 and E3, consistent with its non-dissociable binding to the ER; 4) females do not show prolonged action of 16OHE1. Since estrogens are eliminated more slowly in females than in males, two possible explanations for the prolongation of LS in males with 16OHE1 are (a) 16OHE1 may stabilise brain ER only in males, or (b) brain ER turnover may be slower in males than in females.

193.2

CO-MIGRATION ON 2-D GELS OF AN ESTROGEN-INDUCED BRAIN PROTEIN, AN LHRH-INDUCED PITUITARY PROTEIN, AND AN UNCOATING ATPASE/HEAT-SHOCK 70KD PROTEIN. Charles Mobbs, George Fink, Melanie Johnson#, William Welch#, and Donald Pfaff. (SPON: Z. Wenzel) Edinburgh University, Edinburgh, UK, and Cold Spring Harbor Laboratory, Cold Spring Harbor, NY., Rockefeller University, New York, NY 10021.

Estradiol induces a protein (EI70), MW 70Kd and pI 5.9, synthesized in the female rat ventromedial hypothalamus (VMH) and transported to the midbrain central gray, suggesting a role in estrogen-regulated lordosis; we have hypothesized that this protein may be an uncoating ATPase/heat shock protein and may facilitate vesicle release by removing clathrin from coated vesicles (Mobbs et al., J. Neurosci. 8:113-118). Luteinizing hormone releasing hormone (LHRH) facilitates lordosis and primes the release of LH secretory vesicles and induces a 70Kd protein in pituitary (Curtis et al., J. Endo 105:163-168). We now report that 8.5 or 85 nM LHRH incubated with pituitaries in vitro induces a protein with MW 70Kd and pI 5.9 which precisely co-migrates with EI70 on 2-dimensional gels. Furthermore, these proteins co-migrate on 2-D gels with a protein recognized after Western blotting by antibodies to the uncoating ATPase/heat-shock family. Thus the induction of an uncoating ATPase by hormones may facilitate release of secretory or synaptic vesicles.

193.3

SEX DIFFERENCE IN THE REGULATION OF 70KD HYPOTHALAMIC PROTEINS BY ESTROGEN. Isabel Fraile*, Charles Mobbs and Donald Pfaff. Rockefeller University, New York, N.Y.

Estradiol induces a protein (EI70), MW 70Kd and pI 5.9, synthesized in the female rat ventromedial hypothalamus (VMH) and transported to the midbrain central gray, suggesting a role in estrogen-regulated lordosis; another protein (MW 70Kd, pI 5.8) is decreased by estrogen (Mobbs et al., J. Neurosci. 8:113-118). In the present work we investigated the regulation of these proteins in estrogen-treated males and females. Fisher rats were castrated and one week later given s.c. injections of estradiol benzoate or vehicle. VMH proteins were labeled in conscious rats with (^{35}S) Met/Cys targeted to VMH with cannulae using minipumps. Proteins were separated on 2-D gels and quantitated by densitometry, including extensive analyses of linearity and reproducibility. Of fourteen spots analyzed ten were extremely stable, showing no more than 5-10% variation between groups. In females, but not in males, estrogen treatment increased EI70 and decreased its two immediately acidic neighbors. Thus the estrogen regulation of these 70Kd proteins in female rats does not seem to be present in the males. In females estrogen also induced a previously unreported protein (69Kd, pI 5.7) (no estrogen:58+11; estrogen:94+20). This protein responded in the opposite way to estrogen treatment in the males (no estrogen:79+5; estrogen:50+1).

193.4

DO PROGESTERONE TARGET NEURONS PROJECT TO THE MIDBRAIN? L.L. DonCarlos and J.I. Morrell. Inst Animal Behav, Rutgers Univ, Newark, NJ 07102.

Progesterone (P) affects the genome of cells by binding to P-receptors in these target cells. We hypothesize that, in the nervous system, P-target neurons communicate hormonal status to other neuronal populations via axonal projections. We have used steroid hormone autoradiography combined with midbrain injections of the retrograde tracer, Fluorogold (FG), to determine the location and number of P-target neurons that project to the midbrain in rats. Ovx, adx rats were given a large midbrain injection of FG (3 days before sacrifice) and estrogen treatment to induce P receptors. Injections of 250uCi ^{3}H-ORG 2058 (a synthetic progestin) were made IP 1-1.5 hrs before sacrifice which was by perfusion with 4% paraformaldehyde. Autoradiograms were prepared and exposed 8 mo. ^{3}H-ORG-concentrating neurons were detected in the preoptic area and in the ventromedial, lateral, dorsomedial, arcuate and medial tuberal nuclei of the hypothalamus. FG-labeled neurons were observed in the same, and other, cell groups. ^{3}H-ORG-concentrating neurons that project to the midbrain were generally rare. Supported by HD22983 and NS07997.

193.5

ANDROGEN-CONCENTRATING NEURONS OF PREOPTIC, HYPOTHALAMIC, AND LIMBIC REGIONS PROJECT TO THE MIDBRAIN. C.A. Lisciotto* and J.I. Morrell. Inst Animal Behavior, Rutgers Univ, Newark, NJ, 07102.

Projections of preoptic, hypothalamic, and limbic structures to the midbrain are important for androgen-mediated behaviors. Using steroid autoradiography combined with retrograde tracing, we investigated whether androgen-concentrating neurons in the forebrain project to the ventral midbrain. GDX/ADX adult male rats received 0.3 ul injections of fluorogold into the ventral midbrain. Four days later, 3H-testosterone or 3H-dihydrotestosterone (1.2nM/100g BW; SA=148-168 Ci/mmol) was injected iv, 1-1.5hrs prior to sacrifice. Autoradiograms were prepared and exposed for 3-10 months. Androgen-concentrating neurons that also contained retrograde label were detected in the bed nucleus of the stria terminalis and ventromedial nucleus of the hypothalamus, and were abundant in the medial preoptic area and diagonal band of Broca. Androgen-concentrating neurons were also present in the lateral septal, paraventricular, arcuate, and medial amygdaloid nuclei, but none of these contained retrograde label. Projections of androgen-concentrating neurons may be a route by which steroid target sites influence neural circuits relevant for behavior.

193.6

BOTH FATTY ACID AND GLUCOSE METABOLISM MEDIATE THE EFFECTS OF STARVATION ON REPRODUCTION IN HAMSTERS.
J.E. Schneider* and G.N. Wade., Dept. of Psychology and Neuroscience and Behavior Program, Universtiy of Massachusetts, Amherst, MA 01003.

It has been suggested that a minimum or threshold of body fat is required for reproduction in females of many species, including human beings. We have found that the effects of starvation on hamster estrous cycles depend on prior body weight, such that lean hamsters are highly susceptible, while fat hamsters are less susceptible to starvation-induced anestrus.

We hypothesize that the cessation of reproduction is signalled by changes in the overall availability of metabolic fuels, rather than by a critical body weight per se. Eighty-five per cent of female hamsters immediately stopped estrous cycling when treated with both methyl palmoxirate (MP, an inhibitor of fatty acid oxidation) and 2-deoxy-d-glucose (2DG, an inhibitor of glucose utilization) during the first 2 days of the estrous cycle. However, females treated with either MP or 2DG during the same time period continued cycling. Thus, the cessation of reproduction may be signalled by changes in the general availability of metabolic fuels, rather than by one specific fuel.
(Supported by NIH grants NS 10873, AM 32976 and by RSDA MH 000321)

193.7

ELECTROPHYSIOLOGICAL PROPERTIES OF IDENTIFIED SEROTONERGIC NEURONS IN THE LOBSTER. PoKay M. Ma* and Edward A. Kravitz (SPON: M. Segal). Department of Neurobiology, Harvard Medical School, Boston, MA 02115. Injection of serotonin into freely moving lobsters produces a long-lasting postural change that mimics the naturally occurring posture assumed by dominant animals in a hierarchy. We have recently identified two pairs of large serotonergic neurons, one pair in the last thoracic and one in the first abdominal ganglion that may be important in postural circuits. Intracellular recording shows that these cells receive prominent inhibitory inputs, which generate IPSPs of various sizes; EPSPs, on the other hand, are rarely seen. The IPSPs in the left and right cells within a ganglion are synchronized, suggesting that the two cells receive the same inhibitory inputs. These cells are spontaneously active, firing large action potentials at a frequency of about 1 Hz. Following each action potential, the membrane potential increases slowly through a depolarizing ramp until the next firing threshold is reached. The oscillation of membrane potential is probably endogenous, like the pace-making properties of cardiac myocytes. The oscillation is also temperature-dependent: as the temperature is raised, cell firing changes from irregular through steady to bursting. The bursts of pairs of cells within a ganglion are synchronized. Flexor command neurons excite, while extensor commands inhibit, the firing of the serotonergic cells. When cell firing frequency is increased by direct intracellular stimulation, little effect is seen in the motor output. However, when flexor command neurons are activated the motor output is enhanced or diminished by allowing or preventing the serotonergic cell from firing. These physiological properties will be explored in dominant and subordinate lobsters to see if any changes can be correlated with differences in behaviors exhibited by these animals. (supported by NIH and MDA)

193.8

Pattern of ^{14}C-2-Deoxyglucose (2DG) Concentration Associated With Prostaglandin E_2 Administration and Sexual Behavior of the Female Frog, *Rana pipiens*. C. Diakow, W. Woicyk*, D. McEachron, and N.T. Adler. Adelphi U., Garden City, NY, and Drexel U. and U. Penn., Phila., PA 19104.

An indication of receptive behavior in female *R. pipiens* is absence of a vocalization, the release call, during tactile stimulation of the trunk. Prostaglandin E_2 (PGE_2) silences the female. We compared the pattern of 2DG concentrations in brains of 5 females receiving 1μg PGE_2/g with that of 3 vocalizing controls injected with deionized water. There was high activity relative to the rest of the brain in the PGE_2-treated females in the posterior part of the anterior preoptic nucleus, in the dorsal habenula, and in the dorsal tegmental area of the medulla lateral to the pretrigeminal nucleus. These results are consistent with the ideas that the activity of these areas is modified by PGE_2 action and that these structures participate in inhibition of the release call, hence, reproductive behavior of the female frog.

193.9

DO GLUCOCORTICOIDS INFLUENCE AUDITORY PERCEPTION IN NORMAL SUBJECTS? G. Fehm-Wolfsdorf, B. Oergel*, D. Nagel*, H. Zenz* and H. L. Fehm*. Depts. of Medical Psychology, HNO and Internal Medicine I, University of Ulm, D-7900 Ulm, F.R.G.

Glucocorticoids have been reported to influence brain functions related to stimulus perception and processing (Henkin R. I. et al, J. Clin. Invest., 46:419, 1967). Moreover, two different types of glucocorticoid receptors have been identified in rat brain, leading to the speculation that specific receptors in hippocampal regions might be involved in behavioral regulation (de Kloet E.R. & Reul J.M.H.M., Psychoneuroendocrinology, 12:83, 1987). We studied differential effects of hydrocortisone and dexamethasone on auditory perception. 15 male volunteers were treated with 50 mg hydrocortisone, 2 mg dexamethasone or placebo in a double-blind cross-over design. Sessions indcluded measurement of stapedial reflex, pure tone audiogram, speech audiogram and brainstem evoked response audiometry. Reflex and audiogram measures as well as brain stem potentials did not differ between treatment conditions. Only performance in speech audiogram was enhanced after glucocorticoid intake with no difference between hydrocortisone and dexamethasone. From our results we conclude that physiological doses of glucocorticoids modulate cortical processing rather than perceptual thresholds.

193.10

HYPERPROLACTINEMIA INHIBITS OPEN FIELD DEFECATION BUT NOT ANDROGEN-DEPENDENT URINE MARKING. Paul C. Doherty Department of Anatomy, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Hyperprolactinemia inhibits open-field defecation. To determine if this effect represents a general decline in marking behavior, four groups of animals (CG, castrated, pituitary-grafted males; CS, castrated, sham-transplanted males; TG, castrated, testosterone-treated pituitary-grafted males; TS, castrated, testosterone-treated sham-transplanted controls) were examined for defecation and urine marking responses to a novel environment. Each animal was placed in a ten gallon aquarium lined with filter paper for 5 min, and the number of fecal boluses and area of urine deposition were measured. Both castration and pituitary grafting inhibited defecation (CG, 0.06 $\pm$ 0.06; CS, 1.31 $\pm$ 0.48; TG, 1.50 $\pm$ 0.64; TS, 2.29 $\pm$ 0.61 boluses). In contrast, while castration led to significant inhibitory effects on urine marking (CG, 10.68 $\pm$ 1.86; CS, 11.80 $\pm$ 1.75; TG, 26.15 $\pm$ 5.15; TS, 30.76 $\pm$ 6.39 cm^2), pituitary grafting had no significant effect. These results suggest that: 1. Inhibitory effects of hyperprolactinemia on open-field defecation are unrelated to androgen-dependent urine marking; 2. Inhibitory effects of hyperprolactinemia on male sexual behavior do not represent a global inhibition of the effects of testosterone on behavior. (Supported by a Research Challenge Grant from the Ohio Board of Regents.)

193.11

THE OXYTOCIN FRAGMENT PROLYL-LEUCYL-GLYCINAMIDE FACILITATES SEXUAL BEHAVIOR IN THE FEMALE RAT. B. B. Gorzalka*, K. A. Luck* and G. L. L. Lester* (SPON: R. Tees). Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., V6T 1Y7 Canada

The structure of prolyl-leucyl-glycinamide (PLG) is identical to that of the C-terminal tripeptide of oxytocin. Whether or not PLG is a natural brain metabolite of oxytocin is a matter of controversy. Some investigators have claimed evidence that a hypothalamic peptidase stepwise degrades oxytocin to PLG. Others have been unable to confirm this, and have noted the existence of PLG in non-oxytocinergic neurons. Tyrosine-prolyl-leucyl-glycinamide (TPLG), a biologically active peptide present in the brain, might also function as a precursor to PLG.

In the present series of experiments, we compared the effects of PLG and TPLG with previously observed effects of oxytocin on sexual receptivity. Administration of oxytocin facilitates sexual receptivity in rats provided that they are primed with adequate quantities of both estradiol and progesterone (Gorzalka and Lester, Neuropeptides, 10, 55-65, 1987). In the present study, ovariectomized rats received estradiol and 0, 200, 250 or 300 µg progesterone. Intraperitoneal administration of 10 mg/kg PLG or TPLG significantly facilitated receptivity at the 200 and 250 µg progesterone doses only. Doses of 0.1 mg/kg PLG, 1.0 mg/kg PLG and 1.0 mg/kg TPLG produced similar effects. The progesterone-dependent effects of PLG and TPLG are reminiscent of results with oxytocin.

193.12

PERIPHERAL VS. PREOPTIC AROMATIZATION OF ANDROGEN TO ESTROGEN AND ACTIVATION OF MALE SEXUAL BEHAVIOR IN JAPANESE QUAIL. J. T. Watson, M. Abdulnabi*§, S. Wersinger*, M. A. Ottinger§ and E. A. Regan. Field of Neurobiology and Behavior and Dept. of Psychology, Uris Hall, Cornell Univ., Ithaca, NY, 14853, and §Dept. of Poultry Sci., Univ. of Maryland, College Park MD, 20742.

Male-typical copulatory behavior (mounting) can be restored in castrated male Japanese quail (*Coturnix japonica*) with implants of testosterone (T) or estradiol in the preoptic area (POA) (Watson et al., Soc. Neurosci. Abstracts 12: 835, 1986). Three experiments were conducted to determine if aromatization of T to estrogens (E) in the POA is required to activate mounting. In the first experiment, blood samples were drawn every four hours from groups of sexually active male quail (N=78, 92% mounted) housed under a 16L:8D light-dark cycle, and assayed for estradiol concentration (E2). Mean ± S.D. serum E2 was 52.6± 25.8 pg/ml (median=49.7 pg/ml), and no diurnal cycle in E2 was seen. In the second experiment, 51 males were castrated and implanted with Silastic tubes containing estradiol benzoate (EB) designed to produce 5 different levels of serum E2. At 2, 3, and 4 weeks after implantation, quail were tested for sexual behavior and a blood sample was taken. The range of E2 in quail which mounted was 93.5-527.9 pg/ml (median=263 pg/ml). Only 7.4% of implanted males with E2 in the range of intact males mounted, and only 9.5% of intact males had serum E2 ≥ the minimum associated with mounting in implanted males. These results suggest that serum E2 in intact males is insufficient to activate mounting without aromatization in the brain to produce local E levels higher than those provided by serum E2. In the third experiment, castrated males were each given a POA implant of: testosterone propionate (TP) + cholesterol (CH), TP + the aromatization inhibitor 1,4,6-androstatrien-3,17-dione (ATD), EB+ATD, or CH. Birds with implants containing TP or EB mounted (TP+CH: 4/8, TP+ATD: 3/5, EB + ATD: 3/7, CH: 0/6). These data suggest that POA aromatization is not necessary for T activation of mounting, but simultaneous application of TP and ATD may have reduced the effectiveness of ATD as an aromatization inhibitor. In further studies, ATD treatment will precede POA/TP stimulation. NSF BNS-84-12083.

193.13

ESTROGEN RECEPTORS IN QUAIL BRAIN: A FUNCTIONAL RELATIONSHIP TO AROMATASE AND AGGRESSIVENESS. B.A. Schlinger* and G.V. Callard. Department of Biology, Boston University, Boston, MA 02215.

Estradiol (E) mediates many of the activational effects of testosterone (T) on masculine reproductive behaviors. Using Japanese quail as an animal model and a newly devised procedure for quantifying aggressiveness, we recently showed that aggression is E-dependent and that individual differences in behavioral intensity are correlated with aromatase in the hypothalamus/preoptic area (HPOA). In this study we characterized estrogen receptors (ER) in quail brain and tested the hypothesis that aromatase governs T-induced responsiveness by regulating the quantity of E available for ER binding. Based on standard binding assays and LH-20 chromatography, quail brain ER was E-specific; of high affinity (Kd=0.88nM) and of limited capacity (Bmax 23-37 fmoles/gm HPOA). The quantitative relationship between aromatization, ER, and aggressiveness was tested in reproductively inactive (non-aggressive) males by treatment with T± the aromatase inhibitor 4-hydroxyandrostenedione (OHA). After five days, T markedly stimulated aggressiveness and elevated aromatase and nuclear ER in HPOA. Simultaneous treatment with OHA blocked T effects on aggressiveness and aromatase, lowered nuclear (occupied) ER but increased cytosolic (empty) ER. Total ER (nuclear + cytosolic) was higher after T-treatment whether or not OHA was administered suggesting androgen per se induces ER in quail brain. These data provide good evidence that activation of aggressiveness by T in quail is dependent on neural production of E and subsequent ER occupancy but also suggests that T increases aromatizing potential and total ER abundance. (Supported by NSF DCB 85-19737)

194.1

A NOVEL IN VITRO MODEL OF FOCAL EPILEPSY: FREEZE LESION OF TURTLE VISUAL CORTEX. D. Adams*, M.G. Blanton, and A.R. Kriegstein. (SPON: J. Hotson) Dept. of Neurology, Stanford University, Stanford, CA 94305-5300.

In order to study the cellular changes associated with cortical injury and epileptogenesis we have developed an in vitro model that allows stable, long-term intracellular recordings during the transition from normal to focal epileptic activity. A dry ice cooled brass probe (2 mm diameter) is applied for 45 seconds to an intact slab of turtle cerebral cortex, placed in a recording chamber. Within two hours spontaneous periodic epileptiform discharges emanate from a peri-lesion focus and propagate throughout the cortex. Pyramidal neurons within the area of the focus exhibit abnormal burst discharges coincident with the epileptiform potential. Intracellular recordings reveal a depolarizing envelope accompanied by a conductance increase. In areas that surround the focus, the epileptic discharge often leads to a burst of partial spikes, thought to represent dendritic action potentials (APs) recordable in the pyramidal cell somata. In uninjured areas of cortex, the discharge coincides with summated Cl^--dependent IPSPs in pyramidal cells and repetitive firing of GABAergic nonpyramidal neurons. These data provide evidence for pathological cellular discharge in the region of the focus with recruitment of activity in uninjured cortex consisting of both excitatory and inhibitory PSPs.

Supported by NIH grants NS00887 and NS21223.

194.2

DISTRIBUTION OF INCREASED SYNAPTIC ACTIVITY DURING FOCAL AND GENERALIZED EPILEPTIFORM ACTIVITY REVEALED BY PRESYNAPTIC UPTAKE OF FLOURESCENT DYES. A.R. Kriegstein, J.G. Avilla*, and M.G. Blanton. Dept. of Neurology, Stanford University School of Medicine, Stanford, CA 94305.

Because of the possibility of maintaining large portions of the CNS of the pond turtle in vitro, we have begun to study the participation of cortical and subcortical regions in the generation of focal and generalized epilepsy. To complement physiological recording methods, we used a technique for labelling active presynaptic terminals, introduced in studies of snake skeletal muscle by Lichtman et al (Nature 314:357, 1985). After initiation of seizure activity by cortical freeze lesion (see Adams et al, this meeting) or by application of the $GABA_A$ antagonist bicuculline, the tissue was bathed in a 1% solution of sulfarhodamine 101 or flourescein-5-sulfonate (Molecular Probes), followed by a wash and fixation. Well labelled terminals and occasional faintly labelled neurons were seen in both epilepsy paradigms, while no specific labelling was detectable under conditions of normal spontaneous activity or if synaptic transmission was blocked by omitting Ca^{2+} or adding TTX to the bath. In bicuculline, profound terminal labelling was found in dorsomedial (hippocampal) cortex, the medial septum and basal telencephalon, while the freeze lesion focus was surrounded by a ring of increased activity. This technique should be useful in identifying functionally active regions, with subcellular resolution.

194.3

SPONTANEOUS HIGH $[K^+]_o$-INDUCED ELECTROGRAPHIC SEIZURES IN HIPPOCAMPAL SLICES ARE SUPPRESSED BY HYPEROSMOTIC MEDIA. S.F.Traynelis and R.Dingledine, Dept. of Pharmacology, The University of North Carolina, Chapel Hill, NC 27514.

Rat hippocampal slices in 8.5 mM $[K^+]_o$ displayed two types of spontaneous epileptiform activity: ~50 ms duration synchronous bursts that arose in CA3 and propagated to the CA1 region, and longer (~50s) electrographic seizures restricted to CA1. An increase in $[K^+]_o$ should induce cell swelling and subsequent reduction in extracellular space (ECS). The resulting tighter cellular pack might exaggerate extracellular ion transients and enhance electric field effects, possibly providing a synchronizing trigger for seizure initiation. We have assessed the contribution of ECS to seizure initiation by osmotic manipulations of ECS. Media made 5-30 mOs hyperosmotic by addition of agents that alter transmembrane osmotic pressures rapidly abolished seizures in 48/56 slices. However, hyperosmotic changes with compounds that access intracellular space were ineffective (21/25 slices). Osmotic manipulations might arrest spontaneous seizures through reduction of required interictal input to CA1. While hyperosmotic manipulations did not alter burst frequency, burst intensity was attenuated in CA3 (~85% control) and CA1 (~70% control). Likewise, excitatory synaptic transmission in CA1 in high $[K^+]_o$ was attenuated (n=3) by hyperosmotic media. This suggests that the volume of ECS is indeed a critical determinant of seizure initiation in this model (supported by NS17771).

194.4

REDUCTION BY CNQX, A NON-NMDA RECEPTOR ANTAGONIST, OF EVOKED AND SPONTANEOUS SYNAPTIC POTENTIALS IN RAT HIPPOCAMPAL SLICES. **N.L. Chamberlin and R. Dingledine, Dept. of Pharmacology, Univ. N. Carolina at Chapel Hill; Chapel Hill, N.C. 27599.**

To investigate the role of synaptic excitation in the initiation of spontaneous epileptiform bursts in hippocampal slices perfused with elevated (8.5 mM) $[K^+]_o$, we carried out the following experiments. First, population spikes and field epsps in CA1 and CA3b/c were evoked by stimulating the Schaffer collaterals and dentate granule cells, in normal (3.5 mM) K^+. Synaptic potentials, which were discerned from fiber-volleys by their sensitivity to low (0-.2 mM) Ca^{2+} solutions, were reversibly and greatly reduced by 1-5 μM CNQX (FG 9065). These results suggest that non-NMDA receptors participate in excitatory synaptic transmission in the hippocampus. Second, the effect of CNQX (1-5 μM) was examined on spontaneous interictal field bursts recorded in CA3b/c of hippocampal slices bathed in 8.5 mM K^+. CNQX reversibly reduced burst intensity (58±13% of control, n=8) and frequency (42±15% of control, n=11). Third, intracellular recordings from 3 CA3b/c cells in 8.5 mM K^+ suggested that frequency of spontaneous epsps (≥ 1 mV in amplitude) was reduced by 2 μM CNQX. Therefore, synaptic activation of non-NMDA receptors appears to be required for spontaneous interictal bursting in hippocampal slices bathed in 8.5 mM K^+. CNQX was a gift from T. Honore. Supported by NS-17771.

194.5

BRAIN ASPARTATE DECREASES AND GABA INCREASES DURING STATUS EPILEPTICUS IN THE RAT. **D.M. Treiman, N.Y. Walton,* and S. Gunawan.* Neurology and Research Services, VA West Los Angeles Medical Center and Dept. of Neurology, UCLA School of Medicine, Los Angeles, CA.**

We have described a series of predictable EEG changes during generalized convulsive status epilepticus (SE) in both patients and in experimental SE in the rat (Neurology 37;Suppl 1:244, 1987), which allow us to identify early, mid- and late SE by EEG criteria. We measured whole and regional concentrations of selected amino acids (AAs) during the course of SE induced by IP pilocarpine (25 or 30 mg/kg) in rats given 3 mmol/kg LiCl 24 hours earlier. After decapitation, brains were rapidly removed and frozen in acetone and dry ice. Concentrations of aspartate, glutamate, serine, glutamine, glycine, taurine and GABA were determined by HPLC for whole cerebral hemispheres and 14 specific brain regions after homogenization, extraction in 80% ethanol, and reaction with phenylisothiocyanate to form phenylthiocarbamyl derivatives. Glutamate, serine and taurine did not change in whole brain. The table lists levels of the other AAs acids in whole cerebral hemispheres in umol/gram brain wet weight.

Condition\AA	ASP	GLN	GLY	GABA
saline + saline	2.54	4.78	0.80	2.13
LiCl + saline	2.52	4.29	0.77	2.15
saline + pilo.	2.42	4.75	0.72	2.07
Before onset SE	2.22	4.43	0.74	2.00
After 1st sz.	2.17	4.29	0.77	2.00
Continuous EEG	1.83	4.94	0.84	2.63
PEDs on EEG	1.76	6.12	0.92	3.34
ANOVA: p <	.05	.001	.001	.001

Regional AA concentrations will also be presented and the significance of these observations will be discussed.

194.6

PHENCYCLIDINE ANALOGS AS ANTICONVULSANTS: NOVEL COMPOUNDS WITH ENHANCED ANTI-SEIZURE ACTIVITY RELATIVE TO MOTOR TOXICITY. M.A. Rogawski, A. Thurkauf, K.C. Rice, A.E. Jacobson, J.M.H. ffrench-Mullen and S. Yamaguchi, Medical Neurology Branch, NINCDS and Section on Drug Design and Synthesis, NIDDK, NIH, Bethesda, MD 20892.

Phencyclidine (PCP) is a powerful anticonvulsant in a wide variety of animal seizure models, however, undesirable side effects (such as ataxia, hallucinations and cognitive impairment) which occur in the same dosage range as seizure protection limit its practical usefulness in the treatment of seizure disorders. In the present study, we compared the anticonvulsant and ataxia-inducing activities of a series of novel PCP analogs in an attempt to obtain drugs that maintain PCP's anticonvulsant activity but are less toxic. Drugs were screened in mice for protection against maximal electroshock (MES) seizures; motor toxicity was determined with the rotorod ataxia test. PCP was equipotent at protecting against seizures (ED_{50}) and in inducing ataxia (TD_{50}) so that its "therapeutic index" (TI=TD_{50}/ED_{50}) equaled 1. Many PCP-related compounds [dexoxadrol, *m*-amino-PCP, (+)-PCMP] had a similar profile with TI~1. However, we found that certain PCP analogs [phenylcyclohexylamine (PCA), *trans*-(R)-3-methyl-PCA, 3,4-dehydro-PCP] maintained PCP's anticonvulsant activity but caused ataxia only at higher doses, so that the TI was enhanced (~2). Moreover, several compounds (*m*-nitro-PCP, 1-phenylcyclopentylamine, 1-phenylcycloheptylamine, 1,1-pentamethylenetetrahydroisoquinoline) exhibited moderately reduced anticonvulsant potency, but these drugs had an even greater diminution in toxicity so that TI's as high as 3-4 were obtained. In comparison, currently used anticonvulsant drugs have TI's of 1.6-8.1.

194.7

BRAIN DAMAGE FROM PILOCARPINE-INDUCED SEIZURES IS AMELIORATED BY AN N-METHYL-D-ASPARTATE ANTAGONIST. D.G. Fujikawa. Exp. Neurol. Lab., V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. of Neurology, UCLA Sch. of Med., Los Angeles, CA 90024.

Pilocarpine (PC)-induced seizures in the rat can be prevented with bilateral intracerebral injections of the N-methyl-D-aspartate (NMDA) antagonist 2-amino-7-phosphonoheptanoic acid (2-APH). However, the mechanisms of neuronal damage in this seizure model are unknown. We injected 2-APH unilaterally into the basolateral amygdala of adult Wistar rats to study the effect on seizure-induced neuronal necrosis.

Bilateral guide cannulae were implanted in the basolateral amygdaloid nuclei. One week later 20 ug (89 nmol) of 2-APH in 1 ul of buffer was injected through one cannula; the other side received buffer alone. Rats were then given 400 mg/kg PC i.p., and after 3 h seizures they received diazepam and phenytoin or phenobarbital i.p.

Brain perfusion-fixation was performed 3 d later. Neurons beneath the cannula tip on the side of 2-APH injection were protected from cell loss (total neurons 164±13 vs. 92±7 contralaterally [mean ± S.E.M.], $p<0.02$, $n=4$), and of the remaining neurons, fewer were necrotic (0.2± 0.2% vs. 21.0±6.6% contralaterally, $p<0.05$). Brain damage from PC seizures may be at least in part NMDA-receptor-mediated. 2-APH is not only an anticonvulsant, it may also protect against brain damage from ongoing seizures.

194.8

AFTERPOTENTIALS OF PENICILLIN-INDUCED SYNCHRONIZED DISCHARGES IN THE MOTOR CORTEX OF THE RAT IN VIVO. O.W. WITTE, S. UHLIG* and E. VALLE*, Neurologische Universitätsklinik, Moorenstr. 5, 4000 Düsseldorf, F.R. Germany

Penicillin-induced synchronized epileptic discharges are followed by de- or hyperpolarizing afterpotentials. In the present series of experiments different types of afterpotentials were separated. The experiments were carried out on the motor cortex of anaesthetized rats using conventional intracellular recording techniques.

Four different types of afterpotentials could be differentiated: 1. A fast afterhyperpolarization which in seven typical experiments had an average duration (100% to 10% decay time, measured from begin of PDS) of 614 ± 96 ms. 2. A fast afterdepolarization (703 ± 103 ms, $n = 9$). 3. A slow afterhyperpolarization (1.9 ± 0.33 s, $n = 6$). 4. A slow afterdepolarization (1.8 ± 0.23 s, $n = 6$).

Afterhyperpolarizations were especially pronounced in neurons with a low resting membrane potential while neurons with a high resting membrane potential showed afterdepolarizations. Intracellular current injection revealed that the fast afterpotentials reversed polarity at −60 to −70 mV while the slow afterpotentials inverted between −80 and −90 mV.

194.9

A GABA-MEDIATED, DEPOLARIZATION INDUCED BY 4-AMINOPYRIDINE (4AP) IN HUMAN NEOCORTICAL AND HIPPOCAMPAL NEURONS MAINTAINED "IN VITRO". M. Avoli, P. Perreault, A. Olivier and J.G. Villemure, MNI, McGill University, Montréal, Que., CANADA

Intracellular recordings were performed in neocortical or hippocampal neurons in slices obtained from human epileptogenic brain tissue excised during surgical treatment of epilepsy. Bath application of 4AP (50 uM) induced spontaneous excitatory and inhibitory post-synaptic potentials which were blocked by Tetrodotoxin (1 uM). Most of the neurons (12/16) also generated in the presence of 4 AP a long-lasting (up to 1.5s) depolarizing potential which: (i) behaved as expected for a synaptic potential during alteration of the resting membrane potential; (ii) prevented the generation of action potentials induced by intracellular depolarizing current pulses and (iii) was blocked by bath application of bicuculline (20 uM). In the presence of bicuculline spontaneous hyperpolarizations, which inverted near the K^+ equilibrium potential, were recorded. It is concluded that like pyramidal or granule cells of the rat hippocampal slice, human neocortical or hippocampal neurons obtained from either spiking or non-spiking areas are capable of generating a 4AP-induced long-lasting depolarization which is mediated through GABA receptors. Supported by the MRC of Canada and FRSQ.

194.10

BEHAVIOR AND NEUROCHEMICAL CHANGES 6 MONTHS AFTER KAINIC ACID. H. Baran, H. Lassmann and O. Hornykiewicz. Inst. Biochem. Pharmacol. and Neurol. Inst. Univ. Vienna, Austria.

Behavioral, neurochemical and histopathological changes were investigated in rats 6 months after systemic application of kainic acid (KA; 10 mg/kg, sc). KA induced long lasting generalized seizures up to 6 h after injection. One month later, the animals seemed to have recovered but spontaneous seizures lasting 4-10s occurred once or twice a month thereafter.

Six months after KA injection, significant increases in GAD activity were noted in temporal (62%), parietal (56%), occipital (57%), frontal (39%) and cingulate (37%) cortex and in nucleus caudatus (37%). No significant GAD changes were found in hippocampus, amygdala/pyriform cortex and substantia nigra. CAT activity was normal in all cortical areas, hippocampus and nucleus caudatus but declined significantly (40%) in amygdala/pyriform cortex. Noradrenaline levels remained unchanged in all areas examined. Changes in glutamine levels were restricted to an increase (50%) in the nucleus caudatus and a reduction (30%) in amygdala/pyriform cortex. GABA levels were increased in the hippocampus, nucleus caudatus (70%) and substantia nigra (40%) and decreased in amygdala/p. cortex (40%). Altered GABA-ergic function in the brain 6 months after KA may reflect compensatory responses of GABA neurons to spontaneous seizure activity. (Supported by the Austrian Science Research Fund projects P5647 and S 25/06).

194.11

INDUCTION OF C-FOS PROTO-ONCOGENE PRODUCT mRNA BY ELECTROCONVULSIVE SEIZURES (ECS) IN MOUSE BRAIN. [1]P.J. Marangos, T. Nakajima, J.L. Daval and [2]C.H. Gleiter. [1]Gensia Pharmaceuticals, San Diego, CA 92121-1207, Unit on Neurochemistry, BPB, NIMH and [2]NIAAA/LCS, Bethesda, MD 20892.

Recent studies have shown that the c-fos proto-oncogene product is induced in response to chemically-induced seizures. C-fos is a nuclear DNA binding protein that has been shown to be involved in the induction of transcription in certain cell types. In normal brain tissue, it has been postulated that the c-fos proto-oncogene product may be a nuclear response to various membrane events that involve disturbances in membrane calcium fluxes. In this study, mice were exposed to a single ECS (80mA, 0.5s) and the brains were rapidly removed and frozen in liquid nitrogen at various time intervals following the seizure. Extracted total RNA was resolved by electrophoresis, blotted and analyzed for c-fos mRNA using a ^{32}P labeled DNA probe. A rapid, transient, 5-fold expression of c-fos mRNA was observed at 15 minutes post-ECS and returned to baseline values at 240 minutes. Also, at 15 minutes, a 3-fold significant expression of c-fos mRNA was observed in the control animals that were ear-clipped but not shocked. Our results show that c-fos upregulation is observed with ECS and, perhaps more interestingly, that a much more subtle stimulus associated with the stress of ear-clipping is also capable of inducing c-fos.

194.12

SUPPRESSION AND FACILITATION OF EPILEPTIC ACTIVITY IN THE DENERVATED HIPPOCAMPUS BY NEURAL GRAFTS. G. Buzsáki, G. Ponomareff,* F. Bayardo,* and F.H. Gage. Dept. of Neurosciences (M-024), UCSD, La Jolla, CA 92093, USA.

Spontaneous and stimulus-evoked neuronal activity were studied in the subcortically denervated (fimbria-fornix, FF) hippocampus of the freely moving rat. Spontaneous interictal spikes (< 40 ms, 3-8 mV; IIS) were observed in the hippocampus up to the termination of the experiments (8 mo). Depth profile and unit recording experiments revealed that the trigger zone of IIS was the CA3 region. The threshold for inducing afterdischarges was significantly lower, and the seizures lasted longer in FF rats. Cell suspensions prepared from the fetal locus coeruleus region and transplanted into the denervated hippocampus decreased the incidence of IIS and protected against picrotoxin-induced behavioral seizures. In contrast, in rats with fetal hippocampal cell suspensions the incidence of IIS was significantly higher than in FF controls and spontaneous behavioral seizures occurred in half of the rats. We suggest that the subcortically denervated hippocampus is a useful chronic preparation to study the mechanisms of epilepsy, and replacement of the missing transmitters by means of neural grafts may elucidate the nature of seizure-suppressant control of subcortical afferents.

194.13

THE IMPORTANCE OF CORTICOFUGAL CONNECTIONS IN THE DEVELOPMENT OF SEIZURE-DAMAGE AS EVALUATED BY CO-GRAFTS OF SUBSTANTIA NIGRA AND CEREBRAL CORTEX IN OCULO. M Ingvar°, M Eriksdotter-Nilsson°, L Olson (SPON: O. Johansson). Dept Histology and Neurobiology, Karolinska Institute 104 01 Stockholm

Sustained experimental seizure activity causes cell-necrosis in the substantia nigra pars reticulata (SNPR) in the rat. We have shown that depriving the substantia nigra (SN) of its input from the frontal cortex prior to the seizures diminishes the tissue necrosis in the SNPR. We transplanted fetal rat neuronal tissue from either SN-area alone or in combination with frontal cortex to the anterior chamber of the eye in adult rats. After allowing the grafts to mature for 3-5 months the host rats were subjected to 60 min of status epilepticus (controlled ventilation) and allowed to recover for one week. In all animals subjected to seizures, the expected necrosis in the SNPR was seen in situ. In animals with single intraocular transplants of SN the exposure to seizure activity did not affect the transplants as evaluated by immunohistochemistry of glial fibrillary acidic protein (GFA, a marker for astrocytes) and laminin (a marker for blood vessels). In co-transplants the seizure activity caused a total loss of GFA immunofluorescence within a circumscribed area in the nigral portion of the double grafts, while the cortical portion was unaffected. This change was identical to the findings in situ. The corticofugal fibers to the SNPR contain glutamatergic fibers. This study provides further evidence for the importance of glutamatergic input to the SNPR for the development of a necrosis in the SNPR.

GENETIC MODELS I

195.1

ADRENAL CHROMAFFIN TUMORS IN TRANSGENIC MICE. E.E. Baetge, R.R. Behringer*, A. Messing, A. Sved, R.L. Brinster* and R.D. Palmiter*. HHMI Univ. of Wash. Seattle, WA.; Sch. of Vet. Med. Univ. of Wisc. Madison, WI.; Dept. Behav. Neurosci., Univ. of Pittsburgh, Pittsburgh, PA.; Lab. of Reproductive Phys., Univ. of Penn., Philadelphia, PA.

The human gene for phenylethanolamine N-methyltransferase (hPNMT), responsible for the biosynthesis of epinephrine from norepinephrine has been isolated and the complete nucleotide sequence determined. Transgenic mice containing the hPNMT gene resulted in expression of hPNMT mRNA in adrenal gland (Baetge et al. Proc. Natl. Acad. Sci. USA. Vol. 85, No. 11 (June 1988). Transgenic mice expressing a chimeric gene consisting of 2 kb of the hPNMT 5' flanking region fused to the SV40 early region resulted in T-antigen (T-Ag) protein and mRNA expression in adrenals. By 6-8 wks. of age, the adrenals had become enlarged but maintained normal cortical/medullary structure. By 10-12 wks. of age adrenals had grown 200-500 fold, and cortical cells were no longer apparent. Plasma catecholamine levels in control and transgenic animals were similar up to 10 wks. of age at which time dopamine and norepinephrine levels in the transgenic animals increased approximately 10 fold. Blood pressure was normal through 9 wks., but by 10-12 wks. blood pressure increased 68% in the transgenic animals. These animal lines transmit this phenotype in a dominant manner and may serve as a model system for the study of pheochromocytoma in mice.

195.2

DERIVATION OF NEURONAL CELL LINES FROM RETINAL AND ADRENAL TUMORS IN PNMT-SV40 TRANSGENIC MICE. J.P. Hammang, R.R. Behringer*, E.E. Baetge, R.D. Palmiter*, R.L. Brinster*, and A. Messing. Sch. Vet. Med, Univ. Wisc., Madison, WI 53706, Sch. Vet. Med., Univ. Penn., Phila., PA 19104, H.H.M.I., Univ. Wash., Seattle, WA 98195.

A hybrid gene containing 5' flanking sequences from the human phenylethanolamine N-methyltransferase (PNMT) gene fused to the SV40 early region was used to target oncogene expression to epinephrine-synthesizing cells in transgenic mice. We developed three lines of mice carrying the PNMT-SV transgene that express SV40 T-antigen in adrenal medulla and retina. In all three lines, bilateral adrenal pheochromocytomas develop in 100% of the mice by 10-12 weeks of age. A variable percentage of mice in each line also develops retinal tumors. The retinal tumors arise in the inner nuclear layer of the peripheral retina. Ultrastructural examination revealed numerous neurosecretory dense core vesicles in the adrenal medullary tumor cells, but no secretory vesicles or membrane specializations in the retinal tumor cells. Tissue cultures were established from both adrenal and retinal tumors; T-antigen-expressing retinal cells developed a neuronal-like morphology with small cell bodies (10-15 μm diameters) and long neuritic processes. Both adrenal and retinal cultures have now been growing for over 6 passages and T-antigen positive cells continue to proliferate. Clonal cell lines are currently being developed from each tumor. These cell lines may be useful for comparative studies on catecholamine biosynthesis and regulation of PNMT gene expression in neuroendocrine and CNS-derived cells.

195.3

MYELINATION DEFECTS ASSOCIATED WITH EXPRESSION OF SV40 T-ANTIGEN IN SCHWANN CELLS AND OLIGODENDROCYTES OF TRANSGENIC MICE. A. Messing, E.P. Sandgren*, D. Paulson*, R.L. Brinster*, and R.D. Palmiter*. Sch. Vet. Med, Univ. Wisc., Madison, WI 53706, Sch. Vet. Med., Univ. Penn., Phila., PA 19104, H.H.M.I., Univ. Wash., Seattle, WA 98195.

Transgenic mice carrying an SV40-metallothionein growth hormone fusion gene develop a demyelinating-hypomyelinating peripheral neuropathy (Messing et al., Nature, 316:461, 1985). In order to identify the cell types expressing the SV40 T-antigen, we performed immunocytochemical studies. T-antigen was detected in thick sections of nerve by post-embedding staining, and the T-antigen positive cells were identified in serial thin sections by electron microscopy. Sixty-five T-antigen positive cells were evaluated from the sciatic nerves of two transgenic mice: 77% were Schwann cells either associated with myelinated axons, associated with demyelinated or hypomyelinated axons, or free in the endoneurium, while 23% were unidentified or fibroblasts. None of the non-myelinating Schwann cells associated with small diameter axons were T-antigen positive. No T-antigen positive neurons were found in the peripheral or central nervous systems. Expression of T-antigen consistently occured in CNS white matter, where ultrastructural studies showed mild demyelination. We identified the T-antigen positive cells in the CNS as oligodendrocytes by double-immunolabeling for T-antigen and glial fibrillary acidic protein (GFAP) or cyclic nucleotide phosphohydrolase (CNPase). No cells were positive for both T-antigen and GFAP, while many cells were positive for both T-antigen and CNPase. These results show that expression of T-antigen in two myelin-forming cells fails to induce neoplasia but has the similar consequence of hypomyelination or demyelination.

195.4

THE CEREBELLUM IN PURKINJE CELL DEGENERATION* WEAVER (pcd/pcd*wv/wv) DOUBLE MUTANT MICE. B. Ghetti, C.J. Alyea*, L.C. Triarhou, W.C. Low, S.R. Dlouhy* and R.C. Karn*. Depts. of Pathology (Neuropathology), Physiology & Biophysics and Medical Genetics, and Program in Medical Neurobiology, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

The aim of this study was to produce pcd/pcd*wv/wv double mutant mice and to analyze their cerebellar cortex. Homozygous pcd females were crossed with wv/+ males. Double carriers (pcd/+*wv/+) were identified in the F1 generation and intercrossed. Five F2 mice, obtained from several litters and aged 19, 27, 36, 74 and 199 days, had a neurological dysfunction that was indistinguishable from the wv condition. Histologically, all of them showed severe gc loss; in addition, the 19, 27 and 36 day-old mice had Pc with a basal polysomal mass, the 27 and 36 day-old animals had numerous Pc clearly undergoing degeneration, and the 74 and 199 day-old mice had only a few cortical macroneurons. Cerebellar slices from the 19 and the 74 day-old mice were processed for immunocytochemistry against 28-kDa Ca-binding protein (CaBP), which is selectively localized to Pc in normal and wv cerebellum, and in pcd before Pc death. The 19 day-old mouse contained CaBP immunoreactive Pc with abnormally oriented dendrites. The cerebellum of the 74 day-old mouse did not contain CaBP immunoreactive cells. These findings suggest that (a) pcd/pcd*wv/wv double mutants are viable and (b) the anatomical phenotype of such mice is an additive expression of the component phenotypes.

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195.5

THE MAUDSLEY REACTIVE AND NON-REACTIVE STRAINS OF RAT: EVIDENCE FOR POSSIBLE CONTAMINATION IN THE MNR/Har STRAIN. R.L. Commissaris*, G.M. Harrington*, H.J. Altman and H.W. Kunz* (SPON: D.M. Koester). College of Pharmacy & School of Medicine, Wayne State Univ., Detroit, MI 48202, Dept. of Psychology, Univ. Northern Iowa, Cedar Falls, IA and Univ. Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Open field, conditioned suppression of drinking and acoustic startle behaviors were assessed in male and female Maudsley Reactive (MR/Har) and Non-Reactive (MNR/Har, MNRA/Har) rat strains. While MNRA/Har and MR/Har rats exhibited dramatic strain differences in these behaviors, MNR/Har rats behaved more like Reactive than Non-Reactive rats. Histocompatability testing indicated that the MNR/Har strain may have been genetically contaminated. Thus, while comparisons between the MR/Har and MNRA/Har strains remain viable and relatively clear-cut, the usefulness of the MNR/Har strain as an additional resource with which to study behavioral, physiological and genetic determinants of stress and anxiety may have been significantly undermined. These results underscore the importance of continued periodic validation of the integrity of the genetic determinants which underly differences in selectively inbred strains of laboratory animals used for research. (MH #42501-01; protocol conforms with NIH guidelines)

195.6

GENETIC AND HORMONAL MODELS OF DEVELOPMENTAL LANGUAGE/READING DISORDERS. P. Tallal, R. Ross* and S. Curtiss*, Dept. of Psychiatry, Univ. CA San Diego, La Jolla, CA 92093.

There is an increased propensity of boys to be afflicted with developmental language disorders and for these disorders to run in families. Family history questionnaires from families of language-impaired (LI) children were analyzed to determine whether data would support any known models of sex-influenced genetic transmission. Results demonstrated that LI children with an affected parent differed from those without an affected parent in sex-ratio among probands and rate of sibling impairment. The expected higher male:female sex-ratio in language-impaired children occurred primarily in families with affected parents. Affected mothers, but not affected fathers, demonstrated this sex-ratio difference among probands as well as a significantly increased frequency of affected offspring compared to non-affected parents. However, results failed to support sex-linkage hypotheses in that male and female probands had equal propensity to have an affected mother or father and also did not differ in rate or sex of affected siblings. An unexpected finding was a significantly altered sex-ratio in siblings of LI children (65 brothers, 35 sisters, ratio = 1.9:1). Analyses demonstrated that, whereas non-affected parents and affected fathers had approximately equal male and female offspring ratios, affected mothers had a 3:1, male:female offspring ratio and a 5:1 ratio of affected offspring. Genetic and hormonal influences that might affect both sex-ratio and neuro-developmental disorders are discussed.

195.7

IMPAIRED SPLENIC T-CELL ASSOCIATED RESPONSES IN AN EPILEPSY-PRONE SUBSTRAIN OF BALB/C MICE. Dolina, S.,Tzory, S., Herishanu, Y. and Segal, S., Depts. of Neurology, Microbiology and Immunology, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel.

Patients suffering from epilepsy have been recently shown to exhibit various immune disorders. Since conflicting data have been reported and since the possible mechanism underlying these disorders is obscure, it was necessary to test the existence of a possible association between genetical predisposition to epilepsy and immune impairment in a suitable experimental system. For that purpose, we have bred epilepsy-prone substrain (EPS) of Balb/C mice. The animals were selected by their sensitivity to sound-induced convulsions. Immunocytes originating in the spleens of 8-10 week old EPS and appropriate non-EPS control mice (matched for age and sex) were tested for their ability to respond in vitro to T-cell specific polyclonocal mitogens (Con A and PHA) as assessed by the degree of ^{3}H-thymidine incorporation following 72 hrs of incubation in the presence of the appropriate mitogen. The data obtained demonstrate a severe suppression of the mitogenic response in both sexes of EPS, (especially regarding response to PHA), in comparison to control mice. These results strongly suggest the existence of a possible association between genetical predisposition to epilepsy and immune impairment, at least in the T-cell compartment of the murine lymphoid system. These findings may support the long standing hypothesis implicating the CNS as playing a pivotal role in the modulation of the various arms of the immune system.

SENSORY SYSTEMS II

196.1

THE DEVELOPMENT OF ASCENDING SPINAL PATHWAYS IN XENOPUS LAEVIS. H.J.ten Donkelaar, R.de Boer-van Huizen, J.A.M.van der Linden (SPON: European Neuroscience Association). Dept. of Anatomy and Embryology, University of Nijmegen, P.O.Box 9101, 6500 HB Nijmegen, The Netherlands.

The development of ascending spinal pathways has been studied in the clawed toad, Xenopus laevis. From stage 35 (hatching) on HRP was applied at the spinomedullary border. Several populations of spinal neurons with ascending projections at least as far as the spinomedullary border were successively labeled. In early (up to stage 50) ascending spinal projections arise from Rohon-Beard cells and ascending interneuron populations located at the margins of the gray and white matter, i.e. marginal neurons.

Around stage 50 (shorly after the appearance of limb buds) spinal ganglion cells could be labeled from the spinomedullary border. Gradually all spinal ganglia send ipsilaterally ascending afferents to the brain stem. The number of cells of origin of secondary spinal afferents to the brain stem increases during development and their distribution becomes more extensive. A preponderance of contralaterally projecting neurons present during early development, is superseded by the presence of an increasing amount of ipsilaterally projecting spinal neurons, particularly from the dorsal horn. By applying HRP to the dorsal column nuclei, cerebellum, reticular formation, mesencephalon and thalamus, respectively, the development of spinal projections to these structures has been studied.

196.2

EFFECTS OF NEONATAL INFRAORBITAL NERVE SECTION ON TRIGEMINAL BRAINSTEM CELL NUMBER AND DENDRITIC ORIENTATION. T.A. Henderson*, P.D. Kaszubski*, J.A. Yelon* & M.F. Jacquin (SPON: L.C. Massopust). Dept. of Anat. & Neurobiol., St. Louis University School of Medicine, St. Louis MO 63104.

Retrograde tracing and computer-assisted morphometric techniques were used to assess the effects of left infraorbital nerve section at birth upon projection cell # and dendritic polarity in the trigeminal (V) brainstem complex (TBNC) of adult rats. Bilateral HRP injections spanning all of thalamus (N=4), cerebellum (N=4), or upper cervical spinal cord (N=3) labeled significantly fewer cells in left vs right TBNC (ANOVA: $F=18.7$, $df=21$, $p<.01$). In total, the lesion produced 23.0±12.6%, 27.0±8.2%, and 29.7±17.1% fewer labeled V-thalamic, -cerebellar, and -spinal cells, respectively (mean±SD, corrected for split nuclei). Corrected mean # of V-thalamic cells in left vs right subnuclei principalis (P), oralis (O), interpolaris (I) and caudalis (C) were 9956 vs 12838, 1007 vs 1472, 2280 vs 2917, and 362 vs 499, respectively. Mean # of V-cerebellar cells in P,O,I and C were 985 vs 1084, 619 vs 856, 2401 vs 3323, and 95 vs 132. Mean # of V-spinal cells in P,O,I and C were 65 vs 93, 549 vs 770, 286 vs 346, and 263 vs 364. Dendritic tree centers for each projection group were, on average, in the region of somata, and therefore did not differ from normal. Thus, neonatal denervation results in fewer TBNC projection cells, yet the dendrites of surviving cells are not polarized toward regions of spared mandibular or ophthalmic primary afferent inputs. Support: NIH DE07662, DE07734; AOA.

196.3

CERVICAL RECEPTIVE FIELD SUBSTRATE IN TRIGEMINAL BRAINSTEM CELLS DEAFFERENTED AT BIRTH. W. Anzilotti*, N.L. Chiaia, R.W. Rhoades & M.F. Jacquin (SPON: E.R. Shuter). Dept. Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104 & Dept. Anat., Med. Coll. Ohio, Toledo, OH 43699.

In adult rats with infraorbital nerve section at birth, 519 cells were functionally characterized in trigeminal (V) subnucleus interpolaris (SpVi). Of these, 41 (7.9%) had receptive fields (RF) which included V surfaces and ipsilateral areas innervated by cervical primary afferents (ear, neck, arm, forepaw). Such non-V convergence was never seen in 373 normal cells or in 641 V ganglion cells ipsilateral to the lesion. These atypical RF's must therefore reflect post-synaptic reorganization. Anatomical experiments were carried out to assess possible anatomical substrates. 8 cells with cervical RF's were stained with HRP. Although all had dendrites which were unusually polarized towards SpVi regions containing spared mandibular and/or ophthalmic primary afferents, none had dendrites which extended out of SpVi. Bulk HRP injections into deafferented SpVi (N=5) labeled cells in appropriate regions of cortex, as well as 46±20 cells in ipsilateral C1-3 dorsal root ganglia, and 24±8 cells in C4-8 ganglia. In 3 controls, labeled cells occurred only in C1-3 ganglia (32±9). Bulk HRP injections into lower cervical spinal cord produced weak anterograde labeling in deafferented SpVi, but not in normal SpVi. These data indicate that cervical primary afferent sprouting into SpVi may account for cervical RF's in deafferented SpVi. Support: NIH DE07662, DE07734, NSF BNS8515737.

196.4

FETAL VISUAL CORTEX TRANSPLANTED TO SOMATOSENSORY REGION OF NEWBORN RATS DEVELOPS BARREL-LIKE FEATURES. B.L. Schlaggar and D.D.M. O'Leary (Spon: S.Goldring), Dept of Neurosurgery & McDonnell Ctr for Studies of Higher Brain Function, Washington Univ Sch Med, St. Louis, MO 63110

"Barrels" are aggregations of granule cells and thalamic afferents characteristic of the primary somatosensory region of parietal cortex of rodents. To determine if the potential to form barrels is unique to parietal cortex, or if other "granular" cortices share in this ability, we have assayed for the presence of barrels in transplants of occipital cortex to the barrel field of rats. Donor fetuses, exposed to ^{3}H-thymidine in utero on E15, had a piece of occipital cortex removed at E18 and placed in a cavity aspirated in the prospective barrel field region of newborns. The host rats were perfused on P12 and brain sections processed for AChE histochemistry which reveals barrel formations in normal cortex (Robertson, 1987, NSL 75:259). The transplants, delineated from Nissl-stained autoradiograms of adjacent sections, develop barrel-like morphologies resembling those seen in normal parietal cortex, although the overall patterns are not identical. We are investigating whether the abnormal pattern is due to disruptions resulting from transplantation, or to inherent differences between occipital and parietal cortex. These results suggest, though, that the ability to develop barrels is not a property exclusive to parietal cortex. (D.O'L. is a McKnight Scholar and a Sloan Fellow.)

196.5

A QUANTITATIVE ANALYSIS OF THE EFFECTS OF NEONATAL INFRAORBITAL NERVE TRANSECTION ON TRIGEMINAL PRIMARY AFFERENTS. W.Renehan, R.Rhoades and M.Jacquin. University of Louisville, UMDNJ and St. Louis University

The present report represents a quantitative analysis of 28 individually labeled primary afferents in trigeminal subnucleus interpolaris of rats subjected to neonatal transection of the infraorbital nerve. Data obtained to date suggest that this lesion results in a decrease in the number of boutons/collateral and bouton density for axons reinnervating mystacial vibrissae as well as a decrease in the circularity of mystacial guard hair terminal arbors (pertinent numbers in boldface type below; p<.01, t test).

	A	FF	P	C	B/C	BD
1	.0120±.0093	.56±.2	.556±.48	10±2	**32.4±9.2**	**2695.1**
2	.0179±.0185	.52±.2	1.11±1.1	6.5±2	82.5±88.6	4596.4
3	.0084±.0069	**.38±.2**	.564±.48	4.0	58.25	6963.5
4	.0062±.0041	.55±.2	.397±.22	7.0±1	40.5±5.3	6432.9
5	.0147±.0079	.53±.2	.719±.30	7.5±2	**74.4±56.2**	**5058.8**
6	.0084±.0049	**.64±.2**	.431±.28	8.0	76.4±52.4	9071.3
7	.0069±.0078	.57±.2	.396±.36	6.0±1	30.1±26.5	4333.1

A=area, FF=form factor, P=perimeter, C=# collaterals, B/C=boutons/collateral and BD=bouton density. 1=mystacial vibrissae, 2=nociceptive, 3=maxillary guard hairs, 4=no receptive field, 5=non-maxillary sinus hairs, 6=non-maxillary guard hairs and 7=non-maxillary skin.

196.6

FUNCTIONAL CONSEQUENCES OF NEONATAL DEAFFERENTATION IN RAT TRIGEMINAL SUBNUCLEUS INTERPOLARIS. M.F. Jacquin. Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. Med., MO 63104.

Single unit recording, electrical stimulation, and receptive field (RF) mapping techniques were used to assess the responses of cells in interpolaris after infraorbital nerve section at birth. Of 904 cells, 385 were from normal adults, 519 were from neonatally lesioned adults. Deafferentation produced 1) A reduction in areas containing cells with infraorbital RF's, and only a minor increase in areas solely responsive to non-infraorbital surfaces. 2) An absence of topography within cells expressing regenerate inputs. 3) A slight increase in mean discharge latency to V ganglion or thalamic shocks. 4) An increased % of cells orthodromically activated by diencephalic or cerebellar shocks. 5) A decreased % of vibrissa local circuit cells, with increased %'s of local circuit nociceptors and non-responsive cells. 6) An increased % of nociceptors, vibrissae, guard hair and/or skin sensitive cells projecting to thalamus and/or cerebellum. 7) An increased % of local circuit cells responsive to >1 whisker, whereas projection cells did not differ from normal in the # of whiskers in their RF. 8) An increased % of cells expressing interdivisional and intermodality convergence, split RF's, spontaneous activity, directional, high velocity and neuroma sensitivity. Although many of these effects can be attributed to altered peripheral projections of axotomized primary afferents, others must reflect central reorganization. Support: NIH grants DE07662 and DE07734.

196.7

DISTRIBUTION OF SKIN AFFERENTS IN THE SPINAL CORD AND BRAIN STEM AFTER NERVE SECTION AND REGENERATION: RELATION TO CORTICAL MAPS. S.L. Florence, J.T. Wall, P.E. Garraghty, and J.H. Kaas. Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240.

We compared the fidelity of regeneration of a cut and repaired median nerve to the cortical map of the hand in the same adult macaque monkey (Macaca fascicularis). In normal macaque monkeys, transganglionic transport of an injected tracer from the glabrous skin of a single digit demonstrates discrete and orderly zones of terminations in the spinal cord and cuneate nucleus. For example, in the spinal cord, afferents from D1 normally terminate at C5 and span half a spinal cord segment. In contrast, an injection into digit 1 of a monkey one year after median nerve repair resulted in labeled terminals extending from C4 - C6.5 in regions of the dorsal horn of the spinal cord normally representing skin between the radial forearm and digits 1 - 3. Labeled afferents also distributed over a wider than normal portion of the cuneate nucleus. Thus, many afferents in the cut nerve incorrectly regenerated into the thumb. A detailed microelectrode map of the representation of the hand in the primary somatosensory cortex (area 3b) of the same animal revealed an abnormal organization that included: (1) representations of digits 1 - 3 that were abnormally distributed in the hand map with apparent discontinuities, and (2) recording sites with large or multiple receptive fields. The results indicate that disorderly reinnervation can follow nerve section and repair. While some aspects of the altered cortical map of the hand clearly relate to the peripheral disorder, other aspects may reflect central reorganization.

Supported by NIH grants NS16446 and NS21105.

196.8

ONTOGENY OF AUDITORY BRAINSTEM EVOKED POTENTIALS IN THE MONGOLIAN GERBIL: STIMULUS RATE EFFECTS. N.K. Woolf, H. Gompers-Foster*, and A.F. Ryan. Dept. of Surgery/Otolaryngology, UCSD School of Medicine and VA Medical Center, La Jolla, CA 92093.

The effect of varying stimulus repetition rate on auditory brainstem evoked potential responses (ABR) of mongolian gerbils (*Meriones unguiculutus*) was investigated at 10, 12, 14, 16, 18, 30 and 45-60 days after birth (DAB). This age range included the onset of neonatal hearing through the attainment of mature auditory system characteristics. Developmental trends for ABR, auditory nerve compound action potentials and auditory nerve single unit responses were computed and compared to those for adults.

Click evoked ABR was first observed at 12 DAB, with thresholds exceeding 110 dB SPL (peak). Only 18% of the subjects exhibited ABR at this age and ABR was limited to Peaks I and IV at this time. Between 12 and 30 DAB, ABR thresholds declined approximately 100 dB. Adult threshold, latency, amplitude, waveform morphology and interpeak relationships for the five ABR peaks were not achieved until 30 DAB.

Developmentally, ABR rate adaptation was most pronounced in the youngest subjects. ABR was abolished at 40 clicks per second in neonatal gerbils. For animals 18 DAB or younger, ABR Peak I amplitude declined slower than Peak IV amplitude with increasing stimulus rate. For subjects 30 DAB or older, ABR Peak I amplitude declined faster than Peak IV amplitude with increasing stimulus rate.

The data indicate that the generators of the ABR peaks matured in a hierarchical order, with the peripheral regions of the auditory system reaching maturity before those in the more central regions.

Supported by grants from NIH/NINCDS (NS14945) and the Research Service of the Veterans Administration.

196.9

DEVELOPMENT OF THE BARREL PATTERN IN EARLY POSTNATAL MOUSE SOMATOSENSORY CORTEX AS REVEALED BY THE DISTRIBUTION OF MAP2 IMMUNOREACTIVE DENDRITES AND RC2-LABELED RADIAL GLIA. J.E. Crandall, D. Butler and J.P. Misson. Dept. of Developmental Neurobiology, E.K. Shriver Ctr., 200 Trapelo Rd., Waltham, MA 02254

It has been previously reported that certain lectins can reveal a barrel pattern in mouse somatosensory cortex prior to the development of Nissl-stained cells into the characteristic pattern of cell-dense sides and cell-poor hollows (Cooper and Steindler, J. Comp. Neurol. 1986, 249:157). We have investigated the development of two other components of the maturing neuropil, dendrites and radial glia, using monoclonal antibodies selective for each. We have combined immunocytochemical labeling for MAP2 and the antigen detected by RC2 (Misson et al., Neurosci. Abst. 1987, 13:1465) in the same section to discern the relative role of dendrites and glia in early barrel formation. Both dendrites and radial glia appear to be asymmetrically distributed in a barrel-like fashion by postnatal day 4 (P4; the day of birth = P0). There is a higher density of dendrites and radial glia in the barrel walls compared to barrel centers. Thus, during a similar time period in which the distinctive cell pattern of the barrel cortex forms, radially oriented dendrites and radial glia likewise appear to become similarly organized. This arrangement continues to become more distinctive by the end of the first postnatal week. The age at which these distinctive constellations of radial glia and dendrites first emerges remains under investigation.

196.10

DIETARY DETERMINANTS OF RESPONSES FROM REGENERATED TASTE NERVES. David L. Hill. Dept. Psychology, Univ. Virginia, Charlottesville, VA 22903.

Important determinants of functional responses seem to occur as the first generation of taste receptors form in rat (16 days gestation). The following study was designed to mimic the developmental events by exploiting the trophic influence of taste nerves on receptors. Adult rats had the left chorda tympani/lingual nerves surgically cut; the right nerves remained intact. Rats were then placed on a low-salt diet of 0.03% NaCl for the duration of the experiment. Control rats underwent the same surgical and dietary procedure with the exception of nerve sectioning. When neural recordings were begun 120 days following surgery, taste responses to concentration series of NaCl and sodium acetate from the regenerated nerve was approximately 50-60% of that obtained from nerves in control rats, suggesting that responses from newly-formed receptors are abnormal. Responses to NH_4Cl were similar between regenerated nerves and those in control rats. Surprisingly, responses from the right chorda tympani nerve (uncut nerve) were similar to the abnormal regenerated nerve and not like those in control rats. Thus, functional responses from regenerated and intact nerves were attenuated. It is possible that the intact nerve invaded territory originally occupied by the cut nerve, thereby innervating receptors less sensitive to sodium. Current research seeks to characterize such morphological correlates. (Supported by NIH NS24741 & NS01215).

NEURAL PLASTICITY IN ADULT ANIMALS: INDUCED EFFECTS I

197.1

MECHANISMS UNDERLYING SEIZURE-ELICITED ODC INDUCTION IN ADULT RAT HIPPOCAMPUS. Amy Arai*, Kavian Shahi*, Gary Lynch, Michel Baudry and Christine Gall† (SPON: M. Kessler). Center for the Neurobiology of Learning and Memory, and †Dept. of Anatomy and Neurobiology, Univ. of Calif., Irvine, CA 92717.

Recurrent limbic seizures result in the rapid induction of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis, as well as alterations in the synthesis of several neuroactive peptides in rat hippocampus. In the present study, we examined the involvement of specific neurotransmitter or neuromodulator systems in the seizure-induction of hippocampal ODC.

A small unilateral electrolytic lesion was placed in the dentate gyrus hilus of all experimental rats; this treatment induces bilateral limbic seizures which recur over the period from 1.5 to 10 hrs postlesion. Three groups of hilus lesion (HL) rats were prepared that received either the opiate antagonist naltrexone (2 mg/kg; i.p. every 3 hrs starting at the time of the lesion), the NMDA antagonist D,L-aminophosphoheptanoic acid (AP-7) (75 mg/kg, i.p.), or saline (i.p.). ODC activity was measured in hippocampus and other brain regions at 8 hrs postlesion. In saline injected animals, the HL produced about a 120-fold increase in ODC activity in hippocampus as compared to untreated controls. Repeated administration of naltrexone did not modify the increase in ODC activity elicited by HL. In contrast, AP-7 injection markedly reduced the HL-induced increase in ODC activity.

These results indicate that while opiate receptor stimulation is not critically involved in the seizure-dependent increase in ODC activity, the activation of NMDA receptors might be a necessary step for either this effect or possibly for full seizure expression. Studies with other NMDA receptor antagonists are now in progress to further evaluate this conclusion.

Supported by NINCDS grant NS24624 to CG and MB.

197.2

REGIONAL ANALYSIS OF GENE EXPRESSION IN AMYGDALA-KINDLED RATS. K. Spanknebel*, L. Dawes, C. Applegate, C-A Ohmstede*, N. Sahyoun, R. Neve. The Children's Hospital, Boston, MA 02115; Wellcome Research Laboratories, Research Triangle Park, NC 27709. (SPON: F. Duffy)

Quantitative analysis of RNA from regions of amygdala-kindled rat brains with a variety of cDNA probes provides a means of identifying both the anatomical sites and the molecular basis of the response to kindling. Total RNA was isolated from neocortex, hippocampus, striatum, brainstem and cerebellum of rats each receiving one of four treatments: (1) naive control, (2) seizure induced in a naive animal, (3) amygdala-kindled, or (4) seizure induced in an amygdala-kindled animal. The RNAs were analyzed by Northern and dot blots with cDNA probes for calcium/calmodulin dependent kinase II, G3PD, v-fos, sodium ATPase, GAP-43, GAD, MAP2 and the Alzheimer amyloid precursor. In both kindled and naive animals with recent seizure activity, RNA levels for most genes were elevated compared to naive or kindled animals. This elevation was more pronounced in naive animals induced to seizure; and this pattern was seen in all regions examined but hippocampus. In the hippocampus, expression of all RNAs analyzed was equivalent for all treatments except for that of seizure induced in a kindled animal, in which expression of all genes tested, except for fos, amyloid and G3PD, was reduced several-fold. In situ hybridization will be used to define more precisely the changes in gene expression that we observed.

197.3

RAPID INCREASE IN 4 TRANSCRIPTION FACTOR mRNAs FOLLOWING SEIZURES: TEMPORAL AND ANATOMICAL CHARACTERIZATION. D.W. Saffen*, A.J. Cole, P.F. Worley, B.A. Christy*, K. Ryder* and J.M. Baraban. Depts. of Neuroscience and Molecular Biology, Johns Hopkins Univ., Balto., MD 21205

Transcription factors (TF) are proteins that bind to DNA and regulate gene transcription. mRNA levels for 4 putative TF genes, zif/268, c-jun, jun-B and c-fos increase rapidly in rat brain following pentylenetetrazole (PTZ)- or picrotoxin-induced seizures, becoming maximal by 60- and returning to baseline by 120 minutes after seizures. In situ hybridization shows that mRNA increases are localized to specific neuronal layers in dentate gyrus and entorhinal cortex. By contrast, apomorphine, a dopamine agonist, and p-chloroamphetamine, a serotonin releasing agent cause no detectable changes in mRNA levels in any brain region. Zif/268 encodes a protein with three zinc finger sequences characteristic of a class of TFs. c-Jun encodes the mammalian TF AP-1; jun-B shows extensive homology with c-jun. v-Fos protein stimulates transcription in vivo, and c-fos protein interacts with AP-1. Our observations extend those of Morgan et al. (Science 237:192, 1987) that c-fos mRNA and protein are induced in rat brain following PTZ seizures. TF gene mRNA increases following seizures suggests that specific neuronal populations manifest a complex, highly regulated genomic response to intense stimulation.

197.4

ACTIVATION OF NMDA RECEPTORS INDUCES PROTEOLYSIS OF SPECTRIN IN HIPPOCAMPUS. P. Seubert*, J. Larson*, M. Oliver*, M.W. Jung*, M. Baudry and G. Lynch (SPON: D. Arst). Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA 92717.

Activation of N-methyl-D-aspartate (NMDA) receptors has been implicated both in the induction of synaptic plasticity and the development of certain neuropathologies. Although the calcium conductance associated with NMDA receptor activation is thought to be crucial to its function, the biochemical events triggered by this calcium flux remain controversial. We have suggested that such calcium influxes are capable of initiating proteolytic events which alter cytoskeletal proteins and thus neuronal structure and function.

To examine this hypothesis directly we have exposed hippocampal slices to NMDA and examined the calcium dependent proteolysis of brain spectrin, a major cytoskeletal protein found in neuronal processes including synaptic regions. Exposure of hippocampal slices to ~50 μM NMDA resulted in the reversible loss of synaptic responses and caused a marked increase in the amount of a 150 kDa spectrin breakdown product (BDP) detected on immunoblots. Fifteen minutes after NMDA application, BDP levels were increased 84% ± 21 (mean ± S.E.M.) over levels prior to treatment, while control slices showed only a 16% ± 13 increase. The inclusion of 200 μM 2-amino-5-phosphonovalerate (AP5) prior to NMDA addition resulted in BDP increase of only 28% ± 12. Depolarization induced by the addition of 50 mM KCl caused only a 17% ± 6 increase. NMDA added to slices maintained in calcium-free medium caused the loss of antidromic responses but no increase in BDP. Thus, the effect is blocked by an NMDA antagonist, not reproduced by KCl depolarization and dependent upon extracellular calcium. The demonstration of NMDA receptor activation coupled to irreversible cytoskeletal alterations supports the hypothesis that this mechanism functions in the biological processes linked to this receptor.

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197.5

FUNCTIONAL CHOLINERGIC SUPERSENSITIVITY IN DENERVATED HIPPOCAMPAL PYRAMIDAL NEURONS. D.M. Benson, E.M. Landau, V. Haroutunian and R. Blitzer. Dept. of Psychiatry, Bronx VA Med. Cntr., Bronx, N.Y. 10468.

Acetylcholine excites hippocampal pyramidal cells, causing depolarization and increases in firing rates. The major source of cholinergic input to the hippocampus is from the medial septal nucleus, via the fimbria-fornix (F-F) pathway. We compared the effects of carbachol (CCh), a cholinergic agonist, on normal and denervated hippocampal pyramidal neurons in vitro. Hippocampal slices were prepared from normal adult rats and from rats 3-6 wks following aspirative lesions of the F-F. Slices were superfused with oxygenated artificial CSF at room temperature, and voltage-clamp recordings obtained from CA_1 neurons. CCh (0.5 to 5μM) induced a dose-dependent inward current in all cells tested, which was easily reversible upon washout of the drug. This CCh-induced current was considerably larger in the denervated (n=20) than in the control (n=22) condition, increasing by 105 ± 37%. The differences between the CCh effect on control and denervated cells was significant ($p<.05$) at all doses tested. The apparent K_D for CCh shifted from 2.1μM in control to 0.9 μM in denervated cells. Furthermore, the effect of CCh on the K^+ current, I_{AHP}, was greater in denervated than control cells. CCh (0.5μM) reduced I_{AHP} by 70±6% (n=7) in control but completely reversed the current in all denervated cells tested (n=6). All slices from F-F lesioned rats showed markedly decreased acetylcholinesterase staining compared to controls. Thus, there appears to be a functional supersensitivity to CCh in the denervated hippocampus. Since reports of alterations in receptor numbers following denervation have been inconsistent, this increased sensitivity may involve enhanced coupling, possibly mediated through G-protein activation. (Supported by NIH-NIA Grant #5P50AG05138.)

197.6

DEPOLARIZATION BLOCKADE IN NORMAL AND DENERVATED HIPPOCAMPUS INDUCED BY CHOLINERGIC RECEPTOR ACTIVATION. G. Omri, D. M.Benson, R.D. Blitzer and E.M. Landau. (SPON. E. Heyer). Depts. of Psychiatry and Pharmacology, Mt. Sinai School of Medicine and the Bronx VA Medical Center, New York, NY.

The effect of carbachol (CCh) on neuronal firing was investigated intra-cellularly in hippocampal slices. CCh (20 to 25 uM) depolarized the cells and produced a cessation of neuronal firing. During the depolarization phase, slow calcium spikes were often observed. Similar spikes were also seen when CCh was applied in 0.5 uM of tetrodotoxin. The depolarization blockade could be relieved by applying 100 uM of clonidine which repolarized the cells. When firing was induced with depolarizing current pulses the concentration of CCh leading to blockade of firing was much reduced (0.5 uM) as was the concentration of clonidine offering relief from it (1 uM). In rats where the hippocampus was denervated by sectioning the fimbria-fornix, the threshold for depolarization blockade fell dramatically, CCh as low as 0.5 uM causing the cessation of neuronal firing. These findings suggest that depolarization blockade induced by cholinergic agents may play a major role in determining their pharmacological response, especially in the presence of cholinergic denervation.

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197.7

Novelty Stress Inhibits the Induction of PB Potentiation in the Hippocampus of Awake Rats. D.M. Diamond, M.C. Bennett and G.M. Rose. VAMC and Dept. of Pharmacology, UCHSC, Denver, CO.

In previous work, we demonstrated that electrical stimulation patterned to mimic hippocampal physiological activity reduces the threshold for LTP *in vitro* and *in vivo* (Neurosci. Lett., *69:244*, 1986; J. Neuroscience, in press). This effect, termed primed burst (PB) potentiation, requires only 5 pulses to be induced. In the present study, we analyzed the role of novelty stress as an inhibitor of PB potentiation in the awake rat.

The major finding is that PB potentiation rarely occurred when the subjects were naive to the recording procedures. Over repeated sessions, the incidence of PB potentiation increased to 100% as the animals acclimated to the procedures. Two additional groups were tested to determine whether this effect was specific to acclimation or it resulted from a kindling-like reduction in threshold. In one group, rats were acclimated to the recording procedures without receiving electrical stimulation. PB stimulation then induced potentiation on the first attempt. In a second group, naive rats were stimulated 3 times in their first recording session. PB potentiation did not occur in any of these rats. *Thus, a critical precondition to inducing PB potentiation is acclimation to the environment.* To study the hormonal basis of the effect, naive rats were adrenalectomized and then stimulated. All of these animals showed PB potentiation in the first attempt. These data suggest that adrenal hormones released during novelty stress exert an inhibitory influence on the induction of synaptic plasticity.

197.8

EARLY AND SELECTIVE STRESS-INDUCED CHANGES IN SPECIFIC FOREBRAIN PROTEINS. G. Shanker, V. H. Gilad and G.M. Gilad. Dept. of Neurobiology, Coriell Inst. for Med. Res., Camden, NJ, USA.

We sought to identify specific stress-induced changes in proteins of selective forebrain regions. Rats were subjected to immobilization stress and subcellular fractions, prepared from the hippocampus and frontal cortex, were studied by two dimensional gel electrophoresis. Selective prominent changes occurred after 2 hrs of stress: 1) a protein with a molecular weight of 59,000 (59K) was increased in the soluble fraction but decreased in the synaptosomal fraction, only in the hippocampus. The amount of this protein was higher in an inbred rat strain that is hyper-reactive to stress: 2) a drastic decrease in two 63K proteins differing in pI values, was observed in the soluble fraction of the hippocampus, and 3) a 79K protein increased prominently only in the nuclear fraction of hippocampus where it was enriched, and a 68K protein was decreased in nuclei of both hippocampus and frontal cortex. We conclude that rapid and region-selective changes in brain protein metabolism can be induced by acute application of strong stressors. We now seek to examine whether the magnitude and duration of changes in these proteins may reflect a plastic compensatory metabolic response that may be related to the severity of the stress.

197.9

REGULATION OF CCK AND NPY IMMUNOREACTIVITY WITHIN THE ADULT RAT OLFACTORY BULB BY PRIMARY AFFERENT INPUT. I.A. Scarisbrick* and C.M. Gall (Spon: M. Nieto-Sampedro). Dept. of Anatomy and Neurobiology, University of California, Irvine, C.A. 92717.

Previous investigations have demonstrated that tyrosine hydroxylase immunoreactivity (TH-I) within olfactory bulb periglomerular and tufted neurons is reduced following olfactory nerve ablation or odor deprivation. The present study addresses the question of whether the regulation of CCK immunoreactivity (CCK-I) within periglomerular and middle tufted neurons, which appear distinct from those containing TH-I, and NPY immunoreactivity (NPY-I) within large neurons and axonal arbors in the deep granule cell layer (GR), are similarly dependent upon primary sensory input.

The avidin-biotin immunocytochemical technique was used to examine CCK-I, TH-I and NPY-I in the main olfactory bulb of adult male Sprague Dawley rats. Animals were sacrificed 3 weeks after unilateral deafferentation by reversible chemical (1.5% Triton X-100) nasal epithelial lesion or following olfactory nerve transection and removal. In a third group, primary sensory input was reduced without afferent degeneration by unilateral nares closure. In each experimental group, CCK-I and TH-I were dramatically reduced within the ipsilateral olfactory bulb while NPY-I appeared normal. There was a dramatic reduction in the number of CCK-I neurons in both the external plexiform and glomerular layers (GL) and a striking loss of CCK-I within processes extending into the GL. In addition, there was a complete loss of CCK-I puncta within the internal plexiform layer. The density of CCK-I elements within the granule cell layer was unaffected. As reported by others, TH-I was absent or greatly reduced within periglomerular and tufted cells following a complete first nerve lesion or odor deprivation.

These findings are consistent with the concept that the regulation of CCK within olfactory bulb periglomerular and middle tufted neurons is dependent on sensory afferent activity whereas that of NPY is not. Studies are currently in progress to determine whether the loss of CCK-I following Trition X-100 is reversible upon olfactory bulb reinnervation and to determine if neuropeptide levels are being regulated at the level of genomic transcription.

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197.10

SPINAL AFFERENTS REGULATE THE EXPRESSION OF MOLECULES IN SPINAL CORD CELLS. E. Paige,* P. Levitt, M. E. Goldberger. (SPON: E.H. Murphy). Medical College of Pennsylvania. Phila. PA, 19129.

Unilateral deafferentation (dorsal rhizotomy L1-S2) results in a change in input to lamina II and Clarke's nucleus (CN) due to reactive reinnervation. Although there is no net change in terminal number, behavioral impairments occur, some of which are persistent and might be related to post-synaptic changes as well as altered input. Two markers were used to study post-synaptic effects of unilateral deafferentation of cat spinal cord from 12 days to 3 months post-operatively. LAMP (limbic system associated membrane protein) is a cell-surface glycoprotein which, in the spinal cord, is located only postsynaptically, (i.e. on dendrites and somata) on neurons in lamina II and the IML; cytochrome oxidase (CO) is located both pre- and post-synaptically, and not restricted to specific laminae or nuclei in the spinal cord. After chronic deafferentation LAMP is substantially reduced in lamina II but not in the IML; CO is reduced in lamina II, in the large cells of CN (as well as in the neuropil) and in the IML. In contrast, after chronic hemisection at T13, LAMP is depleted in the IML but unchanged in lamina II and CO is not consistently changed. In Clarke's nucleus, decreases in CO were seen first in the neuropil and later in the large neurons. Changes in LAMP immunoreactivity, seen at 12 days, were moderate but became more dramatic through the 3 month post-operative period. Thus levels of production and\or activity of certain cell-surface and metabolic proteins are regulated by specific afferent inputs or patterns of neuronal activity that depend on those inputs.

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197.11

DISTRIBUTION OF NEUROFILAMENT ANTIGENS FOLLOWING INTRARETINAL LESION IN ADULT HAMSTERS. K.E. Sloan * and J.A. Stevenson. Dept. of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Perikarya of axotomized peripheral neurons accumulate phosphorylated (P) epitopes of neurofilament proteins (NFP) with no apparent increase in the nonphosphorylated epitopes (Rosenfeld et al., J. Neuropathol Exp Neurol 46:269). As axons regenerate, the number of cells containing P-NFP declines. Although somata of retinal ganglion cells (RGCs) also accumulate P- epitopes following a lesion of the optic nerve or tract (Drager and Hofbauer, Nature 309:624), it is unclear whether there is a change in the distribution of NP- epitopes. In addition, it is unknown how the transient and limited regrowth of RGC axons, known to take place within the retina (Goldberg and Frank, Exp Neurol 70:675), affects the NFP content within perikarya. To further characterize the changes in neurofilament content following axotomy, antibodies against P or NP epitopes were used to examine components of RGCs 1-28 days following an intraretinal lesion. At 4-7 days, chromatolytic somata peripheral to the lesion demonstrated increased reactivity to antibodies against NP-NFP with no comparable increase in P-NFP immunoreactivity. Effects of longer survival times are being examined to determine when P epitopes appear and whether the distribution patterns change if axons begin regrowth.

197.12

INDUCTION OF NEUROFILAMENT PHOSPHORYLATION IN BOVINE CHROMAFFIN CELLS. N.J. Grant* & B. Demeneix* (SPON : K. Takeda), Centre de Neurochimie & Inst. Physiologie Générale, Univ. Louis Pasteur, 67084 Strasbourg, France

Chromaffin cells from the adrenal medulla can be induced to transdifferentiate from an endocrine to a neuronal phenotype in culture. We have recently described the induction of neurite outgrowth in bovine chromaffin cells by hypophyseal intermediate lobe (IL) cells (FEBS Lett. 226, 337, 1988). In this study, the expression of neurofilaments (NFs) was examined in order to evaluate the neuronal nature of the phenotype displayed by cultured chromaffin cells. Immunofluorescent studies were done using monoclonal antibodies against nonphosphorylated (np) or phosphorylated (p) epitopes of the 200kD NF subunit. Under standard culture conditions, most chromaffin cells did not display a neuronal phenotype and did not express pNFs. However, npNFs were detected in the cell bodies. In contrast, when chromaffin cells were maintained in IL-conditioned medium for 10 days, the npNF form was also observed in neurites of about 25% of the cells with extensions, but there was little or no immunoreactivity for pNFs. By 17 days in conditioned medium, over 50% of the cells with neurites had extensions showing immunoreactivity for both pNFs and npNFs. The pNFs were restricted to neurites. The results suggest that certain soluble factors released by IL-cells can cause chromaffin cells to develop a more typical mature neuronal phenotype as judged by the appearance and phosphorylation of NFs in neuronal processes.

197.13

SYNAPTIC PLASTICITY IN THE MATURE CNS INVOLVES INCREASED LEVELS OF GAP-43. L.I. Benowitz, W.R. Rodriguez, W.F. White and R.L. Neve. Depts. of Psychiatry, Neuropathology and Pediatrics, Harvard Medical School; McLean Hospital, Belmont, MA 02178; Children's Hosp., Boston, MA 02115

When the projections to the rat dentate gyrus from the entorhinal cortex are damaged, other neurons sprout axon collaterals and form new functional synapses upon dendritic segments denervated by the lesion (Cotman et al, 1984, Science 225:1287). Using this well-characterized model system, we investigated whether the growth of new synapses in the mature CNS requires an increased expression of the neuron-specific, growth-associated phosphoprotein GAP-43 (equivalent to B-50, F1, pp46, P-57). At times varying from two days to one month after unilateral entorhinal lesions, rats were sacrificed and their brains processed to visualize GAP-43 by immunocytochemistry as described (Benowitz et al, 1988, J. Neurosci. 8:339). As early as two days after surgery, marked increases were seen in the intensity and extent of GAP-43 immunostaining in the segment of the dentate granule cell dendrites in which the projections arising from hippocampal pyramidal neurons are known to be sprouting; this increase persisted for at least a week. Dendritic segments known to receive sprouting septal projections, however, did not show large GAP-43 increases. We are currently using in situ hybridization to determine whether the differences between the sprouting of hippocampal and septal afferents reflect differences in transcription of the GAP-43 gene or changes in turnover at the synapse. **[NS 25830]**

197.14

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE GROWTH-ASSOCIATED PROTEIN, GAP-43, IN THE HUMAN CEREBRAL CORTEX. N. I. Perrone-Bizzozero, D. Weiner*, J. Winickoff*, and L.I. Benowitz. Dept. Psychiat., Harvard Med. School, McLean Hospital, Belmont, MA 02178

The presynaptic membrane phosphoprotein, GAP-43, has been implicated in both the development and modulation of neuronal connections. By the use of a monospecific antibody raised against rat GAP-43, we have studied the distribution of the protein in cortical and subcortical areas of the human brain. On Western blots, the antibody reacted with a synaptosomal plasma membrane protein which had a molecular weight and isoelectric point similar to GAP-43 of other species. In postmortem human brain, the densest immunoreactivity was found in associative areas of the neocortex, particularly within layers 1 and 6, in the molecular layer of the dentate gyrus, the caudate-putamen, and the amygdala. In contrast, primary sensory and motor regions of the cortex, portions of dorsal thalamus, and cerebellum showed only light staining. Staining was generally confined to the neuropil, which showed punctate labeling, whereas most neuronal somata and fiber bundles were unreactive. These results are consistent with the distribution of GAP-43 mRNA in the human brain (Neve et al, PNAS 85 [May 1988]) and may reflect regional variations in the potential for functional and/or structural remodeling. [Support: **NEI EY 05690 and NINCDS NS 25830**]

197.15

FUNCTIONAL SIGNIFICANCE AND LOCALIZATION OF PHOSPHOSITE(S) IN THE NEURON-SPECIFIC PROTEIN B-50/GAP43. L.H. Schrama*, P.N.E. de Graan*, L.V. Dekker*, A.B. Oestreicher*, H. Nielander*, P. Schotman* and W.H. Gispen* (SPON: A.J. Schecter). Div. Mol. Neurobiol., Rudolf Magnus Inst. Pharmacol, Lab. Physiol. Chem., Inst. Molec. Biol. & Med. Biotechnol., Univ. of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

The neuron-specific phosphoprotein B-50/GAP43 is an endogenous substrate of protein kinase C (PKC) in presynaptic terminal membranes. Phosphorylation of purified B-50 by PKC followed by extensive digestion with S. aureus protease V8 (SAP) revealed that all phosphosites are limited to the small neutral 15 kDa peptide. SAP digestion of the full-length cRNA in vitro translation product after labeling with either [^{35}S]-Met or [^{35}S]-Cys yielded a 15 kDa immunoreactive peptide containing all the label incorporated in the undigested protein. This suggests that the phosphosite(s) in B-50 are confined to the N-terminal portion of the molecule. PKC-mediated B-50 phosphorylation was studied by in situ phosphorylation of synaptosomes or hippocampal slices with $^{32}P_i$ followed by quantitative immunoprecipitation. K^+-depolarization induces neurotransmitter release and a concomitant increase in B-50 phosphorylation. Both the release and the increase in B-50 phosphorylation can be inhibited by PKC inhibitors in a dose-dependent manner and by omittance of extracellular Ca^{2+}. The effects of K^+ can be mimicked by Ca^{2+}-ionophore A23187. Our data suggest a role of B-50 phosphorylation in transmitter release.

197.16

REGIONAL DISTRIBUTION OF MAP 2 IN AGING RAT BRAIN. E.M. Grollman* and P.D. Coleman (SPON: I. Shoulson). Department of Neurobiology and Anatomy. University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642.

When thermostable extracts of fresh mammalian brain are subjected to polyacrylamide gel electrophoresis (PAGE), two high molecular weight bands corresponding to isoforms A and B of microtubule-associated protein 2 (MAP 2) are easily identifiable after Coomassie staining and can readily be quantitated by laser densitometry. Since MAP 2 is a neuron specific molecule, primarily localized to dendrites (Huber and Matus, J. Neurosci. 4:151-60,1984; De Camilli et. al., Neurosci.11:817-46,1984), the amount of MAP 2 present in a given brain region can be taken as a measure of the dendritic compartment in that region.

An issue important to the mammalian CNS is whether dendritic proliferation (or regression) accompanies normal aging. Previous studies on this topic have relied primarily on quantitative morphological analyses to determine changes in dendritic extent with aging in specific neuronal populations. Marked growth in the dendritic tree of olfactory bulb mitral cells of the Sprague-Dawley rat was observed at 27 months of age followed by a precipitous decline (Hinds and McNelly, J. Comp. Neur. 171:345-68,1977). On the other hand, no age related changes in the complexity of the dendritic network was found in either Purkinje cells (Pentney, Neurobiol. Aging 7:241-8,1986) or layer II pyramidal cells of entorhinal cortex (Coleman and Buell, Soc. Neurosci. Abs.,1983) of the F344 rat or in layer IV stellate neurons in the posteromedial barrel subfield of C56Bl/6 mouse somatosensory cortex (Coleman et. al., Neurobiol. Aging 7:101-5,1986).

We have used laser densitometry of Coomassie stained polyacrylamide gels to quantify MAP 2 levels in cerebellum, entorhinal cortex, hippocampus, olfactory bulb, and frontal cortex in the F344 rat at five ages from 3 to 30 months. This technique provides a nonhistologic means of quantitating the dendritic compartment by brain region.

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197.17

EFFECT OF TESTOSTERONE ON CALCITONIN GENE-RELATED PEPTIDE LIKE IMMUNOREACTIVITY IN THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS. P. Popper and P. E Micevych. Department of Anatomy and Lab. of Neuroendocrinology, Brain Res. Inst., UCLA School of Medicine, Los Angeles, CA 90024.

Changes in the hormonal milieu of an animal induce changes in the molecules expressed by neurons of its central nervous system. The spinal nucleus of the bulbocavernosus (SNB) is a sexually dimorphic nucleus of about 200 androgen accumulating motor neurons in the lumbar segments 5 and 6 of the rat spinal cord. In the present work we have used immunohistochemistry to investigate the change in calcitonin gene-related peptide like immunoreactivity (CGRP-LI) in the motor neurons of the SNB induced by long term castration. Rats were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, the spinal cord removed, postfixed for 24 hours in the same fixative. 30 um thick serial sections were processed according to the avidin-biotin peroxidase antiperoxidase method using polyclonal antiserum directed against synthetic rat (Tyr)CGRP$_{23-37}$ (gift from Dr Sternini, CURE, UCLA). The nuclei of CGRP-LI containing neurons and unstained neurons from alternate sections were counted. In the intact animals 53+/-7.4 % (n=4) of the SNB neurons had CGRP-LI and in the castrates 80+/-1.7% (n=9, p<0.001). In animals castrated for four weeks and then implanted with testosterone containing silastic capsules for four weeks the percentage of the SNB motor neurons containing CGRP-LI was not significantly different from that in intact animals. These results demonstrate that change in testosterone level regulates the number of SNB motoneurons that express CGRP-LI. Supported by NS23468.

EXCITATORY AMINO ACIDS IV

198.1

Na^+- AND Ca^{++}-CHANNEL AGONISTS STIMULATE ENDOGENOUS AMINO ACID RELEASE FROM PRIMARY CEREBELLAR NEURONAL CULTURES. G.R. Dutton, K.L. Rogers and R. Philibert Dept. of Pharmacology, University of Iowa, Iowa City, IA 52242.

We have used HPLC to quantify the release of endogenous amino acids from primary cerebellar neuronal cultures in response to Na^+- and Ca^{++}-channel agonists.

Veratridine (50 µM) evoked release of aspartate, glutamate, GABA, taurine, adenosine, alanine, proline and serine. The onset and peak of release of glutamate, aspartate, GABA, alanine, proline and serine coincided with the onset and peak of stimulation, whereas the peak release of taurine and adenosine was delayed relative to maximal stimulation by approximately 5-7 min. Release of all amino acids was completely antagonized by preincubation with 2 µM tetrodotoxin.

The dihydropyridine Ca^{++}-agonist BAY K 8644 (1 µM) markedly augmented the 30 mM K^+-evoked release of glutamate, aspartate, GABA and adenosine, while increasing the release of the other amino acids only to a minor extent. However, the addition of nifedipine (5 µM) to these stimulating conditions decreased the evoked release of all amino acids well below the levels of release elicited by 30 mM K^+ alone.

These findings demonstrate that a wide variety of amino acids are released from cerebellar neurons, voltage-dependent Na^+- and L-type Ca^{++}-channels are functionally coupled to amino acid release in these cultures, and L-type Ca^{++}-channels may differentially regulate the release of amino acids in response to neuronal depolarization. Supported by NS 20632 and MH 15172.

198.2

FAST HPLC METHODS FOR "ON LINE" AND "OFF LINE" MONITORING OF ASPARTATE AND GLUTAMATE IN MICRODIALYSIS PERFUSATES: J. Kehr* and U. Ungerstedt* (SPON: D. Ottoson). Dept. Pharmacology, Karolinska Institute, Stockholm, Sweden.

Fully automated reverse phase high-performance liquid chromatography with precolumn o-phthaldialdehyde (OPA) derivatization and flourescence detection has been modified for the rapid scanning of Asp and Glu levels in microdialysis perfusates of the rat striatum. The method applies an autosampler provided with two 6-port valves, where the separation column (60x4 mm Nucleosil C18, 5 um) is installed in the position of the second valve. The robotics program for the injector enables to optimize the run time in such a way, that after the elution of Asp and Glu (80 sec) the second valve is attenuated and the rest of amino acids is flushed out of the column in the opposite direction within 40 sec. Another 30 sec is needed to reequilibrate the column before the next injection. "Off line" method implies automated derivatization procedure by the autosampler, whereas in the "on line" setup the sample from the microdialysis probe and OPA reagent (both delivered at flow rate 2 ul/min by use of a syringe pump) are mixed before being loaded into the valve. The procedure is used for the monitoring of Asp and Glu outflow under different neuropathological states (ischemia, hypoglycemia), in pharmacology (effects of varying concentrations of K^+, Ca2+, Mg2+ ions in the perfusion medium) and in toxicology.

198.3

HIGH SENSITIVITY OF NMDA-STIMULATED CALCIUM UPTAKE TO INHIBITION BY ETHANOL IN PRIMARY CULTURES OF CEREBELLAR NEURONS. Carolyn S. Rabe and Boris Tabakoff. Lab of Physiologic and Pharmacologic Studies, NIAAA, Rockville, MD 20852.

Recent work in this laboratory has shown that glutamate-stimulated cGMP accumulation in cultured rat cerebellar cells is quite sensitive to inhibition by ethanol (Hoffman et al., submitted). In an attempt to understand the mechanism underlying the inhibition, we have begun examining the effect of ethanol on $^{45}Ca^{2+}$ uptake into cultured cerebellar cells in response to stimulation with agonists of the various glutamate receptor subtypes. Both NMDA (100 µM, in Mg^{2+}-free buffer) and kainate (100 µM) produce detectable increases in $^{45}Ca^{2+}$ uptake within 10 sec in these cells. The increase stimulated by NMDA is completely inhibited by DL-2-amino-5-phosphonovaleric acid (200 µM). The NMDA-stimulated increase in $^{45}Ca^{2+}$ uptake also is highly sensitive to inhibition by ethanol. Threshold inhibition is observed between 2.5 and 5 mM ethanol. The EC_{50} for inhibition occurs between 10 and 25 mM and maximal inhibition is observed at 100 mM ethanol. Inhibition of NMDA-gated currents by similar concentrations of ethanol in neurons acutely isolated from dorsal root ganglia also has been observed using patch clamp techniques. (D. Lovinger, personal communication). In contrast, kainate stimulated $^{45}Ca^{2+}$ uptake in cerebellar cells is an order of magnitude less sensitive to inhibition by ethanol. Threshold inhibition of kainate-stimulated $^{45}Ca^{2+}$ uptake is observed between 25 and 50 mM ethanol and 100 mM ethanol causes approximately a 30% inhibition of kainate stimulated uptake. The sensitivity of the kainate-stimulated uptake is consistent with values reported for the ability of ethanol to inhibit voltage-dependent $^{45}Ca^{2+}$ uptake in clonal neuronal cultured cells (PNAS 83:6213, 1986). The relatively high sensitivty of NMDA-stimulated Ca^{2+} uptake to inhibition by ethanol suggests that inhibition of NMDA-mediated responses may have an important role in acute ethanol intoxication.

198.4

CHLORPROMAZINE (CPZ) AND IMIPRAMINE (IMI) METABOLITES ARE NMDA ANTAGONISTS Ian J. Reynolds and Richard J. Miller Dept. Pharm. Physiol. Sci. Univ. Chicago, Chicago, IL 60637.

The NMDA-selective subtype of the glutamate receptor consists of an NMDA recognition site coupled to a cation selective ion channel. The activity of the channel is subject to modulation by a variety of agents. Magnesium and zinc decrease channel activity, while glycine enhances the ability of NMDA and glutamate to open the channel. Using [3H] MK801 we have been able to distinguish between the different binding sites which regulate NMDA receptor activity (Reynolds and Miller, 1988, Mol. Pharm. in press). We have recently found that CPZ and IMI derivatives inhibit binding in a zinc-like fashion. In an attempt to develop specific tools to probe the function of the zinc binding site in vivo we have examined the binding activity of several CPZ and IMI metabolites. Mono- and di-desmethyl IMI are more potent than IMI (7, 5 and 25µM respectively), while didesmethyl CPZ is more potent than CPZ (13 and 40µM). Methoxylation of the phenothiazine ring significantly decreases potency, as does sulfoxidation. However 7- and 2-hydroxy derivatives are more potent than the parent compounds. Based on dissociation kinetic assays these compounds appear to be zinc-like. However CPZ and chlorimipramine are more effective zinc-like drugs than their non-halogenated counterparts. Several of the modifications to the structure of CPZ and IMI that increase the potency at the NMDA receptor are associated with a decrease in their activity at catecholamine uptake sites, dopamine and muscarinic receptors suggesting that it may be possible to separate the respective actions and develop a specific ligand for the zinc binding site of the NMDA receptor.

198.5

COMPARISON OF REGIONAL ^{14}C-2-DEOXYGLUCOSE (2-DG) UPTAKE IN RAT BRAIN FOLLOWING PARENTAL ADMINISTRATION OF EITHER D-2-AMINO-5-PHOSPHOHEPTANOATE (APH) OR KETAMINE. J.E. Mathisen[1]*, S.M. Rothman[1], P.C. Contreras[2] and R.K.Deuel[1]. [1]Department of Pediatrics and Neurology, Washington University School of Medicine, St. Louis, MO 63110. [2]Searle Research and Development, Chesterfield, MO 63198.

Several investigators, using the 2DG method, have observed striking increases in the cerebral metabolic activity in limbic structures following the administration of ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist (Crosby et al., 1982). This has raised the question of whether the excitatory effects are mediated entirely by the NMDA receptor. We compared the regional uptake of ^{14}C-2-DG using standard quantitative autoradiographic techniques in 21 loci following the intravenous administration of either the competitive NMDA antagonist APH (600 mg/Kg) or ketamine (20 mg/Kg).

The APH animals had lower global mean cerebral gray matter activity (.207 ± .054 µCi/gm) compared to ketamine animals (.467 ± .238 µCi/gm) ($p < 0.10$). The APH group showed higher relative 2DG uptakes in lateral geniculate and superior colliculus ($p < 0.05$) and sensory motor cortex ($p < 0.01$), while ketamine animals had higher uptake in the anterior thalamus ($p < 0.01$). The lower global gray matter activity in the APH group suggests that increased activity with noncompetitive NMDA antagonists must represent action at a site different from the NMDA receptor complex.

198.6

GUANYLATE CYCLASE ACTIVATION BY EXCITATORY AMINO ACIDS AND ATRIAL NATRIURETIC PEPTIDE IN RAT CEREBELLAR GRANULE CELLS IN PRIMARY CULTURE. A. Novelli, M.A. Vigano'*, R.C. Henneberry* and A. Borsellino*. Lab. Mol. Neurobiology, NIH, Bethesda,MD 20892 ,USA and Internatl. School for Advanced Studies, Trieste 34014,Italy.

Excitatory Amino Acids (EAA) and Atrial Natriuretic Peptide (ANP) have both the capability to increase intracellular concentrations of cGMP in cerebellar granule cells in primary culture (Novelli, A. and Henneberry, R.C., Dev. Brain Res., 34:307,1987). We now present evidences for an interaction between EAA receptors and ANP receptors. In the presence of 1mM Mg^{++},Glutamate (GLU) did inhibit,in a dose-dependent manner, up to 40% of the ANP mediated increase of intracellular cGMP levels. This inhibition of ANP action by GLU was non-competitive and appeared to occur at the proximity of the receptor for ANP. ANP elevation of cGMP levels was not affected by D-(-)-2-Amino-5-PhosphonoValeric acid (APV), a specific glutamate antagonist.In Mg^{++}free conditions, GLU dose-dependent stimulation of cGMP increase was not modified by the presence of 10^{-7}M ANP,a maximal concentration for ANP-stimulated cGMP production. Furthermore no additive effect in the elevation of cGMP levels by GLU and ANP was detectable at concentrations of GLU that were unable to inhibit ANP effect. In the absence of Mg^{++}, GLU induced elevation of cGMP levels was Ca^{++} dependent and a similar increase in intracellular cGMP concentration was achieved using the Ca^{++} ionophore A23187. This receptor-independent, Ca^{++}-mediated stimulation of cGMP synthesis was also not additive to the stimulation of cGMP synthesis by ANP. We suggest that Glu receptor and ANP receptor are closely associated and may be coupled to the same guanylate cyclase molecules.

198.7

PCP-LIKE METABOLIC EFFECTS OF THE NMDA-CHANNEL BLOCKING AGENT, MK-801. P.Kazckovsky*, W.E.Hoffmann, and M.F.Piercey, (Spon. P.Ho), The Upjohn Company, Kalamazoo, MI 49001.

MK-801, an NMDA ion channel blocker, has neurocytoprotective and anticonvulsant effects in animals (Iversen et al., Pharmacol. Biochem. Behav. 28:127, 1987). Since PCP is an NMDA-channel blocker, MK-801 may be psychotomimetic. 2-Deoxyglucose (2-DG) autoradiography shows that PCP psychotomimetic effects are elicited in limbic areas, especially Papez' circuit and dopamine (DA) areas (Piercey and Ray, Pharmacol. Biochem. Behav. 28:136, 1987). In the present experiments, rats were injected with 1 mg/kg i.p. MK-801 15 min prior to 2-DG injection using a standard protocol (Sokoloff et al., J. Neurochem. 28:897, 1977). Like PCP, MK-801 produced dramatic increases in metabolism in DA areas (caudate, glob. pall., n. accumbens, olf. tub.) and in Papez' circuit (mamm. body, ant. thal., ent. cortex, mol. layer of hipp). MK-801 produced PCP-like columns in sensorimotor cortex and, like PCP, inhibited metabolism in l. habenula and inf. coll.

198.8

CYSTEINE: RELEASE UPON DEPOLARIZATION OF RAT BRAIN SLICES AND EFFECT ON EXCITATORY AMINO ACID RELEASE H.J. Keller*, K.Q. Do*, M. Zollinger*, +K.H. Winterhalter* and M. Cuénod. Brain Research Institute, Univ. Zürich, CH-8029 Zürich and +Laboratory for Biochemistry I, ETH-Zentrum, CH-8092 Zürich, Switzerland.

We performed a screening of potential neuroactive substances by selecting compounds that are released from rat brain slices upon depolarization (50 mM K^+) in a Ca^{2+}-dependent manner. Lyophilized superfusates were derivatized with 9-fluorenylmethyl-succinimidyl-carbonate (FMOC-ONSu) and analyzed by reversed-phase HPLC combined with fluorescence detection. An HPLC peak, which was increased after depolarization, was identified as cysteine (Cys) by fast-atom bombardment mass spectrometry. In order to improve Cys quantification and to minimize further the oxidation of Cys, superfusates were cooled to 0°C and immediately reacted with N-ethylmaleimide. The two HPLC peaks corresponding to the diastereomeres produced by that reaction were summed up. Potassium-stimulated Ca^{2+}-dependent efflux of Cys was highly significant ($p<0.01$) in cortex (33 pmol · mg protein^{-1} · min^{-1}), meso-diencephalon (24), and striatum (18), significant ($p<0.05$) in cerebellum (13), whereas it was not significant ($p>0.05$) in hippocampus and pons-medulla. Moreover in neocortical slices Cys (10-500 µM) increased in a dose-dependent manner both the basal efflux and the efflux induced by 25 mM K^+ of aspartate and glutamate. This suggests that Cys could be released from a synaptic compartment and could modulate excitatory amino acid release.

198.9

SOURCES OF PRESUMPTIVE GLUTAMERGIC/ASPARTERGIC AFFERENTS TO THE RAT MESIAL PREFRONTAL CORTEX. K.M. Carnes & J.L. Price. Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Small injections of a combined solution of 3H-D-aspartate (3H-D-Asp) and wheat germ agglutinin-horseradish peroxidase (WGA-HRP) were placed in the rat mesial prefrontal cortex in order to compare the pattern of presumptive glutamergic/aspartergic afferents with the overall pattern of afferents to this region. The afferents labeled by 3H-D-Asp were in general a subset of those labeled by WGA-HRP.

Cells retrogradely labeled by an injection of 3H-D-Asp into the prelimbic and anterior cingulate cortical areas were primarily found in the dorsal peduncular, infralimbic, insular and perirhinal cortical areas, the parataenial, mediodorsal, ventromedial (principal division) and intralaminar/midline thalamic nuclei, the amygdaloid complex, and the claustrum. In the contralateral hemisphere, labeled cells were found in the mesial prefrontal and agranular insular cortices.

Fewer neurons were retrogradely labeled by 3H-D-Asp than by WGA-HRP in all of the regions except the claustrum, and the parataenial and ventromedial thalamic nuclei. In addition, neurons in the dorsal peduncular cortex were labeled only with 3H-D-Asp. Except for an occasional cell, neurons along the lateral edge of cingulate cortex, and in the anterior olfactory nucleus (pars medialis), the magnocellular basal forebrain (MNBF), the hypothalamus, the hippocampal formation, and the brainstem were labeled by WGA-HRP but not by 3H-D-Asp.

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198.10

EXCITATORY AMINO ACID-EVOKED RELEASE OF ENDOGENOUS ADENOSINE FROM RAT CORTICAL SLICES K. Hoehn and T.D. White, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7

Adenosine may have a protective role in the CNS in situations of excess excitatory neurotransmission due to its ability to inhibit the release of neurotransmitters and decrease firing of central neurons. We have studied excitatory amino acid-evoked release of adenosine in vitro by a continuous superfusion method. Slices were superfused at 0.75ml/min and samples collected at 2.5 min intervals. Glutamate released adenosine in a dose dependent manner. Whereas K^+-evoked release was decreased by about 50% in the absence of Ca^{2+}, glutamate -evoked release was not decreased. Exposure to the specific glutamate receptor agonists, NMDA, kainate, and quisqualate at 100 and 500 µM also released adenosine. Kainate-evoked release was diminished by about 40% by 1mM γ-D-glutamyl-glycine. 500µM NMDA-evoked release was blocked by 1mM DL-APV. 1mM APV also decreased 1.5mM glutamate-evoked release by about 50%, suggesting that NMDA receptors are involved in glutamate-evoked adenosine release. During ischemia or seizures, adenosine may be released to act as an endogenous inhibitory modulator to dampen the firing of neurons.

Supported by the Medical Research Council of Canada.

198.11

RELEASE OF [^{3}H]NOREPINEPHRINE (NE) FROM HIPPOCAMPAL SLICES BY N-METHYL-D-ASPARTATE: COMPARISON OF THE INHIBITION BY MK-801 AND Mg^{2+}. C.J. Schmidt* and V.L. Taylor* (SPON: R.J. Dinerstein). Merrell Dow Res. Inst., 2110 E. Galbraith Road, Cincinnati, OH 45215

[^{3}H]NE release from rat hippocampal slices was studied as a functional response to activation of the NMDA receptor. NMDA-induced [^{3}H]NE release was inhibited by the competitive NMDA antagonist, CPP, and was completely blocked by 1.15 mM Mg^{2+}. Predepolarization with 10 mM K^+ or 50 μM kainic acid eliminated this effect of Mg^{2+}. Concentration-response curves for MK-801 and Mg^{2+} yielded IC_{50} values of approximately 35 nM and 35 μM, respectively. Using consecutive pulses of NMDA, MK-801 demonstrated use-dependency in that the degree of inhibition increased with time. In contrast, the degree of inhibition by Mg^{2+} was constant. Kinetic analysis of MK-801-induced inhibition indicated an uncompetitive mechanism while inhibition by Mg^{2+} was noncompetitive. The inhibition of [^{3}H]NE release by MK-801 was a cumulative process which persisted even after removal of the drug. The effect of Mg^{2+} was immediately reversed upon its removal from the media. The results are consistent with both MK-801 and Mg^{2+} inhibiting NMDA receptor-mediated events at a site distinct from the receptor. The voltage sensitivity of Mg^{2+} and the agonist-dependency of MK-801 are consistent with this site(s) being at the cation channel gated by NMDA. Differences in use-dependency, kinetic mechanism and reversibility suggest the two antagonists may act at different sites within the channel.

198.12

TRANS-2-CARBOXY-3-PYRROLIDINE ACETIC ACID (CPAA), A NOVEL AGONIST AT NMDA-TYPE RECEPTORS. C. Tsai, J. A. Schneider* and J. Lehmann. Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901.

Although 2-carboxy-3-pyrrolidine acetic acid analogs are associated with activity as agonists at kainate-type receptors, some compounds in this series possess activity at the NMDA-type receptor. For instance, beta-kainate has been found by some workers to be an antagonist of NMDA-type receptors. Here we report that CPAA is an agonist at NMDA-type receptors.

CPAA evoked the release of [^{3}H]acetylcholine (ACh) from striatal slices with the same efficacy as N-methyl-D-aspartate (NMDA), and an EC_{50} of 20.0 μM, compared to an EC_{50} of 45.8 μM for NMDA. CPAA-evoked [^{3}H]ACh release was inhibited by tiletamine (IC_{50} = 0.53 μM), MK-801 (IC_{50} = 0.12 μM), and $MgCl_2$ (IC_{50} = 26 μM). CPAA produced a tachyphylaxis when applied continuously for 18 min or more, and a cross-tachyphylaxis to NMDA. Similarly, NMDA generated a cross-tachyphylaxis to CPAA. All of these data suggest that CPAA is an agonist at NMDA-type receptors.

198.13

4-PHOSPHONOPROPYLPIPERIDINE-2-CARBOXYLIC ACID (PPPC), AN ANTAGONIST OF THE N-METHYL-D-ASPARTATE (NMDA)-SENSITIVE GLUTAMATE RECEPTORS. D. T. Wong, P. G. Threlkeld*, P. L. Ornstein* and J. W. Chambers*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

PPPC exhibits high affinity for the NMDA receptors labeled with ^{3}H-CPP in rat hippocampal membranes which have been treated with 1% Triton. The IC_{50} value of PPPC to inhibit ^{3}H-CPP binding is 66 nM, and is about equally potent as the known NMDA antagonists: 91 nM for d-AP5 and 67 nM for CPP. The Hill coefficients for the compounds are not significantly different from unity. Recently, specific ^{3}H-MK801 binding has been described and is functionally linked to the NMDA-sensitive glutamate receptors in vitro (A. C. Foster and E. H. F. Wong, Br. J. Pharmacol. 91:403, 1987). Glutamate (10 μM) and in combination with glycine (30 μM) significantly increased the affinity of ^{3}H-MK801 binding in membranes of forebrain by lowering the dissociation constant from 53.5 to 29.5 and 20.4 nM, respectively, but without changing the density of binding sites (3 pmole/mg protein). On the other hand, PPPC, d-AP5 and CPP inhibited ^{3}H-MK801 binding with IC_{50} values of 2.3, 1.4 and 2.5 μM, respectively. Thus, PPPC behaves like an NMDA receptor antagonist. The relative potencies of PPPC homologs will be described.

198.14

IONIC AND RECEPTOR MECHANISMS INVOLVED IN GLUTAMATE-STIMULATION OF DOPAMINE NEURONS IN CULTURE H. Mount, S. Welner, J. Diorio, R. Quirion and P. Boksa. Depts. Pharmacology and Psychiatry, Douglas Hospital Res. Ctr., McGill Univ., Verdun, Quebec, Canada H4H 1R3.

We have previously shown glutamate (GLU) stimulated Ca^{2+}-dependent, Na^+-independent release of [^{3}H]dopamine ([^{3}H]DA) from rat dissociated mesencephalic cells in culture (Mount et al. 1987, Soc. for Neurosci.). We now report that GLU-stimulated [^{3}H]DA release from these cells was not inhibited by tetrodotoxin, Zn^{2+}, lidocaine, or Co^{2+}, indicating that Na^+ or Ca^{2+} action potentials are not necessary for release. This suggests that GLU stimulates DA release directly from dendrites or nerve terminals of DA neurons. N-methyl-D-aspartate (NMDA), quisqualate (QUIS) and kainate, the preferred agonists for 3 GLU receptor subtypes also stimulated [^{3}H]DA release. A specific NMDA antagonist, d,l-APV (100 μM) completely inhibited NMDA-stimulated release, but not QUIS or kainate responses. GLU-stimulated release was inhibited by a broad spectrum antagonist (cis-2,3-PDA), but not by 100 μM d,l-APV, suggesting that GLU acts via non-NMDA receptors. The endogenous amino acid transmitters, homocysteate, aspartate, also stimulated [^{3}H]DA release; this release was inhibited by 100 μM d,l-APV suggesting that these amino acids interact with NMDA receptors. Thus, both NMDA and non-NMDA preferring GLU receptor subtypes may mediate stimulation of DA release from these cells.

198.15

ACTIVATION OF GLUTAMATE RECEPTORS INCREASES INTRACELLULAR FREE ARACHIDONATE IN PRIMARY CULTURES OF CEREBELLAR GRANULE CELLS. E. Aronica*, P.L. Canonico and F. Nicoletti* (SPON: P. Strocchi). Institute of Pharmacology, Catania Univ. Sch. of Med., Catania, Italy.

Cerebellar neurons in primary culture express specific classes of ionotropic and metabolotropic excitatory amino acid receptors, associated with Ca^{2+} influx and inositol phospholipid hydrolysis, respectively. Cultures were prelabeled for 16 h with ^{3}H-arachidonic acid (1 uCi/ml). Glutamate (100 uM) produced a small and transient increase in free ^{3}H-arachidonate, and a consistent reduction of ^{3}H-arachidonate-labeled phospholipids. Glutamate stimulated also the formation of unidentified arachidonate metabolites. When cultures were incubated in the presence of cyclo- and lipoxygenase inhibitors (indomethacin and NDGA, 10 uM), glutamate significantly increased (by about 35-45%) intracellular levels of ^{3}H-arachidonate after a 5 min-incubation. The incorporation of ^{3}H-arachidonate into membranal phospholipids was not reduced, suggesting that, in the presence of inhibitors of arachidonic acid metabolism, the released ^{3}H-arachidonate is rapidly recycled back into phospholipids. These results indicate that activation of glutamate receptors stimulates arachidonate release and metabolism in neurons.

198.16

TRANSMEMBRANE SIGNALLING AT QUISQUALATE RECEPTOR IN CULTURED NEURONS. A.Ambrosini* and J.Meldolesi* (SPON: F.Clementi). Dept.Pharmacol.,S.Raffaele Inst., CNR Ctr.Cytoph., Milano, ITALY.

In neurons, excitatory amino acids activate membrane phosphoinositides hydrolysis mainly via receptors (R) of the quisqualate (Q) subtype. Experiments in neuronal primary cultures from striatum and hippocampus revealed: 1. an Ins-1,4,5P_3 increase detected as soon as 15 sec after Q (10 μM) addition, with accumulation of total inositol phosphates proceeding for up to 10-15 min; 2. 80% inhibition of these Q-induced responses in cells pretreated for 3 hr with pertussis toxin (PTx). This result demonstrates that transmembrane signalling at QR is mediated by a PTx-sensitive G protein; 3. an even larger (90%) inhibition by short (3min) pretreatment with phorbol dibutirate (PDBu,100nM), suggesting a negative feed-back role of protein kinase C in the modulation of QR. These effects of Q were compared with those induced by carbachol (0.5mM), working through the activation of a muscarinic (M) R. MR responses were unaffected by PTx and inhibited by only 40-50% by PDBu. Thus, the generation of the same intracellular signal at two distinct R types occurs by different coupling mechanisms, and is differently regulated in the same in the same neuronal preparation.

198.17

POLYAMINE SPIDER VENOM COMPONENTS AS EXCITATORY AMINO ACID ANTAGONISTS IN THE RAT CNS. M. J. Pagnozzi*, N. A. Saccomano*, M. F. Gullak*, R. A. Volkmann* and E. E. Mena, (SPON: L. S. Reynolds). Departments of Neuroscience and Medicinal Chemistry, Central Research Division, Pfizer, Inc., Groton, CT 06340.

Spiders paralyze or kill their prey by injecting venoms which have potent actions on invertebrate physiological systems. Polyamine compounds recently isolated from the venoms of the *Argiope aurantia* (Arg-659 and Arg-636) and *Joro* spider (JSTX) potently block excitatory transmission at invertebrate neuromuscular junctions. Since glutamate is the neurotransmitter at these synapses, we examined the effects of these compounds on glutamergic activity in the rat CNS.

The venom components blocked both NMDA-induced (100 μM) and Kainic acid-induced (100 μM) elevation of cGMP in newborn rat cerebellum (IC50's of 10, 50 and 120 μM vs. NMDA and 30, 80 and 250 μM vs. KA for Arg 659, Arg-636 and JSTX respectively). Arg 659, Arg-636 and JSTX also inhibited NMDA-stimulated release of norepinephrine from rat hippocampal slices with IC50's of 1, 32 and 50 μM, respectively. Thus these compounds are functional antagonists at both NMDA and non-NMDA glutamate receptors. Arg-659, Arg-636 and JSTX also inhibited [^{3}H]TCP binding (IC50's of 15, 25 and >100 μM, respectively). The venom components, however, enhanced [^{3}H]Glycine binding. Arg-659 increased [^{3}H]Glycine binding to 285% of control values at 32 μM. The results of this study indicate that the polyamine spider venom components are glutamate antagonists at mammalian synapses. However, the characteristics of this antagonism are inconsistent with either the competitive or noncompetitive type.

198.18

COMPARISON OF MLV-6976 AND NMDA ANTAGONISTS IN THE RAT CORTICAL WEDGE. L. J. Robichaud, T. Malone* and P. A. Boxer. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

A novel oxazolidinone derivative (MLV-6976) has been reported to be a centrally acting muscle relaxant, with non-competitive glutamate blockade proposed as a mechanism of action. In the rat cortical wedge model, we compared the actions of MLV-6976 with the competitive NMDA antagonist CPP and the non-competitive NMDA antagonist MK-801. Wedges were placed in chambers with fluids separately bathing the cortex and white matter, allowing differential DC recordings of membrane potentials in Mg++ free Krebs buffer. MLV-6976 (100 μM) had no effect on the spontaneous epileptiform discharge rate. CPP (3μM) reversibly blocked the discharge rate, while MK-801 (235 nM) irreversibly reduced the rate. Repeatable depolarizations induced by NMDA (10-30 μM) in the presence of tetrodotoxin (0.5 μM) were compared before and after a test agent. CPP (10 μM, 15 min) and MK-801 (235 nM, 120 min) completely inhibited responses to NMDA. MLV-6976 (10-100 μM, 30 or 120 min) failed to inhibit depolarizations induced by NMDA, kainate or KCl. Cortical wedges previously depolarized by NMDA (30 μM) were hyperpolarized by MLV-6976 in a dose-dependent manner. Our data show no evidence that MLV-6976 is an NMDA or kainate antagonist. The mechanism of the hyperpolarization by MLV-6976 remains to be explored.

198.19

GLUTAMINASE-LIKE IMMUNOREACTIVE NEURONS IN RAT LOWER BRAINSTEM. T. Kaneko*, H. Akiyama and N. Mizuno. Dept. of Anatomy (1st Div.), Faculty of Medicine, Kyoto Univ., Kyoto 606, Japan.

Identification of glutamatergic neurons in the lower brainstem of the rat was made immunohistochemically by using a monoclonal antibody MAb-120 raised against phosphate-activated glutaminase (PAG; J. Neurosci. 7:302, 1987). Many brainstem neurons were labeled for PAG. Neuronal cell bodies with the most intense immunoreactivity were densely distributed in the precerebellar nuclei sending the mossy fibers to the cerebellum; in the pontine nuclei, pontine tegmental reticular nucleus of Bechterew, lateral reticular nucleus, and external cuneate nucleus. In the cerebellum, PAG-labeled possible axon terminals were seen in the deep nuclei as well as in the granule cell layer. These findings suggest that many brainstem neurons sending mossy fibers to the cerebellum are glutamatergic. Neuronal cell bodies intensely labeled for PAG were also scattered in the main sensory and spinal trigeminal nuclei, medial and superior vestibular nuclei, regions around the superior olive, posterior ventral cochlear nucleus, paracochlear glial substance, prepositus hypoglossal nucleus, and pontine and medullary reticular formation.

198.20

DEVELOPMENTAL INDUCTION OF GLUTAMINASE IN PRIMARY CULTURES OF CEREBELLAR GRANULE CELLS. C. Banner*, J.W. Thomas, A. Novelli, H.H. Smith*, R.C. Henneberry*, and E. Freese. Laboratory of Molecular Biology, NINCDS, National Institutes of Health, Bethesda, MD 20892

We have used a recently isolated glutaminase cDNA probe (Banner et al., Mol. Brain Res., in press) to examine glutaminase mRNA levels in differentiating granule cell cultures. Between days 3 and 8 after plating neonatal rat cerebellar granule neurons, glutaminase mRNA levels increased over 3-fold relative to total RNA, poly(A$^+$) RNA, and two other mRNAs. Glutaminase protein levels increased over 50% between days 3 and 8 and over 100% by day 10. The total amount of glutamate per cell did not change during this period. Glutaminase induction paralleled the development of calcium-stimulated glutamate release, and the formation of synapses and synaptic vesicles. These results suggest that neuronal glutaminase is involved in the synthesis of a neurotransmitter pool of glutamate.

EXCITATORY AMINO ACIDS V

199.1

PURIFICATION AND CHARACTERIZATION OF THE GLUTAMATE RECEPTOR FROM THE NEMATODE CAENORHABDITIS ELEGANS. Susan Pietrzak Rohrer* (SPON: M. Cascieri). Merck Sharp & Dohme Research Laboratories, Rahway, N.J. 07065

The C. elegans glutamate receptor originally described by Schaeffer et al. (1988, J. Neurochemistry), has been solubilized and purified to homogeneity. In the course of stabilizing the glutamate binding activity of C. elegans membrane preparations, an endogenous ligand, capable of displacing glutamate in the filter binding assay, was discovered. This ligand was removed from tissue by dialysis through tubing with a MW cutoff of 3500. The ligand in the dialysate was lyophilized to dryness, passed over a Sephadex G-25 gel filtration column and recovered as a single active peak absorbing at 206 nM. The membrane bound glutamate receptor was solubilized in 30 mM octyl-B-glucoside. The soluble receptor-ligand complex can be assayed by trapping on glass fiber filters pretreated with 0.3% polyethyleneimine or it can be precipitated from solution with 10% polyethylene glycol and then trapped on glass fiber filters. The K_d for glutamate binding to the soluble receptor is of the same order of magnitude as that for the membrane bound receptor. The soluble receptor has been purified by a combination of ion exchange, hydrophobic, and gel filtration high pressure liquid chromatography (HPLC).

199.2

THE PHENCYCLIDINE ANALOG, [^{3}H]TCP, LABELS TWO BINDING SITES IN GUINEA PIG BRAIN DISTINGUISHED BY (+)-MK801. **K.C. Rice[1], M.V. Matteon[1]*, A.E. Jacobson[1],* J.A. Monn[1]*, A.A. Reid[2] and R.B. Rothman[2]. [1]Section on Drug Design and Synthesis, NIDDK and [2]Unit on Receptor Studies, LCS, NIMH, Bethesda, MD 20892.**

Recent studies suggest that NMDA excitatory amino acid receptors and phencyclidine (PCP) receptors coexist in a receptor complex. In this study we begin to test the hypothesis that not all PCP receptors are associated with the NMDA receptor. One prediction of this hypothesis is that [^{3}H]TCP should label two binding sites distinguished by MK801, a potent ligand at the PCP receptor, and in humans, a drug generally devoid of the psychotomimetic effects generally associated with the ingestion of PCP. This clinical observation suggests that MK801 may "mark" the PCP receptor associated with the NMDA receptor.

Large batches of frozen membranes were prepared from 20 to 40 frozen guinea pig brain (including cerebellum). Incubations with [^{3}H]TCP and test drugs proceeded for 2 to 4 hr at 0° C in 5 mM TRIS buffer as described (Neuropeptides 10:261-264, 1987). Using these assay conditions, neither glutamate (100 uM) nor the NMDA antagonist CPP (10 uM), had any effect on binding. To characterize [^{3}H]TCP binding sites, binding surfaces were fit to a one or two site binding model using nonlinear least squares curve fitting methods. The results indicated that the data were best fit by a two site binding model with the following parameter estimates:

	Units	Site 1	Site 2
Bmax	(fmol/mg protein)	428±82	433±52
TCP	(Kd, nM)	47±9	15±1.2
PCP	(Kd, nM)	55±11	102±11
(-)Cyclazocine	(Kd, nM)	566±104	38±4
Dexoxadrol	(Kd, nM)	383±108	5.2±0.6
(+)-MK801	(Kd, nM)	854±750	6.6±2.2

These data demonstrate the existence of two PCP binding sites distinguished by MK801, consistent with the prediction of the hypothesis. Additional studies will test other aspects of the hypothesis.

199.3

TRANSIENT INCREASED DENSITY OF NMDA BINDING SITES IN THE IMMATURE HUMAN AND RAT HIPPOCAMPUS.
E. Tremblay*, A. Represa*, M.P. Roisin*, C. Marlangue and Y. Ben-Ari. INSERM U-29. 123 Bd Port-Royal. Paris 14. FRANCE

Recent studies performed in the visual system suggest a preferential involvement of NMDA receptors in developmental plasticity. In the present report, we have examined the developmental changes in the density of NMDA binding sites in the hippocampus.

Human - Using quantitative autoradiography, we found a highly significant postnatal reduction in ^{3}H Glut. binding sites. Most of this reduction is due to NMDA subtypes of receptors. Thus, in CA1 the density of these sites was of 103 ± 8, 105 ± 3, 41 ± 6 at E (embryonic age) 23-27 weeks E40 weeks and adults respectively. A similar reduction was found in CA3 and the fascia dentata.

Rat - With membrane preparation, a highly significant reduction in NMDA binding sites was found post nataly. This was not associated with a change in affinity. With quantitative autoradiography a 50 % reduction of NMDA sites occured between P8 and P12 in CA1. There is therefore an abrupt transient increase in the density of NMDA binding sites in developping human and rat hippocampus. A parallel report from this laboratory shows the presence of spontaneous recurrent giant epep in hippocampal neurons before, but not after P8. These giant epsps are readily blocked by specific NMDA receptors in immature hippocampal synapses (Coradetti et al. ENA meeting).

199.4

GLUTAMATE IN THE SYMPATHETIC INTERMEDIOLATERAL NUCLEUS OF THE RAT THORACIC SPINAL CORD: SOURCES AND ULTRASTRUCTURAL CHARACTERIZATION *J. Callaway*, S. Morrison, T.A. Milner, P. Petrusz, A. Rustioni and D.J. Reis.* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021 and Dept. of Anat. and Physiol., Univ. North Carolina at Chapel Hill, Chapel Hill, NC 27514

Stimulation in the medullary nucleus reticularis rostroventrolateralis (RVL) produces an excitation of sympathetic preganglionic neurons (SPNs) in the spinal intermediolateral nucleus (IML) that is blocked by iontophoresis of the glutamate blocker, kynurenate (Soc. Neurosci. Abstr. 13:808, 1987). To identify the anatomic substrate for these results, a polyclonal antiserum against hemocyanin-conjugated glutamate (J. Neurosci.7:1887-1901, 1987) was localized in the IML using the peroxidase-antiperoxidase method. By light microscopy, glutamate-like immunoreactivity (Glu-LI) was observed in cells and varicose processes within the IML which were unevenly distributed throughout the rostrocaudal extent of the thoracic cord. Glu-LI in the IML was eliminated after preabsorption of the antiserum with low concentrations of bovine serum albumin(BSA)-conjugated glutamate but not with BSA-conjugated aspartate. Six days following T3 spinal transection, Glu-LI in the IML was greatly reduced caudal but remained unchanged rostral to the transection. By electron microscopy, Glu-LI was found in perikarya, dendrites, axons and axon terminals in the IML. The amount of Glu-LI in perikarya and dendrites varied, however, depending on fixation conditions. Terminals with Glu-LI (0.2-1.5μm) contained numerous small clear and 1-3 large dense core vesicles. Out of 89 terminals, 80 were synaptically associated with labeled or unlabeled perikarya and dendrites. Synaptic junctions on perikarya were rare (1%) whereas, those on proximal (large) dendrites were more numerous (13%) and included asymmetric as well as symmetric contacts. However, the majority of terminals with Glu-LI formed asymmetric junctions on distal (small) dendrites (81%) and dendritic spines (5%). These data indicate that glutamate, possibly released by a descending spinal pathway, could play a significant role as an excitatory (asymmetric synapses) transmitter regulating the discharge of SPNs in the IML. (Supported by NIH grants HL 18974 and NS22721.)

199.5

AMPA BINDS TO A SUBSET OF QUISQUALATE-SENSITIVE GLUTAMATE BINDING SITES. J. J. Cha, E. Ø. Nielsen*, T. Honoré*, J. B. Penney, and A. B. Young. Neuroscience Program and Department of Neurology, Univ. Michigan, Ann Arbor, MI 48104-1687.

Calcium (Ca^{2+}) and chloride (Cl^-) ions stimulated [^{3}H]glutamate ([^{3}H]Glu) binding to quisqualate (QA) - sensitive [^{3}H]Glu binding sites in rat brain fourfold, as measured by quantitative autoradiography, whereas potassium thiocyanate (KSCN) had no additional effect. In contrast, Ca^{2+} and Cl^- had little effect on the binding of [^{3}H]AMPA, but 100 mM KSCN stimulated binding fourfold. In the absence of KSCN, AMPA displaced little [^{3}H]Glu binding from QA-sensitive binding sites but displaced up to 40% of [^{3}H]Glu binding in the presence of KSCN. The regional distributions in brain of QA-sensitive [^{3}H]Glu binding sites and [^{3}H]AMPA binding sites correlated highly (r = 0.94), suggesting that these binding sites are the same or closely related. Given the differential effects of ions on [^{3}H]AMPA and QA-sensitive [^{3}H]Glu binding and AMPA's limited ability to displace [^{3}H]Glu from QA-sensitive sites, the results suggest that AMPA binds to a subclass of QA-sensitive [^{3}H]Glu binding sites that are highly influenced by ionic environment or that QA-sensitive binding sites exist in several states. Supported by NIH NRSA 5T32 and UHPHS grant NS 19613.

199.6

SOLUBILISATION AND CHARACTERISATION OF QUISQUALATE A RECEPTOR FROM CHICK BRAIN J.M. Henley* and E.A. Barnard*, (spon. S. Simasko) MRC Molecular Neurobiology Unit, MRC Centre, Hills Road, Cambridge CB2 2QH, U.K.

The binding of the quisqualate receptor selective agonist [^{3}H]AMPA (α-amino-3-hydroxy-5-methylisoxazole-propionate) to 1-day old chick brain membrane homogenates in the presence of 100 mM KSCN revealed a single class of specific high affinity [^{3}H]AMPA binding sites with a B_{max} of 1.3 pmol/mg protein and a Kd of 51 nM. Solubilisation of viable binding sites was optimal with the anionic detergent sodium cholate. The affinity of the solubilised binding sites for [^{3}H]AMPA in the presence of KSCN was increased (Kd = 21 nM) and the B_{max} was 0.9 pmol/mg protein. The pharmacology of the solubilised and membrane bound receptors was similar with quisqualate ≃ AMPA > CNQX DNQX > L-glutamate being the most potent competitive ligands for [^{3}H]AMPA displacement. The dissociation rate of [^{3}H]AMPA from solubilised binding sites was 0.013 min^{-1} and the kinetically determined Kd was 20 nM. [^{3}H]AMPA binding activity was not enriched by ion exchange chromatography but an approximately 25 fold increase in the specific [^{3}H]AMPA binding per mg protein was obtained using wheat germ lectin chromatography. The results demonstrate the solubilisation of a stable, viable, quisqualate receptor and suggest that chick brain may provide a useful system for further characterisation and eventual purification of this glutamate receptor subtype.

199.7

GLUTAMATE RECEPTORS ARE TRANSPORTED BIDIRECTIONALLY IN RAT VAGAL AFFERENT NEURONS. P.M. Beart*, D. Lodge*, S. Lewis*, A. Verberne*, B. Jarrott*, R. Summers*† and M. Cincotta* (SPON: A.W. Goodwin). Univ. of Melbourne, Clin. Pharm., Austin Hosp., Heidelberg VIC 3084 and †Dept. of Pharmacol. Parkville VIC 3052, AUSTRALIA.

Receptors for a variety of transmitters are synthesized in perikarya of neurons of the nodose ganglion (NG) and axonally transported in vagus nerve (X). Since L-glutamate (GLU) may be a transmitter of baroreceptor afferents and NG neurons are excitotoxin-sensitive (Verberne, A.J.M. et al., Eur. J. Pharmacol., 139: 365, 1987), we examined GLU receptor dynamics in NG/X system. Sprague-Dawley rats, anaesthetized with amylobarbitone-methohexitone, had their left NG exposed and X singly or doubly ligatured. Controls received sham ligations or operations. At 24 h postsurgery, rats were re-anaesthetized and each NG/X removed. Longitudinal (10 μm), slide-mounted sections were processed for receptor autoradiography using [^{3}H]GLU. In ligated and control NG, binding was identical and punctate. Binding in X was only found after ligation, both proximal and distal to ligature. Double ligation of peripheral X confirmed this finding. GLU receptors also accumulated after ligation of central X. Colchicine prevented receptor accumulation. All receptor subtypes were present (kainate >50%) and NG contained single population of sites (Kd 130 nM, Bmax 0.6 nM.μg^{-1} protein). GLU receptors are localized in NG and undergo bidirectional flow in peripheral and central X.

199.8

ONTOGENY OF EXCITATORY AMINO ACID RECEPTOR SUBTYPES IN RAT BRAIN
T.R. Insel, L.P. Miller, A. E. Johnson, R. Gelhard*. Lab. of Clin. Sci., NIMH, Poolesville, MD 20837; Vet. Adm. Med. Ctr., Wash D.C. 20422

Recent evidence implicates excitatory amino acids (EAA) in experience-dependent changes (e.g. imprinting, olfactory learning, formation of ocular dominance columns) during neural development. To investigate possible sites of EAA action in development, the ontogeny of various glutamate receptor subtypes was examined in rat brain using *in vitro* receptor autoradiography. Slide-mounted 12μ sections from rats at ages 1,7,14,21,28 days, and adult were incubated with ^{3}H-glutamate ± NMDA to label NMDA receptors, ^{3}H-AMPA ± AMPA to label quisqualate receptors, and ^{3}H-kainic acid ± kainic acid to label kainate receptors. Computer-based image analysis using ^{3}H standards was performed on 30 brain regions. The three receptor subtypes showed slightly different developmental profiles. For each of the subtypes, binding appeared to increase gradually with age, peaking at age 21-28 days in most areas, then decreasing to adult levels. In addition, local patterns of markedly increased binding were seen for ^{3}H-AMPA binding in CA3 (days 1-14) and ^{3}H-kainic acid binding in the reticular nucleus of the thalamus (day 7). These regions of transient "overshoot" of EAA receptors may represent sites where EAAs have a specific role restricted to development. Whether EAA receptors are involved in the normal eliminative processes characterizing post-natal neuronal differentiation remains to be determined.

199.9

IMMUNOAFFINITY PURIFICATION OF KAINATE BINDING PROTEIN IN PIGEON CEREBELLUM. A. U. Klein* and P. Streit. Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland.

Kainic acid, an agonist at a certain type of excitatory amino acid receptor, binds in the pigeon cerebellum to a low affinity site which is located in the molecular layer (Henke, H. et al., Brain Res., 219:95-105, 1981) and which can be solubilized by Triton X-100 (Dilber, A. et al., Soc.Neurosci.Abstr. 9:260, 1983). To raise monoclonal antibodies (mAb) with crude solubilized preparations against this binding site, a procedure involving partial immunosuppression (Matthew, W. D. and Patterson, P. H. in: Cold Spring Harbor Symposia on Quantitative Biology, Vol.48, pp.625-631, 1983) had to be used. A mAb (mAb 15A4) precipitating kainate binding activity was detected. Immunoaffinity-precipitated by mAb 15A4, this activity appeared as a single peak with M_r = 220,000 in gel filtration FPLC and as a single band with M_r = 50,000 in SDS-PAGE under reducing conditions. Polyclonal antibodies to purified material and mAb 15A4 labeled selectively as yet unidentified elements in the molecular layer of pigeon and chicken cerbellar cortex. In the pigeon an analogous distribution of labeling had been observed in autoradiographic studies with tritiated kainate (Henke et al., 1981).

199.10

QUANTITATIVE AUTORADIOGRAPHY OF [3H]GLYCINE BINDING TO THE GLYCINE RECEPTOR ASSOCIATED WITH THE NMDA RECEPTOR OPERATED CHANNEL. J.W.McDonald, J.B. Penney, M.V. Johnston, and A.B. Young, MSTP and Neuroscience Programs and Department of Neurology and Pediatrics, University of Michigan, Ann Arbor, MI. 48104

Glycine potentiates NMDA responses and enhances [3H]TCP, [3H]MK-801, and NMDA receptor binding in vitro suggesting that there is a glycine binding site associated with the NMDA receptor operated channel. We have developed an in vitro autoradiographic method for visualizing this glycine binding site. Kinetic studies were performed at 4°C in 50 mM Tris-citrate, pH=7.4, containing 100nM [3H]glycine using 20 um adult rat brain sections. [3H]Glycine binding was saturable and reversible with a K_d of 75-88 nM. Binding equilibrium was reached within 10-15 min. Under these conditions specific binding represented >90% of total binding. The relative distribution of [3H]glycine binding was highly correlated with that of NMDA receptor binding (r=0.94), MK-801 binding (r=0.97), and TCP binding (r=0.89); CA_1 > dentate > cingulate cortex > parietal cortex (I,II) > thalamus > striatum > cerebellar granule layer > inferior colliculus. [3H]Glycine binding was insensitive to the inhibitory glycine receptor antagonist, strychnine. Binding was not altered by most competitive (NMDA, glutamate, CPP) or non-competitive (MK-801, TCP) NMDA receptor agonists and antagonists. In general, D- rather than L-amino acids were more effective inhibitors of [3H]glycine binding with the exception that L-valine was more potent than D-valine. Kynurinic acid and DL-APV were potent inhibitors of [3H]glycine binding. These data suggest that some of the reported competitive NMDA antagonists may actually reduce NMDA responses by blocking the glycine site and preventing glycine receptor mediated enhancement of NMDA responses.

Supported by USPHS grant NS 19613 and MSTP grant 5 T32 6M07863-07

199.11

QUANTITATIVE AUTORADIOGRAPHY OF [3H]MK-801 BINDING IN RAT BRAIN. S. Sakurai, J.J. Cha, J.B. Penney, & A.B. Young Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, MI 48104 - 1687.

MK-801 is a non-competitive antagonist of the NMDA receptor. We have developed a quantitative autoradiographic binding assay for [3H]MK-801. Kinetic experiments were performed at room temperature on 20 µm sections of adult rat brain in 5.0 nM [3H]MK-801 (29.4 Ci/mmol) in Tris-Acetate. The sections were apposed to tritium-sensitive film and exposed for 3 weeks. The binding of [3H]MK-801 was reversible and saturable with a K_d of 1-2 nM as determined by kinetic experiments. The $t_{1/2}$ of association was approximately 50 min. Under these conditions specific binding was greater than 95% of total binding. The relative distribution of [3H]MK-801 binding was: CA1 > dentate gyrus > outer cortex > striatum > cerebellum. The regional distribution of [3H]MK-801 was highly correlated with the distribution of TCP binding sites (r = 0.96) and NMDA receptors (r = 0.96). [3H]MK-801 binding was inhibited by TCP, while NMDA enhanced binding in all areas examined. The NMDA stimulation of [3H]MK-801 binding was inhibited by the competitive NMDA receptor antagonist CPP. In addition, CPP inhibited binding in the absence of NMDA. Finally, glycine stimulated [3H]MK-801 binding in all areas examined. Supported by NS 19613, NIH NRSA 5T32 and a Merck Faculty Development Award (to ABY).

199.12

IN VIVO [3H]-MK 801 BINDING IN THE RAT: ANOMALOUS RESULTS OF BLOCKING STUDIES. B.J. Ciliax, J.B. Penney, and A.B. Young, Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104.

[3H]-MK 801 binds to the high affinity phencylidine (PCP) site, which is coupled to the NMDA receptor and closely matches the anatomical distribution of the NMDA receptor. In vivo [3H]-MK 801 binding was used to investigate this ligand's potential to image the NMDA/PCP receptor complex for ultimate use in PET.

Binding of [3H]-MK 801 after i.v. injection in control animals was heterogeneous and high in cortex, CA1 of hippocampus, and thalamus. The binding distribution was very similar to that seen in in vitro binding assays with [3H]-MK 801 or [3H]-TCP. Surprisingly, a predose of 5 mg/kg cold MK 801 (given from 0 to 240 min prior to the [3H]) *increased* the amount of in vivo [3H]-MK 801 binding. The anatomical distribution of [3H]-MK 801 binding in vivo after cold MK 801 pretreatment was very similar to that in the control animals, but the laminar binding seen in CA1 of the hippocampus and other brain regions was no longer as distinct. These data suggest that the adminstration of the cold drug somehow activated the system to allow for more binding, possibly by recruitment of cryptic sites or feedback activation of the use-dependent PCP site.

Supported by USPHS grant NS 15655, the ADRDA, and a Merck Faculty Development Award.

199.13

PHOTOAFFINITY LABELING AND BINDING STUDIES REVEAL TWO TYPES OF PHENCYCLIDINE (PCP) RECEPTORS IN THE NCB-20 CELL LINE. R. Haring, R.S. Zukin and S.R. Zukin. Depts. of Psychiatry and Neuroscience, Albert Einstein Coll. Med., Bronx, NY 10461

It has recently been shown that [3H]N-(1-[2-thienyl]cyclohexyl)piperidine ([3H]TCP) binds to both PCP-type and to non-PCP-σ type sites on membranes of cultured NCB-20 cells (Kushner *et al.*, submitted for publication). The PCP-type site was shown to be coupled to NMDA receptors. However, its pharmacological selectivity differed somewhat from that established for the brain PCP receptor. We addressed the question of whether PCP receptors of NCB-20 cells are structurally congruent with the brain receptors by comparing their polypeptide compositions using the photolabile PCP derivative [3H]azido-phencyclidine ([3H]AZ-PCP). The IC50 values for AZ-PCP displacement of [3H]TCP binding and for TCP displacement of [3H]AZ-PCP binding in the cell membranes were similar. The pharmacological selectivity of [3H]AZ-PCP binding was similar to that which has been reported for [3H]TCP binding. We concluded that [3H]AZ-PCP is suitable for labeling of the PCP receptors to NCB-20 cells. Competition binding curves for AZ-PCP, dexoxadrol and MK-801 were shallow with Hill coefficient <1. Computer-assisted analysis of MK-801 displacement curves suggested a biphasic mode of inhibition with the apparent K_i values of 13 nM and 2.3 µM for the high (10-15% of sites) and low affinity (85-90% of sites) components, respectively. Photoaffinity labeling was carried out, essentially as described before (Haring et al, Biochem. 26: 5854, 1987). We found that each of the five polypeptides previously shown following labeling of rat brain membranes were present in the membranes from NCB-20 cells (M_r 90,000, 68,000, 49,000, 40,000 and 33,000). Addition of MK-801 (10^{-5}M) to the incubation mixture inhibited the labeling mainly of M_r 90,000 and 68,000 polypeptides with less effect upon M_r 33000. The σ ligand (+)3-(3-hydroxyphenyl)-N-(1-propyl)piperidine (+)3-PPP (10^{-5}M) was found to inhibit [3H]AZ-PCP labeling mainly of M_r 49,000 and 33,000 bands. These results indicate the existence of PCP receptors in NCB-20 cells which have the same polypeptide composition as the brain receptors. One population of sites appears to be linked to the NMDA receptor channel. Our findings indicate that the polypeptides of M_r 90,000 and 68,000 previously identified in rat brain as components of the high-affinity PCP binding sites might be associated with the PCP-NMDA receptor-channel complex. Our results also support the presence of low affinity sites. These may be responsible for the anomalies in the ligand selectivity pattern and relatively low affinity shown by [3H]TCP sites in the cells. Supported in part by grants to SRZ (PHS DA-03383; Ritter Foundation), RSZ (PHS DA-04439, 00069) and RH (Camp David Institute).

199.14

ASPARTATE IMMUNOREACTIVITY IN CORTICOFUGAL NEURONS IN RATS. R. Giuffrida* and A. Rustioni. Dept. Cell Biology and Anatomy, Univ. North Carolina at Chapel Hill, Chapel Hill, NC 27599.

We have previously reported that 50 to 60% of corticofugal neurons are immunoreactive for an antiserum for glutamate (Glu). We have now explored the possibility that corticofugal neurons immunonegative for glutamate (Glu) are immunopositive for an antiserum for aspartate (Asp) raised in rabbits, like the Glu-antiserum, against the amino acid conjugated to hemocyanin by glutaraldehyde. A retrograde tracer was injected in the motor cortex, or ventral thalamic nuclei, or cervical spinal cord of rats. Two to 3 days later these were perfused with carbodiimide and paraformaldehyde and sections of the sensorimotor cortex were processed for immunocytochemistry. In the regions with highest density of retrogradely labeled neurons, 50 to 60% of these were Asp-immunopositive. The percentage of immunopositive corticofugal neurons increased to over 90% when the same section was processed for both Glu- and Asp-antisera suggesting that a large fraction of these neurons are immunoreactive for either one amino acid but not for both. High levels of Glu and Asp may be present in a fraction of neurons which may release both amino acids or a substance closely related to these.

Supported by USPHS grant NS 12440.

199.15

GLUTAMATE-IMMUNOREACTIVE SYNAPTIC TERMINALS OF PRIMARY AFFERENTS TO THE CUNEATE NUCLEUS OF RATS. S. De Biasi and A. Rustioni (SPON: M. Fabri) Dept. Cell Biology and Anatomy, Univ. North Carolina, Chapel Hill, NC 27599.

Glutamate (Glu) meets several criteria as neurotransmitter in dorsal root ganglia (DRG) neurons; only recently has this been tested by immunocytochemistry. Up to 70% of DRG neurons in rats are labeled with an antiserum against Glu conjugated to hemocyanin. We have investigated whether synaptic endings of DRG neurons in the cuneate nucleus are immunopositive for the same Glu antiserum. Rats were perfused with 4% paraformaldehyde and 0.1% glutaraldehyde. Sections were reacted for pre-embedding immunocytochemistry or processed for post-embedding immunogold staining. With both methods: a) terminals with the same morphological features are labeled; b) labeled terminals co-exist with unlabeled ones in the same section; c) terminals with flat vesicles are not labeled. The origin of labeled terminals from DRG neurons is demonstrated in rats with injections of an anterograde tracer. Primary afferent, Glu+ terminals have a scalloped profile, light cytoplasm, and contain many mitochondria and small agranular vesicles homogeneous in size. They contact many profiles some also containing vesicles.

The results support the hypothesis that primary afferent terminals in the dorsal column nuclei release Glu as synaptic transmitter.

199.16

COLCHICINE INJECTION PRODUCES A RAPID DECREASE IN GLUTAMATE RECEPTOR BINDING IN RAT HIPPOCAMPUS. Yuzo Nakagawa* and Michel Baudry. Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA 92717.

Colchicine injection has been widely used to produce selective neuronal death especially in the hippocampal formation where, at low doses, it results in the elimination of dentate granule cells while at higher doses it also eliminates CA_1 pyramidal neurons. Several subtypes of glutamate receptors have been defined and can be labeled by appropriate ligands, i.e., ^{3}H-glutamate and ^{3}H-AMPA for the NMDA and quisqualate receptors respectively. In the present study, we determined the effects of colchicine injection on these two types of receptors in both the dentate gyrus and in CA_1.

Unilateral injection of colchicine (15 µg) in the dorsal hippocampus did not produce any change in ^{3}H-glutamate and ^{3}H-AMPA binding in the dentate gyrus or CA_1 contralateral to the injection site. However, it produced a progressive decrease in the binding of both ligands in both subfields of the injected hippocampus. The changes in binding as a function of time after the injection could be fitted with an exponential function, and the half-life of the binding sites were calculated to be about 7 days for ^{3}H-AMPA and 9 days for ^{3}H-glutamate in the dentate gyrus. Similar values were found in CA_1 although the changes in binding were delayed by about 2 days as compared to the dentate gyrus. Kinetic analysis of the binding at equilibrium were performed 7 days after the injection and indicated that the changes in binding were due to changes in the maximum number of sites but not in affinity for the ligands.

These results indicate that the sites labeled with ^{3}H-glutamate and ^{3}H-AMPA are localized mainly postsynaptically and on neuronal structures. The exponential decay of the receptor binding suggests that the number of glutamate receptors reflects directly the number of surviving neurons in the hippocampal formation.

Supported by grant BNS 12156 from NSF.

199.17

AUTORADIOGRAPHIC VISUALIZATION OF NON-NMDA RECEPTORS USING ^{3}H-CNQX: PHARMACOLOGICAL CHARACTERIZATION AND COMPARISON WITH ^{3}H-AMPA BINDING SITES IN RAT BRAIN. E.Ø. Nielsen*, J. Drejer* and T. Honoré* (SPON: L.H.Jensen) Ferrosan Research Division, DK-2860 Soeborg, Denmark.

^{3}H-CNQX (6-nitro-7-cyanoquinoxaline-2,3-dion) selectively labels the non-NMDA receptors in rat brain. ^{3}H-CNQX binding is highest in CA-1, dentate gyrus and CA-3 of hippocampus followed by cortex and molecular layer of the cerebellum. Apparently, ^{3}H-CNQX binds to a single population of sites with a K_D of 67 nM and B_{max} 3.56 pmol/mg protein (molecular layer of cerebellum). Displacement of ^{3}H-CNQX binding by AMPA revealed a biphasic inhibition curve. Quisqualate, glutamate and kainate had high affinity for ^{3}H-CNQX sites whereas NMDA was without significant activity at 100 µM. Equilibrium studies of ^{3}H-AMPA binding in the presence of SCN^- gave two binding sites with K_D = 9 and 278 nM and B_{max} = 0.15 and 1.54 pmol/mg protein, respectively. The finding that ^{3}H-AMPA binding gave biphasic Scatchard plots and AMPA gave biphasic inhibition of ^{3}H-CNQX suggest that the linear Scatchard plot of ^{3}H-CNQX binding is in accordance with CNQX having the same affinity to the two conformational states of the quisqualate receptor (Honoré and Drejer, J. Neurochem. in press, 1988). The regional distribution of non-NMDA receptors labelled with ^{3}H-AMPA and ^{3}H-CNQX showed a good correlation with the distribution of quisqualate-sensitive ^{3}H-glutamate binding.

199.18

FULL-LENGTH HUMAN GLUTAMATE DEHYDROGENASE cDNA: MITOCHONDRIAL TARGETTING PRESEQUENCE AND ALTERNATE POLYADENYLATION. Y. Nakatani*, M. Schneider*, C. Banner*, B. Lee*, H. Smith*, and E. Freese (SPON: M. Martin). Laboratory of Molecular Biology, NINCDS, NIH, Bethesda, MD 20892

A genetic defect in a glutamate dehydrogenase (GDH) "isozyme" has been suggested to occur in some patients with certain neurodegenerative disorders. Two or more forms of GDH have been previously reported to exist in brain and liver tissue. By hybridization with one human brain GDH cDNA, we have isolated and sequenced part or all of three GDH cDNAs from human brain, three from human liver, and two from fibroblasts. The identical DNA sequence was found in all cases. Several other GDH cDNAs did not differ from these by restriction mapping or oligonucleotide hybridization analysis. We conclude that the same GDH gene is expressed in all three tissues, and that if GDH "isozymes" occurred in these individuals, they probably had a post-translational origin. Two different GDH mRNAs resulted from polyadenylation at two sites on a single transcript. The full-length GDH cDNA encoded an N-terminal amino acid sequence resembling the previously described sequences that enable protein translocation into the mitochondrial matrix. Isolated rat liver mitochondria actually imported and processed the precursor protein which could be synthesized in vitro from the GDH cDNA.

199.19

EFFECTS OF GUANINE NUCLEOTIDES ON GLUTAMATE STIMULATED [^{3}H]TCP BINDING TO RAT BRAIN SYNAPTIC PLASMA MEMBRANES. R.P. Compton, W.F. Hood and J.B. Monahan. CNS Diseases Research, G.D. Searle & Co., Chesterfield, MO 63198.

GTP (along with its non-hydrolyzable analogs 5'-guanylylimidodiphosphate and 5'-guanylylmethylenediphosphate) and GDP inhibit the binding of [^{3}H]1-(1-(2-thienyl)cyclohexyl)piperidine ([^{3}H]TCP) to the N-methyl-D-aspartate (NMDA) receptor coupled phencyclidine recognition site in rat forebrain synaptic plasma membranes. GMP, cyclic GMP and guanosine are significantly less potent. NMDA receptor agonists, which increase the affinity of [^{3}H]TCP for its recognition site, were examined for their effects on the GTP inhibition of [^{3}H]TCP binding. The inclusion of 1µM glutamate in the [^{3}H]TCP assay results in a parallel shift in the inhibition curves of phencyclidine analogs (eg. MK-801) toward higher potencies while no effect on Zn^{+2} induced inhibition is observed. Glutamate induced a rightward shift (lower potency) in the GTP dose-response curve (IC_{50}'s= 34µM and 235µM, respectively) in a manner similar to that seen with the competitive NMDA antagonist, D-2-amino-7-phosphonoheptanoate. These results are consistent with our previous findings that GTP (IC_{50}=28µM) and its analogs competitively inhibit NMDA-sensitive L-[^{3}H]glutamate binding and support the hypothesis that guanine nucleotides may be endogenous antagonists of NMDA receptor function.

199.20

PCP AND SIGMA RECEPTORS: DIFFERENTIAL EFFECTS OF CATIONS AND pH ON LIGAND AFFINITY. Z.W. YANG, G.A. PALEOS, AND J.C. BYRD. Developmental Neurobiology Program, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Phencyclidine (PCP) and benzomorphans bind to at least two sites in the CNS - the PCP receptor, and the Sigma receptor. Using ligands selective for each of these receptors ([^{3}H]TCP and [^{3}H] (+) PPP, respectively) we have compared the binding characteristics of these radioligands for the two receptors. Both ligands achieve equilibrium by 60 min at 21°C. (+) PPP also achieves equilibrium by 120 min at 4°C, but TCP is far from equilibrium under these conditions. TCP shows a pH optimum of 8.0, while (+) PPP has an optimum at pH 9.5. Binding of (+) PPP at pH 9.5 is substantially reduced by both heating and protease digestion. Tris buffer decreases TCP binding by 25% at 5 mM, and 75% at 20 mM. In contrast (+) PPP binding is maximal in 10 mM tris, but decreases 40% in 50 mM Tris. Binding of both TCP and (+) PPP is inhibited by sodium (IC_{50} for TCP = 3 mM; IC_{50} for (+) PPP = 30 mM). 1 mM Ca^{++} virtually abolishes TCP binding, while (+) PPP binding is inhibited 50% by 2 mM Ca^{++}. Other divalent cations (Zn^{++}, Mn^{++}, Ca^{++}, Mg^{++}) also cause marked decreases in TCP and (+) PPP binding. Ca^{++} reduces TCP and (+) PPP binding by different mechanisms. Ca^{++} causes a 2-4 fold increase in the K_d of TCP, but reduces the B_{max} of (+) PPP by 45%. The rank order of potency of several benzomorphans in displacing [^{3}H] (+) PPP is essentially the same at both pH 8.0 and 9.5. However, the stereoselectivity of these compounds is greatly reduced at the higher pH, due largely to a selective decrease in the affinity of the (+) isomer for the Sigma receptor.

199.21

MONOCLONAL ANTIBODIES IDENTIFY A KAINIC ACID RECEPTOR D.R. Hampson, K.D. Wheaton*, C.J.Dechene*,and R.J. Wenthold NIH, NINCDS, Lab of Neuro-otolaryngology, Bethesda, MD 20892

Monoclonal antibodies were produced in Balb/C mice after subcutaneous or intraperitoneal immunizations with a kainic acid receptor isolated from frog (Rana pipiens) brain using ion exchange and ligand affinity chromatography. Ligand affinity chromatography was carried out using the high affinity kainic acid analog, domoic acid (Hampson and Wenthold, JBC 263:2500,1988). Primary screening was done by dot blotting using the affinity purified receptor as antigen. Additional screening assays consisted of western blotting and an indirect immunoprecipitation assay using a solubilized receptor preparation (Hampson, et al., J. Neurochem.49:1209,1987) and antibody-coated microspheres. Hybridoma cloning was via limiting dilution (2X) using hybridoma cloning factor as a feeder cell replacement.

A large number of positive clones were detected in the dot blot assay. Of these, several were capable of immunoprecipitating solubilized kainic acid receptors. The range of immunoprecipitation was from 20% to greater than 80% of the solubilized receptors. For the western blots, crude brain membranes, crude solubilized preparations, and affinity purified receptors were used. Several of the antibodies that reacted on the dot blot and in the immunoprecipitation assay also reacted strongly with a band of M_r=48,000 on western blots. This band corresponds to that observed on silver stained gels of the affinity purified preparations. A number of the monoclonal antibodies displayed a unique pattern of staining on tissue sections that corresponded to the pattern of ^{3}H-KA binding on autoradiograms.

199.22

COMPARISON OF AMINOPHOSPHONOBUTYRIC ACID (AP4)-SENSITIVE EXCITATORY AMINO ACID RECEPTORS MEASURED BY [^{3}H]N-ACETYLASPARTYLGLUTAMATE ([^{3}H]NAAG) BINDING AND IBOTENATE (IBO)-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS. B.G. Johnson * and D.D. Schoepp, Lilly Research Laboratories, Indianapolis, IN 46285.

The binding of [^{3}H]NAAG to brain membranes and the activation phosphoinositide hydrolysis by IBO in brain slices are biochemical parameters which have been previously shown to be selectively inhibited by D,L-AP4. In this study we have further examined if the binding of [^{3}H]NAAG and stimulation of phosphoinositide hydrolysis by IBO are indexing the same or different populations of AP4-sensitive sites in brain. The L-isomer of AP4 selectively inhibited IBO-stimulated phosphoinositide hydrolysis and was 20-times more potent than the D-isomer in competing for [^{3}H]NAAG binding. Likewise, L-serine-O-phosphate, but not D-serine-O-phosphate, selectively inhibits both IBO-stimulated phosphoinositide hydrolysis and [^{3}H]NAAG binding. D,L-aminoproprionic acid (D,L-AP3) was also found to be a relatively potent inhibitor of IBO-stimulated phosphosinositide hydrolysis (IC50% = 113 $\pm$ 11μM). However, at similar concentrations D,L-AP3 does not inhibit [^{3}H]NAAG binding, and concentrations of NAAG up to 1000μM did not stimulate phosphoinositide hydrolysis. Although both assays are sensitive to L-AP4 inhibition they appear to represent disparate excitatory amino acid sites in brain.

199.23

AGE-DEPENDENT LOSS OF NMDA RECEPTORS IN RODENT BRAIN. D.T. Monaghan, K.J. Anderson, C. Peterson and C.W. Cotman, Depts. Surgery and Psychobiology, Univ. California, Irvine, CA 92717.

NMDA receptors play a key role in neuronal plasticity as well as excitotoxic mechanisms. The aged nervous system is selectively vulnerable to excitotoxic insults. In this study we have examined the status of the NMDA receptor complex in the aged rodent CNS. Quantitative *in vitro* autoradiographic techniques were used to estimate the density of NMDA receptors in aged (24-26 month-old Fisher 344), middle-aged (4-6 month-old) and infant (14 day-old) rodent brains. [^{3}H] L-glutamate was used to examine the agonist-preferring NMDA recognition sites, [^{3}H] (3-((±)2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP) to examine the antagonist-preferring NMDA sites, and [^{3}H] glycine to examine the glycine allosteric regulation site. For each of these ligands there was a significant reduction of binding to the NMDA receptor complex in select regions of the rat brain. Areas that were especially vulnerable included the striatum, entorhinal cortex and subiculum. It may be significant that the brain regions showing the greatest loss of NMDA receptor binding are known to be sensitive to age-dependent neuropathologies. Loss of striatal neurons occurs in Huntington's disease while Alzheimer's disease is associated with a loss of entorhinal and subicular neurons. Since NMDA receptors have been implicated in the loss of striatal neurons in Huntington's disease, it is tempting to speculate that the loss of NMDA receptors during normal aging may be acting to protect brain regions especially vulnerable to excitotoxic insults.

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199.24

EFFECTS OF NOVEL ANTAGONISTS AND KINDLING ON NMDA-SENSITIVE ^{3}H-GLUTAMATE BINDING. S.M. Jones, *L.D. Snell and K.M. Johnson, Dept. Pharmacol. & Toxicol., Univ. Texas Med. Br., Galveston, TX 77550.

Buffy coat membranes were prepared from rat cortex according to the method of Kishimoto et al (1981). ^{3}H-glutamate bound with a K_D of 37.2 nM and B_{max} of 2.7 pmol/mg protein. Binding was displaced by NMDA as well as the NMDA antagonists 2-aminophosphonovalerate (APV) and 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) with IC_{50}'s of 2.6μM, 1.1μM, and 0.9μM, respectively. In contrast, kainic acid and α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) had little effect. We feel that this membrane preparation is suitable for screening compounds which act at the NMDA receptor. The NOVA Pharmaceutical Company compounds NPC 12626 (4,5 cyclohexyl-aminophosphonoheptanoate) displaced binding with an IC_{50} of 4.8 μM. This compound also shifted NMDA-enhanced ^{3}H-TCP binding to the right in a competitive fashion. In contrast, 4,5 benzo-aminophosphonoheptanoate (NPC 451) was 100-fold less potent at the NMDA receptor. ^{3}H-glutamate binding was also examined in tissue from amygdaloid-kindled rats three to five weeks following the last stimulation. Preliminary experiments reveal no differences in K_D or B_{max} of ^{3}H-glutamate binding between cortex, hippocampus or amygdala of kindled rats and that of implanted controls. Supported by DA-02073 and NOVA Pharmaceutical Company.

199.25

SYNTHESIS, CHARACTERIZATION AND USE OF (+)[^{3}H]MK801 AND SOME DERIVATIVES. J.F.W. Keana*#, M. Scherz*#, P. Barmettler*#, Y. Kitahara*#, M. Quarum*@,M.S.Sonders*@ and E. Weber@ (SPON: C. Russell). #Department of Chemistry, University of Oregon, Eugene, OR 97403 and @Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

MK801 is the most potent and selective ligand recognized to date for the high affinity PCP binding site, and like PCP, blocks cation flux through the NMDA-preferring glutamate receptor. In order to biochemically characterize and ultimately purify the PCP/NMDA receptor, we have synthesized a high specific radioactivity (+)[^{3}H]MK801 and a photoaffinity derivative of it, as well as several nonradioactive congeners.

(+)MK801 was synthesized, N-trifluoroacetylated and then brominated, predominantly yielding a tribrominated derivative. The N-protecting group was removed, the material was converted to the HCl salt and subjected to a catalytic halogen-tritium exchange reaction. Subsequent purification by reverse phase HPLC gave pure (+)[^{3}H]MK801 with a specific activity of 97 Ci/mmol. In radioreceptor assays using guinea pig brain membranes the radioligand displayed a K_d of 2 nM, B_{max} of 1.3 pmol/mg protein and pharmacological profile quite similar to that of [^{3}H]TCP.

Further data will be presented on the use of (+)[^{3}H]MK-801 in solubilizing the PCP/NMDA receptors and on the synthesis and characterization of other derivatives including a photoaffinity analogue of MK801.

199.26

THE LOW AFFINITY STATE OF THE PHENCYCLIDINE (PCP) RECEPTOR: SIMILARITY TO THAT LABELED BY [^{3}H]DEXTROMETHORPHAN? Matthew A. Sills, Michael Williams and Pat S. Loo, * Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901.

Considerable evidence has accumulated for the existence of an N-methyl-D-aspartate (NMDA)/PCP receptor complex. Previously, multiple states of the PCP receptor were demonstrated (Loo et al., Mol. Pharmacol. 32:820) and compounds such as PCP, MK-801 and dexoxadrol were found to be selective for the high affinity state of this receptor. The present study examined the ability of a series of compounds to inhibit [^{3}H]TCP binding, including the anti-ischemic agents dextromethorphan and trihexyphenidyl, as well as several antipsychotics and antihistaminics. Each compound produced a shallow inhibition curve of [^{3}H]TCP binding in the absence of Mg^{2+} and L-glutamate. In the presence of Mg^{2+} and L-GLU, steep inhibition curves were generated. In contrast to PCP, these compounds were less potent under these conditions, indicating selectivity for the low affinity state of the PCP receptor. A significant ($p < 0.05$) correlation was noted between the activity of these compounds at the low affinity state of the PCP receptor and their ability to inhibit [^{3}H]dextromethorphan binding (Craviso and Musacchio, Mol. Pharmacol. 23:629). These results indicate that [^{3}H]dextromethorphan labels the low affinity state of the PCP receptor and suggests that the two states of the PCP receptor may have functionally distinct roles in the NMDA/PCP receptor complex.

200.1

ELECTRON MICROSCOPIC FEATURES OF TENSOR TYMPANI MOTONEURONS IN THE RAT. K.M. Spangler, C.A. Miller* and G.T. Schneider* Dept. Anat., Creighton Univ. Sch. Med., Omaha, NE, 68178.

Computer aided morphometry was performed on examples of electron micrographs of tensor tympani (TT) motoneurons and their synaptic terminals. The results revealed medium sized multipolar motoneurons which received a modest covering of their somata and proximal dendrites with synaptic terminals. The observed synaptic terminals were of four types based on size and shape of the vesicles and the nature of the synaptic density. The most prevalent type of ending contained medium sized synaptic vesicles which were mostly round (length/width ratio<1.25). Occasional dense-cored vesicles were seen in this type of terminal. A second type of ending had vesicles which were slightly larger and more oval in shape (1.25< l/w ratio <1.5). Two other types of endings were rarely seen, one with small round vesicles (max. vesicle size 30-35 nm.) and another type with vesicles which were mostly flat (1/w ratio > 1.5). These findings indicate that TT neurons receive synaptic inputs from several different, as yet unidentified, sources.

Supported by a grant from the Deafness Research Foundation.

200.2

DIFFERENT TYPES OF MOSSY FIBER TERMINALS IN THE GUINEA PIG COCHLEAR NUCLEI. M.E. Dunn*, D.E. Vetter, A.S. Berrebi, H.M. Krider* and E. Mugnaini. Lab. of Neuromorphology,U154 and Biotechnology Image Applications Facility, U131, The University of Connecticut, Storrs, CT 06268.

In the cochlear nuclei, there is a mossy fiber-granule cell system, similar to that in the cerebellar cortex, that provides a pathway for the activation of neurons with dendrites situated in the molecular layer of the dorsal cochlear nucleus. It becomes important, therefore, to establish the sources of mossy fibers. Their terminals form the central elements of glomeruli, structurally analagous to those in the cerebellar cortex. By standard electron microscopy, we have identified at least four types of mossy fiber terminals which differ in parameters of internal structure such as features of mitochondrial profiles, density and size of synaptic vesicles, and percentage of dense core vesicles. Some of these parameters were determined visually, while others were measured with the aid of a video-microscopy, computer-assisted image analysis system (Olympus CUE-4- Gould). The experimental animal for this study was the adult NIH-strain guinea pig, but the heterogeneity of cochlear mossy fibers was also recognized in mouse, rat, and cat.

This work was supported by NINCDS NRSA Postdoctoral Fellowship NS-08175 (A.S.B.) and PHS grant NS-09904 (E.M.).

200.3

ULTRASTRUCTURE OF THE BAT LATERAL SUPERIOR OLIVE L.K. Owen*, J.M. Zook and R.A. DiCaprio. Dept. of Zoological & Biomedical Sciences and Basic Sciences, COM, Ohio Univ., Athens, OH 54701

The normal ultrastructure of the lateral superior olive (LSO) was examined with the electron microscope in two echolocating bat species: Eptesicus fuscus and Pteronotus parnellii. In general, ultrastructural features of the LSO in both species resemble those seen in other mammals. Synaptic terminals containing large, flattened vesicles form the principal contacts upon the somatic surface of LSO fusiform cells. Synaptic terminals with large, round vesicles contact both the large, proximal dendrites and small, distal dendrites of these cells.

In further experiments, axons of principal cells of the medial nucleus of the trapezoid body were filled intracellularly with horseradish peroxidase (HRP) in an in vitro tissue slice preparation of the brainstem of these two bat species. Preliminary ultrastructural examination of the terminal field of these labeled axons in the LSO suggests that the medial nucleus of the trapezoid body is the source of the synaptic terminals containing flattened vesicles.

Supported by NIH grant NS20986, OURC grant 756, and the OUCOM.

200.4

ULTRASTRUCTURAL STUDIES OF SYNAPTIC TERMINALS IN THE RAT MEDIAL SUPERIOR OLIVARY NUCLEUS. M.A. Casey. Dept. of Cell Biol. and Anat., University of Alabama at Birmingham, Birmingham, AL 35294.

Neurons in the medial superior olivary nucleus (MSO) are binaurally innervated via projections from the left and right ventral cochlear nuclei. The ultrastructure of the cat MSO has been well characterized (Clark, 1969; Lindsey, 1975; Schwartz, 1980; Kiss and Majorossy, 1983), but the ultrastructure of the rat MSO has not been described prior to the present study. In rats only about 36% of the perikaryal surface is apposed to synaptic endings, while in cats the percent coverage is about 84% (Schwartz, 1980). The vast majority of axosomatic synaptic termials in the rat MSO contain pleomorphic synaptic vesicles and exhibit symmetrical membrane specializations (Type 2 terminals), while in cats, terminals containing large spherical vesicles and asymmetrical synapses (Type 1 terminals) predominate on the soma. Type 1 terminals are predominant on dendrites in the neuropil of the MSO in cats and rats. Type 3 terminals, containing densely packed small spherical vesicles, form less than 5% of the axosomatic terminal population in the rat MSO.

While the synaptology of the lateral superior olivary nucleus (LSO) is essentially the same in the rat and cat (Cant, 1984; Casey, 1986), the arrangement of axosomatic synaptic terminals in the MSO differs markedly in the two species. (Supported by NIH grant AG06188)

200.5

ULTRASTRUCTURAL CHARACTERIZATION OF GABA AND GLYCINE IMMUNO-REACTIVE SYNAPSES IN THE GUINEA PIG SUPERIOR OLIVARY COMPLEX. R.H. Helfert, J.M. Bonneau,* R.J. Wenthold, + and R.A. Altschuler. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109, and +Lab. of Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD 20892

Immunoperoxidase immunocytochemistry was performed on aldehyde-fixed semi-thin sections to assess the distribution of GABA and glycine (GLY) in four major superior olivary complex (SOC) nuclei: the lateral superior olive (LSO), medial superior olive (MSO), medial trapezoid nucleus (MTN) and superior paraolivary nucleus (SPN). The fine structure of the immunoreactive (IR) synapses was evaluated using colloidal gold immuno-electron microscopy.

There was little variability in the morphology of GLY IR synapses among the SOC nuclei. They contained pleomorphic "flat" vesicles and possessed punctate symmetrical membrane specializations separated by a 20 nm synaptic cleft. Light microscopic evaluation showed that most of the somal surface (>65%) of the LSO principal neurons, and the SPN radial neurons, was apposed to GLY IR puncta. These puncta were also seen, to a lesser extent, on the proximal dendrites of these cells. Most perikarya in the MSO central cell band possessed GLY IR puncta on >50% of their surface and on >35% of the surface of their proximal dendrites. Small numbers of GLY IR puncta were observed scattered on MTN somata.

GABA IR synapses were found, in small numbers, on most neurons in the SOC. Unlike the GLY IR synapses, variations in the morphology of GABA IR snyapses were observed. In the LSO, all GABA IR presynaptic terminals were packed with pleomorphic "oval" vesicles and were ~1.25 µm in diameter. These terminals were also identified on the somata and dendrites of neurons in the MSO and MTN. A second type of GABA IR terminal was observed less frequently on MSO dendrites; they were smaller (~1µm wide by 0.75 µm in elevation) and contained very few pleomorphic vesicles. A third type of GABA IR synapse, containing a few dense core vesicles in addition to the electron-lucent pleomorphic "oval" vesicles, was encountered on small caliber dendrites in the SPN. This type is similar, morphologically, to a class of synapses contacting neurons of both the lateral and medial olivocochlear system (R.H. Helfert, I.R. Schwartz, and A.F. Ryan, J. Neurosci., in press).

These data suggest that there may be at least three sources of GABAergic input to the SOC nuclei. Whether or not this is the case for glycinergic input remains to be seen.

Supported by NIH grants NS24369 and NS07106.

200.6

STRUCTURE OF SYNAPTIC CALYCES AND THEIR POST-SYNAPTIC TARGETS IN THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS IN THE GUINEA PIG. B. R. Schofield and N. B. Cant Dept. Anatomy, Duke Univ., Durham, NC 27710

We have used the light and electron microscopes to examine synaptic calyces in the ventral nucleus of the lateral lemniscus (VNLL) in the pigmented guinea pig, Cavia cobaya. Calyces as well as many smaller axon terminals are labelled by anterograde transport after injections of Fast Blue into the contralateral cochlear nucleus. Each calyx arises from a large axon that splits into numerous tendrils that encircle a neuronal cell body.

In the electron microscope, calyces are characterized by their large size (spanning up to 9 um) and round synaptic vesicles. They usually contact somas, which also synapse with smaller terminals. Some of these smaller terminals contain round vesicles and may be branches of the calyx; others contain pleomorphic or flat vesicles.

The structure of calyces in the VNLL is similar to that of endbulbs in the anteroventral cochlear nucleus and calyces in the medial nucleus of the trapezoid body, where these terminals ensure fast, secure synaptic transmission. Since virtually every cell in VNLL projects to the inferior colliculus (IC) (e.g., Adams'79, J. Comp. Neurol. 183:519-538), calyces in the VNLL probably participate in fast disynaptic pathways to the IC.

Supported by NIH Grants NS14655 and MH15177.

200.7

THALAMOCORTICAL SYNAPSES WITH GOLGI-IMPREGNATED, GABAERGIC AND PEPTIDERGIC NEURONS IN MONKEY AUDITORY CORTEX. A. Keller, E. L. White and P. B. Cipolloni. Dept. Morphol., Fac. Health Sci., Ben Gurion Univ., Beer Sheva, ISRAEL; and Depts. of Anat. and Neurol., Boston Univ. School of Medicine, Boston, MA 02118.

Thalamocortical inputs to identified neurons in the auditory cortex of the monkey were examined. Bipolar electrodes were inserted into the medial geniculate nucleus (MGN), and electrolytic lesions were placed in the electrophysiologically-identified MGN to label thalamocortical axon terminals, by lesion-induced degeneration. Four days later, the animals were perfused with aldehydes, and blocks of tissue containing the primary auditory cortex (area KA) were Golgi-impregnated. The blocks were then cut at 120µm, and the sections gold-toned and embedded in plastic. Labeled nonpyramidal neurons were drawn and photographed with a light microscope, and then thin sectioned and processed for electron microscopy. The selected neurons were studied in serial thin sections to determine the distribution of thalamocortical synapses they received. A postembedding immunocytochemical procedure was employed to label the somata belonging to the selected neurons, using antisera to GABA, somatostatin, and choleocystokinin (CCK). All of the nonpyramidal neurons were immunoreactive for GABA. Two of the GABA-immunoreactive neurons were also CCK - positive, and one stained with the antiserum to somatostatin. In no instance were GABAergic cells immunoreactive for both peptides. Supported by BSF grant #8600041/1 , and the Institute for Neurologic Research, Inc.

200.8

DISTRIBUTION OF VIMENTIN IN THE GUINEA-PIG COCHLEA. **E.C. Oesterle[1]*, V.J. Sarthy[2], and E.W Rubel. (SPON: R.A. Code). Depts. of Otolaryngology[1] and Ophthalmology[2], Univ. of Washington, Seattle, WA 98195.**

The hypothesis that proteins known to occur in glial cells of the CNS may be present in inner-ear supporting cells was investigated. The main rationale was to determine if vimentin, an intermediate filament protein present normally in certain types of CNS glia, could be used as a marker to identify particular cell types in the auditory end organ. The distribution of vimentin was explored in cochleae of adolescent and adult guinea pigs at the light microscope level using monoclonal antibodies directed against vimentin. Immunocytochemical localization using indirect immunofluorescence and avidin-biotin-peroxidase methods labels a variety of structures in the cochlea. Within the organ of Corti staining is observed in Deiters' cells, one type of supporting cell in the end organ. Immunoreactivity is also observed in the following inner-ear structures: fibroblasts in the spiral ligament, external sulcus cells, some Claudius cells, tympanic border cells, cells lining the perilymphatic surface of Reissner's membrane, satellite-cell processes in the habenular opening, Schwann-cell processes surrounding the cell bodies and peripheral processes of spiral ganglion cells, and areas of the spiral limbus. Results demonstrate that vimentin may be a useful marker for the identification of Deiters' cells from other end-organ cells. (Supported by NIH Grant NS24522 and the Deafness Research Foundation).

200.9

COMPARATIVE AMINO ACID LEVELS IN THE HEARING ORGANS OF RAYS, CHICKENS AND MAMMALS. G.M. Bryant, G.G. Ceasar* and R.P. Bobbin. Kresge Hearing Research Lab., LSU Medical Center, New Orleans, LA 70112.

With the advent of immunocytochemical techniques, there are numerous reports on the presence of putative neurotransmitters in heretofore unsuspected locations. For example, Fex et al. (Brain Res. 366:106, 1986) have found GABA immunostaining in a small number of efferent endings in the upper turns of the cochlea and Usami et al. (Hearing Res. 30:19, 1987) reported GABA-like immunoreactivity in the cytoplasm of hair cells of the basilar papilla of the chicken. In an effort to relate the histological findings to biochemical levels, we tested the hypothesis that the amount of GABA in the guinea pig cochlea is small in comparison to that found in the chick basilar papilla. Whole tissue homogenates were analyzed by gradient-elution, reverse-phase HPLC which allowed the determination of endogenous levels of GABA plus 12 other amino acids. The results show that the guinea pig cochlea has 5 times as much GABA as the chick basilar papilla on a per ug protein basis.

(Work supported by NIH grants T32-NS07058-10 and NS24238, the Kresge Foundation and the Louisiana Lions Eye Foundation.)

200.10

RELEASE OF ENKEPHALINS AND DYNORPHINS AT A MULTITRANSMITTER SYNAPSE IN THE COCHLEA. D.W. Hoffman, R.D. Edkins*, and K.L. Jones-King*. Neurochemistry Lab., Dept. of Psychiatry and Pharmacology, Dartmouth Medical School, Hanover, NH 03756.

The mammalian cochlea is innervated by efferent fibers which arise in the brainstem. These fibers comprise two systems which separately innervate the eighth nerve dendrites under inner hair cells, and the bases of the outer hair cells themselves. The efferent fibers appear to be cholinergic, but the fibers under inner hair cells also contain enkephalins and dynorphins. We use the cochlea as an in situ perfusion chamber to study neurochemical interactions of the neurotransmitters and neuromodulators co-localized in the efferent nerve terminals in the cochlea. Met- and leu-enkephalin, dynorphin B and ACh have all been shown to be released in cochlear perfusate by high potassium or veratridine. Peptides are measured by HPLC-RIA, and ACh by radioenzymatic assay. We are currently assaying the effects of neuropeptides and ACh on each others' release processes.

[Supported by NSF grant BNS8646563 to DWH]

200.11

IDENTIFICATION OF A PROTEIN PRIMARILY LOCALIZED TO THE COCHLEAR NUCLEUS. L. Winsky, and D.M. Jacobowitz, Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

In a previous report (Winsky et al., 1987 Soc. Neurosci. Abs. 250.14), large differences were found in the content of several proteins within the auditory system of rabbits. The present study was aimed at providing a similar comparison of proteins within the central auditory system of guinea pigs. Micropunch samples were obtained from the ventral and dorsal cochlear nuclei as well as the lateral superior olive, nucleus of the lateral lemniscus, inferior colliculus, medial geniculate and auditory cortex. Proteins were separated using two-dimensional gel electrophoresis and were visualized by a silver stain method. Results indicated one protein which was consistently greater in amounts in both the dorsal and ventral cochlear nuclei as compared with other regions examined. Protein separation using more discrete micropunches of the dorsal cochlear nucleus (DCN) suggested that this protein is found predominantly within the principle cell layer of the DCN with much less in the molecular/granule cell region. Finally, preliminary findings suggested that the amounts of this protein may decrease following destruction of the cochlea.

The MW (29 kD), pI (5.2) and appearance of this protein on gels was similar to a cochlear-specific protein identified in both rabbits and rats. Possibly, this protein could be associated with either direct projections from the cochlea via the auditory nerve or cell types which are intrinsic to the cochlear nuclei.

200.12

CALBINDIN LABELS AUDITORY NUCLEI IN THE MUSTACHED BAT. M.L. Zettel, C.E. Carr[1], W.W. Wilson & W.E. O'Neill. Depts. of Physiology, and Neurobiology and Anatomy[1], Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Echolocation places a premium on processing rapid acoustic events, which might correlate with fast recovery in auditory neurons. One proposed function of Calbindin is that of an intraneuronal calcium buffering system, which could enhance temporal coding. We therefore used antibodies against calbindin to examine the auditory pathways in the mustached bat. The primary antibody was a rabbit anti-monkey cerebellar serum monospecific for 28 kD CaBP (courtesy Dr. K. Baimbridge), and the secondary was a biotinylated protein A (Dr. R. Sloviter).

Calbindin D-28k prominently stained auditory nuclei. In Cochlear Nucleus (CN), Anteroventral CN showed staining in spherical cells of the anterior group as well as multipolar cells of the medial group. Posteroventral CN had extensive neuropilar and light somatic labeling throughout while Dorsal CN had label only in lateral superficial cells. Lateral Lemniscus had strong somatic labeling in all nuclei, with stained neuropil and a striking cellular banding pattern in both divisions of Ventral nucleus. The Sup. Olives and Trapezoid Bodies stained darkly and uniformly. Labeled cells were scattered throughout Central Nucleus of Inferior Colliculus except for Medial division, where many cells were darkly stained. Medial Geniculate had darkly labeled neuropil and somata in Dorsal division and to a lesser extent in Medial division. Supported by NSF BNS-8617152 to WEO.

200.13

GABA AND GLYCINE IMMUNOREACTIVITY OF DESCENDING AND COMMISSURAL INPUTS TO THE COCHLEAR NUCLEUS IN GUINEA PIG.

E.-M. Ostapoff, C. Staatz-Benson, D.K. Morest, S.J. Potashner, and R.L. Saint Marie. Department of Anatomy and Center for Neurological Sciences, University of Connecticut Health Center, Farmington, CT 06032.

Retrograde transport of HRP from the cochlear nucleus was combined with pre-embedding immunocytochemistry to determine which inputs might use GABA or glycine as a neurotransmitter. Nearly all of the neurons immunoreactive (+) for GABA or glycine and projecting to the cochlear nucleus were from periolivary regions of the superior olivary complex. Most GABA+ projection neurons were seen in the ventral trapezoid nucleus, bilaterally. Most glycine+ projection neurons were seen in the lateral trapezoid nucleus, ipsilaterally, and in the ventral trapezoid nucleus, bilaterally. Most commissural neurons in the contralateral cochlear nucleus were glycine+, a few were GABA+. Although there were many GABA+ neurons in the inferior colliculus, GABA+ neurons projecting to the cochlear nucleus were rare. Glycine+ neurons were not detected in the inferior colliculus.

The results suggest that some of the projections to the cochlear nucleus in guinea pig may use GABA or glycine as a neurotransmitter. These include projections from the superior olivary complex and the contralateral cochlear nucleus.

(Supported by NIH grants NS14347, NS18391, and NS19036).

200.14

TARGETS AND DISTRIBUTION OF THE CARTWHEEL CELL AXON IN THE GUINEA PIG DORSAL COCHLEAR NUCLEUS.

A. S. Berrebi and E. Mugnaini. Laboratory of Neuromorphology, U-154, The University of Connecticut, Storrs, CT 06268.

We have previously shown that PEP-19, a neuron-specific calcium binding polypeptide, represents an excellent immunocytochemical marker for dorsal cochlear nucleus (DCoN) cartwheel neurons. In guinea pig hindbrain, only cerebellar Purkinje neurons and DCoN cartwheel neurons are densely stained with a rabbit antiserum (gift of Dr. J. I. Morgan). The deeper branches of the cartwheel cell axons demarcate layer 3 of DCoN. By electron microscopic pre-embedding immunocytochemistry, we have found that cartwheel neurons have two primary targets in the superficial layers of DCoN: 1) cells of the same class and 2) the cell bodies and basal dendrites of fusiform cells, the only projection neurons of the superficial layers (which project to the central nucleus of the inferior colliculus). Thus, cartwheel neurons, the most numerous of the inhibitory local-circuit neurons, must be largely responsible for modulation of fusiform cell output. These findings were obtained in adult NIH-strain guinea pigs, but similar observations have been made in mouse and rat.

This work was supported by an NINCDS NRSA Postdoctoral Fellowship to A.S.B. (NS-08175) and PHS grant NS-09904 to E.M.

200.15

LOCALIZATION OF THE GABAA/BENZODIAZEPINE RECEPTOR COMPLEX IN THE GUINEA PIG COCHLEAR NUCLEUS: LM & EM IMMUNOCYTOCHEMICAL STUDIES.

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Immunocytochemical studies using antibodies to GABA and GAD as well as pharmacological, biochemical and receptor binding studies all suggest that the inhibitory amino acid GABA is a major transmitter in the cochlear nucleus. Recent studies showing co-containment of GABA and glycine immunostaining in cells and terminals in the cochlear nucleus as well as GABA and GAD immunostaining terminals apposed by glycine receptor immunoreactivity (Wenthold et al. 1987, Oberdorfer et al. 1988) have raised questions about localization of the GABA receptor complex. As a part of studies to characterize GABAergic synapses in the cochlear nucleus and their relationship to GABA and glycine receptors we have used recently developed monoclonal antibodies to the GABAA/Benzodiazepine receptor complex (deBlas et al. 1988) to study the distribution of this complex in the guinea pig cochlear nucleus at the light and electron microscopic level. Immunoperoxidase and immunofluorescence techniques were used on free-floating and cryostat sections respectively for light microscopic evaluation of the cochlear nucleus, pre-embedding immunoperoxidase and immunogold techniques were used for the ultrastructural evaluation.

GABA receptor complex immunoreactive staining was seen at the light microscopic level in all divisions of the cochlear nucleus with a general distribution similar to that of GABA immunoreactive terminals but with heavier distribution in the granule cell cap. An intracellular cytoplasmic labeling was seen in many small cells and fusiform cells in the dorsal cochlear nucleus. At the ultrastructural level most labeling was associated with the active zone of axo-somatic and axo-dendritic synapses, usually post-synaptic to terminals containing oval/pleomorphic vesicles. A significant amount of cytoplasmic immunostaining was evident, both in dendrites and soma. At some synapses pre-synaptic label was also seen opposite the post-synaptic label. This would suggest that a major synaptic action of GABA is post-synaptic but that at some synapses there may also be a pre-synaptic, perhaps feed-back function.

supported by NIH grant NS 21440 and a Generalitat Valenciana Fellowship.

200.16

RELEASE OF AMINO ACIDS IN THE GUINEA PIG COCHLEAR NUCLEUS AFTER ABLATION OF THE INFERIOR COLLICULUS.

M. Bergman and S. J. Potashner. Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032

This study determined if projections from the inferior colliculus (IC) to the cochlear nucleus (CN) might use amino acid transmitters. Surgical ablation removed approximately 84% of the left IC without damage to other auditory structures in the brain stem. Five days after the ablation, the electrically-evoked calcium-dependent release of [3H]-D-aspartate and [14C]-GABA from the anteroventral, posteroventral, and dorsal CN were unaffected. The release of [14C]-glycine was depressed by 47% in the anteroventral CN bilaterally, but not in the other CN subdivisions. These findings suggest that fibers projecting from the IC to the CN probably do not use glutamate, aspartate or GABA as transmitters. Similarly, collicular projections to the dorsal and posteroventral CN probably do not use glycine as a transmitter. However, glycine might be a transmitter of some collicular fibers projecting to the anteroventral CN. (Supported by NS 19036 from NINCDS).

200.17

CALBINDIN IMMUNOSTAINING OF THE AUDITORY BRAINSTEM,

J. C. Adams, M. Thomasset,* E. Mugnaini and S. Christakos*. ENT Dept., Med. Univ. S.C.; INSERM U.120., Le Vesinet, France; Lab. of Neuromorphology, Univ. Conn., Storrs, CT 06268; Dept. Biochem. N.J. Medical School, Newark, N.J.

Immunostaining in the auditory brainstem of rat and cat using antisera to calbindin shows some staining in all nuclei. The number of cell classes that stain is limited and much about relations of cell classes can be learned from the staining patterns. In the ventral cochlear nucleus a small number of cells in the region of the nerve root are stained. In the dorsal cochlear nucleus the cartwheel cells are stained. Various sized terminals are stained throughout the cochlear nucleus. In the olivary complex all principal cells of the medial nucleus of the trapezoid body are densely stained, as are some cells in the lateral superior olive and in the lateral trapezoid nucleus. Dense pericellular terminals are stained in principal olivary nuclei and in limited periolivary regions. Stained cells are present in the lemniscal nuclei. Many stained cells are present in extracentral portions of the inferior colliculus, the sagulum, and brachium of the inferior colliculus. Large numbers of cells are stained throughout the medial geniculate body. Overall immunostaining in rat and cat is in register but there are exceptions. The most obvious is that some neurons in the dorsal nucleus of the lateral lemniscus stain in rat but not in cat.

200.18

TRANSPORT OF 3H-GLYCINE TO THE SUPERIOR OLIVARY COMPLEX FROM THE COCHLEAR NUCLEUS IN THE GUINEA PIG.

C. Staatz-Benson and S. J. Potashner, Dept. of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

Glycine may be a transmitter of some descending fibers synapsing in the mammalian cochlear nucleus. Possible sources of descending projections to the cochlear nucleus were identified using retrograde labelling with horseradish peroxidase (HRP). HRP (20% w/v) with 1% peroxidase labelled wheat germ agglutinin was injected into the guinea pig cochlear nucleus. After 24 hours labelled neuronal cell bodies were found bilaterally in all the periolivary nuclei, in the inferior colliculus and in the contralateral cochlear nucleus.

Retrograde labelling with 3H-glycine was used to determine which of these structures provide glycinergic projections to the cochlear nucleus. 3H-Glycine (0.3-0.8 ul; 53 Ci/mmol; 19-380 uM) was injected into the guinea pig cochlear nucleus. After 6-48 hours retrogradely labelled neuronal cell bodies were found ipsilaterally in the ventral and lateral nuclei of the trapezoid body, the dorsal and anterolateral periolivary areas, and the vestibular nuclei. These results suggest that some of the glycinergic synaptic endings in the cochlear nucleus may be contributed by fibers projecting from the ipsilateral periolivary area. (Supported by grant NS 19036 from NIH-NINCDS.)

200.19

DISTRIBUTION OF CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE TRAPEZOID BODY OF THE RAT. D.A. Godfrey, L. Carlson*, J.A. Parli* and C.D. Ross. Dept. of Physiology, Oral Roberts University, Tulsa, OK 74171

Since our previous studies suggested that centrifugal cholinergic innervation reaches the rat cochlear nucleus via the trapezoid body, the distribution of choline acetyltransferase (ChAT) enzyme activity was mapped within this tract in 5 albino rats. ChAT catalyzes the synthesis of acetylcholine and is a reliable marker for cholinergic neurons. ChAT activity was measured for contiguous pieces microdissected from freeze-dried sections. A detailed map for one of the rats was based on 31 transverse sections spaced at 60 μm intervals. Adjacent sections histochemically stained for acetylcholinesterase activity were used to identify pieces containing facial nerve root or parasympathetic fibers which cross through the trapezoid body. The rostral and middle parts of the trapezoid body contained higher ChAT activity than the caudal part. At each rostro-caudal level ChAT activity laterally in the trapezoid body was higher in its ventrolateral portion than in its dorsomedial portion, but farther medially the ChAT activity was often higher dorsally than ventrally. These results are consistent with cholinergic fibers mostly entering the trapezoid body in its middle and rostral parts, from a more dorsal location at a point just medial to where it courses under the spinal trigeminal tract, and moving superficially as they move laterally to enter the cochlear nucleus. (Supported by NIH grant NS17176).

SENSORY SYSTEMS: AUDITORY SYSTEMS III

201.1

MULTIPLE CELL TYPES HAVE GABA IMMUNOREACTIVITY IN THE INFERIOR COLLICULUS OF THE CAT. D.L. Oliver, S.C. Nuding, and G. Beckius*. Dept. of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

Neurons in the central nucleus of the inferior colliculus were analyzed after immunocytochemical localization of GABA (Wenthold et al., 1987, Brain Res. 380: 7-18). Several types of immunoreactive cells can be distinguished by the size and orientation of their somata and by the visible portions of the proximal dendrites. For example, small cells in one sample have average diameters of 17-18 μm (range 12-20 μm), while medium-sized cells have average diameters of 24-26 μm (range 20-32 μm). Some labeled neurons have somata and proximal dendrites oriented parallel to the fibrodendritic laminae of the central nucleus. These may be disc-shaped neurons. Other neurons have cell bodies and proximal dendrites oriented orthogonal to the laminae and may be stellate cells.

The results suggest that many of the small disc-shaped and stellate cells in the central nucleus may use GABA as a neurotransmitter. Small cells represent only 10-15% of the cells in the central nucleus but about half of the present sample. The medium-sized immunoreactive cells could be special types of stellate and disc-shaped neurons.

(Sponsored by grant R01 NS18391 from NINCDS.)

201.2

RETROGRADE TRANSPORT OF 3H-GLYCINE FROM INFERIOR COLLICULUS TO NUCLEI OF THE LATERAL LEMNISCUS AND SUPERIOR OLIVE. R.L. Saint Marie, R.A. Baker, and D.K. Morest. Department of Anatomy and Center for Neurological Sciences, Univ. of Connecticut Health Center, Farmington, CT 06032.

Retrograde transport of ^{3}H-glycine was used to identify possible sources of glycinergic inputs to the inferior colliculus in chinchilla and guinea pig. Radiolabeled cells were found only ipsilaterally after survival times up to 26 hours. Cells in the ventral nucleus of the lateral lemniscus, the lateral superior olive, and the dorsomedial periolivary nucleus were heavily labeled after survival times as short as 6 hours. Cells in other periolivary nuclei were less heavily labeled.

Retrograde transport of HRP from the inferior colliculus in each of the cases confirmed additional projections that did not accumulate glycine. These included contralateral projections from the inferior colliculus, dorsal nucleus of the lateral lemniscus, lateral superior olive, periolivary nuclei, and cochlear nucleus, and ipsilateral projections from the medial superior olive and cochlear nucleus.

The results suggest that some of the uncrossed inputs to the inferior colliculus from nuclei of the superior olivary complex and lateral lemniscus may use glycine as a neurotransmitter, whereas crossed inputs from these regions and inputs from the medial superior olive and cochlear nucleus probably do not.

(Supported by NIH grant NS14347).

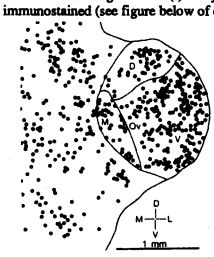
201.3

GLUTAMATE IS A MAJOR TRANSMITTER IN THE RAT MEDIAL GENICULATE BODY. J. M. Popowits*, D.T. Larue, and J.A. Winer (SPON: J.J. Wenstrup). Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

The relative simplicity of the rat medial geniculate body is a model system for the study of neurotransmitters. Few neurons (~1%) are GABAergic (J.A. Winer and D.T. Larue, *J. Comp. Neurol.* [in press]), and most of the others appear to project to the cortex (J.A. Winer and D.T. Larue, *J. Comp. Neurol.* 257:282-315 [1987]). However, the thalamocortical transmitter is unknown, and its identity is the goal of this study.

Anesthetized adult rats were perfused at 4°C with Krebs-Ringer's, followed by carbodiimide-glutaraldehyde, then glutaraldehyde or glutaraldehyde-paraformaldehyde. Vibratomed sections were immunostained using the avidin-biotin technique (S.M. Hsu et al., *J. Histochem. Cytochem.* 29:1349-1353 [1981]) and a monoclonal antibody to a glutamate-keyhole limpet hemocyanin conjugate (see J.E. Madl et al., *J. Histochem. Cytochem.* 34:317-326 [1986]) at a 1:20,000 dilution. Controls processed without the primary antibody showed no specific immunostaining.

The main findings are that (i) many neurons in each auditory thalamic subdivision are immunostained (see figure below of cell plots), including their primary dendrites;



(ii) there are regional differences in the intensity of immunostaining, with the darkest cells in the medial division, the palest in the dorsal division, and the ventral division intermediate; (iii) glutamatergic neurons include the largest medial geniculate cells; (iv) interspersed among, and in the same focal plane as the glutamatergic neurons, are many glutamate-negative neurons. Other transmitters besides GABA and glutamate may be present in the medial geniculate complex.

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201.4

THE GABAERGIC NEURONAL ORGANIZATION OF LAYER I IN RAT AUDITORY CORTEX. D.T. Larue and J.A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

The neurons and puncta (axon terminals) immunostained for glutamic acid decarboxylase (GAD) were studied in layer I of rat area 41. Male rats were perfused with zinc salicylate/formalin (after E. Mugnaini and A.-L. Dahl, *J. Histochem. Cytochem.* 31:1435-1438 [1983]) and 25-50 μm-thick frozen or Vibratome sections were immunostained with GAD-1440 (W.H. Oertel et al., *Neuroscience* 6:2689-2700 [1981]) and the avidin-biotin immunoperoxidase technique (S.M. Hsu et al., *J. Histochem. Cytochem.* 29:1349-1353 [1981]).

More than 90% of layer I cells were GAD positive (GAD+) in semi-thin (1 μm-thick) sections cut from pre-reacted tissue. Members of each of the four types of neurons identified in Golgi material were represented. The mean somatic area of GAD+ neurons (59±21 μm^2) indicates that small and medium-sized cells predominate. Some cells have dendrites immunostained for almost 100 μm, well past their secondary branch points. Control sections incubated in pre-immune serum showed no specific staining.

Clusters of 3 to 9 GAD+ cells/100 μm of cortex are common, usually separated by cell-poor intervals of 100 μm or more. Indeed, most GABAergic neurons are within dendritic proximity to each other. GAD+ cells were twice as numerous in the lower half of layer I compared to the superficial half. Puncta were also non-uniformly distributed, being densest in the upper and lower parts of the layer and sparser in the middle.

Since layer I receives thalamic (D.K. Ryugo and H.P. Killackey, *Brain Res.* 82:173-177 [1974]), callosal (D.W. Vaughan, *Brain Res.* 260:181-189 [1983]), and intrinsic (A. Sousa-Pinto et al., *Brain Res.* 95:443-458 [1975]) input, and extrathalamic axons immunoreactive for serotonin, choline acetyltransferase, tyrosine hydroxylase, and dopamine-β-hydroxylase (M.J. Campbell et al., *J. Comp. Neurol.* 261:209-220 [1987]), the largely GABAergic neuronal population could serve as a common pathway for these diverse influences.

This research was supported by United States Public Health Service Grant RO1 NS16832-08. We thank Drs. D.E. Schmechel and E. Mugnaini for their generous gift of antiserum and technical advice.

201.5

WELL-DEVELOPED BRAINSTEM AUDITORY NUCLEI IN MANATEES TRICHECHUS MANATUS. J. I. Johnson, Anat. Dept., Mich. State U., E. Lansing, MI 48824; R. L. Reep, Dept. Neurosci., U. Fla., Gainesville, FL 32610; R. C. Switzer III, Depts. Pathol., Med. Biol., U. Tenn., Knoxville, TN 37920; J. A. W. Kirsch, Dept. Zool., and W. I. Welker, Dept. Neurophysiol., U. Wis.-Mad., Madison, WI 53706

Sections through brain stems of 5 manatees, in 3 planes, with Nissl, myelin or cytochrome oxidase stains show: Cochlear nuclei as in other auditorily specialized mammals -- the anteroventrals are large, as are the posteroventrals which hang outside the brain stem along the 8th nerve, capped by the rudimentary dorsals. The superior olives show prominent small-celled lateral nuclei, linear medial nuclei bounded by cell free regions, and large-celled nuclei of the trapezoid body. The nuclei of the lateral lemniscus are particularly massive with distinctive subnuclei. The large inferior colliculi resemble those of other audition-oriented species. (Supported by NSF grant BSR 85-03687.)

201.6

CENTRAL PROJECTIONS OF INDIVIDUAL AUDITORY NERVE FIBERS IN THE ALLIGATOR LIZARD (*GERRHONOTUS MULTICARINATUS*).

M.R. Szpir, S. Sento and D.K. Ryugo. Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115; Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA 02114; and Center for Hearing Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The auditory ganglion cells of the alligator lizard give rise to two types of peripheral fibers: tectorial fibers which contact hair cells covered by a tectorial membrane, and free-standing fibers which contact hair cells without a tectorial membrane. We examined the central projections of these ganglion cells after they were labelled with intracellular and extracellular injections of horseradish peroxidase. The tissue was processed with diaminobenzidine which produced a continuous labelling of the axons and terminals, enabling the correlation of the peripheral and central targets of the ganglion cells. Cells whose peripheral processes contact tectorial hair cells project to all three divisions of the cochlear nucleus: N. magnocellularis lateralis (NML), N. magnocellularis medialis (NMM), and the lateral part of N. angularis (NA). Cells whose peripheral processes contact free-standing hair cells project primarily to the medial part of NA, but a few cells also project a single, thin branch to NML. The cells contacting free-standing hair cells do not project to NMM. Tectorial fibers have a greater mean axonal diameter, form a greater number of terminal swellings, and make proportionally more somatic contacts. Electrophysiological studies have shown that tectorial fibers are tuned to low frequencies (100-800 Hz), whereas free-standing fibers are tuned to high frequencies (900-4000 Hz; Weiss, T.F. et al., *Brain Res.* 115:71-90, 1976). Thus, our anatomical results reveal that the different divisions of the cochlear nucleus are associated with particular frequency ranges, and suggest that these frequency ranges are processed differently in the brain. (Supported by NIH grants NS13126, NS20156, and an Albert J. Ryan fellowship to MRS).

201.7

"Holes" in the Gerbil Auditory System Do Not Cause Major Changes in Neuronal Populations in the PVCN. I.R. Schwartz & R. Karnofsky*, Div. Otolaryngology, Yale School of Med., New Haven, CT 06510

To assess possible changes in neuronal populations, size and number of neurons and "holes" (micro-cystic structures first described in the gerbil by Ostapoff et al, ARO Absts. 10:209, 1987) were measured in the posterior ventral cochlear nucleus (PVCN) of gerbils aged 3 & 12 months (M). The 3M gerbil is a fully mature adult & holes are sparse; 12M gerbils are still reproductively active and have attained less than 1/3 of their normal life span, but holes are prominent.

Serial slices (150-250μ) of mixed aldehyde fixed brains were osmicated, uranyl block stained and embedded in Epon. Number and size of neurons (only nucleus containing profiles) and holes were digitized from camera lucida drawings of 1μ plastic sections of the faces of serial transverse slices of the brainstem. Total PVCN volumes were estimated by summing the area occupied by PVCN and multiplying by the slice thickness. Three PVCNs at each age were measured.

Neither the number of neurons/section (3M-132.7±20.8, 12M-144.4±27.2), the % of total area occupied by neurons (3M-8.70±1.8, 12M-7.65±1.85) or total PVCN volumes were significantly different between the two ages. Holes occupy ≈3% of the 3M PVCN (3.17±1.42); ≈9% (8.70±1.84) at 12M. Holes/section at 3M=24.3±9.0; at 12M=64.4±24.2). Since variability within groups and the small sample size could have obscured small differences in neuron numbers and PVCN volume, additional material is being measured.

The absence of major changes in neuronal populations in the presence of substantial increases in the number and volume of holes suggests that hole formation is not directly related to any particular neuronal perikaryal population. Rather, it may be a dynamic process not necessarily resulting in cell loss.

Supported by NINCDS NS14503 and NS14945.

201.8

AXONAL PROJECTIONS OF PHA-L-LABELED NEURONS IN THE MEDIAL NUCLEUS OF THE TRAPEZOID BODY. S.C. Bledsoe, Jr., P.Pandya*, R.A. Altschuler, and R.H. Helfert, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109.

The medial nucleus of the trapezoid body, (MNTB) is a major relay nucleus in the superior olivary complex and is thought to be an important component in the processing of binaural input. Several studies have described the projection of MNTB neurons in the cat (Morest *et al, 1968;* Spangler *et al, 1985;* Glendenning *et al, 1985*); however, a detailed description of the projection of this nucleus is lacking in the guinea pig, which has been the subject of numerous biochemical, physiological, and immunocytochemical studies.

In this study, the anterograde neuronal tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) was iontophoresed into the guinea pig MNTB to study the distribution of the terminal arborizations of its neurons. PHA-L offers advantages over other conventional tracers to elucidate axonal projections, such as the discrete deposition of tracer within a well-defined area (normally <1 mm in diameter), and the small numbers of neurons labeled which allows for greater ease in delineating the projection patterns of individual neurons.

The projection of the guinea pig MNTB appeared to be entirely ipsilateral. There also appeared to be a topographical organization to this nucleus which was preserved in many, if not all, of the nuclei it projected to. Labeled axons were seen terminating within the MNTB. Labeled neurons in the MNTB produced terminal aborizations with beaded expansions and terminal boutons which surrounded the perikarya and/or proximal dendrites in the following nuclei: lateral superior olive, medial superior olive, superior paraolivary nucleus, lateral nucleus of the trapezoid body, ventral nucleus of the lateral lemniscus, dorsal nucleus of the lateral lemniscus.

Further evaluation correlating these results with those we are obtaining from immunocytochemical studies is in progress.

Supported by NS 05785, NS 07106 and a grant from the Deafness Research Foundation.

201.9

PRESERVATION OF FIBERS OF PASSAGE FOLLOWING KAINIC ACID LESIONS OF THE SUPERIOR OLIVARY COMPLEX IN THE FERRET: AN HRP ANALYSIS. B.J. Rooney*, G.L. Kavanagh*, and J.B. Kelly (SPON: Z. Wollberg). Laboratory of Sensory Neuroscience, Carleton University, Ottawa, Canada, K1S 5B6.

Previous studies have shown that microinjections of kainic acid (KA) into the superior olivary complex (SOC) destroy cell bodies but leave fibers of passage intact (Kavanagh and Kelly, 1986; Masterton et al., 1979). Behavioural and electrophysiological studies of the effects of these lesions provide an analysis of the function of the SOC that is not possible with traditional surgical methods. In this study we present further anatomical data on the effectiveness of KA for making auditory brainstem lesions. Animals were anesthetized with pentobarbital sodium and KA (5 nmoles/ul Locke's solution) was injected into the SOC through a micropipette. After periods of up to one year animals were again anesthetized and multiple injections of HRP were made into the central nucleus of the inferior colliculus. Forty-eight to seventy-two hrs later animals were sacrificed and the brains processed with TMB. Destruction of the SOC was verified in Nissl strained material by gliosis and the absence of nerve cells. In cases with unilateral lesions, the contralateral SOC showed heavy HRP labelling, while no labelling was evident at the lesion site. The fibers of passage were shown to be intact by retrograde labelling of neurons within the cochlear nucleus. Our results, besides demonstrating that the SOC is sensitive to the excitotoxicity of KA, also indicate its utility in studies investigating the effects of auditory brainstem lesions by avoiding the disruption of fibers of passage.

201.10

A DISTRIBUTED NEURAL NETWORK FOR CALL DETECTION IN THE FROG'S THALAMUS. **J.E. YOCHIM*, J.C. HALL* AND A.S. FENG (SPON: D. Green). Dept. Physiol. & Biophys. Univ. of Illinois, Urbana, IL 61801.**

We are conducting ongoing studies addressing issues concerning the neural basis of call recognition in the frog's auditory system. Recently, we reported that time and frequency cues are processed independently in the frog's thalamus with separate neural channels for each. Specifically, neurons in the posterior and central thalamic nucleus were shown to be selective for the spectral and temporal features of the species vocalizations, respectively. But, are these the only thalamic structures involved in call detection? We have addressed this question by recording acoustically evoked potentials (AEPs) from thalamic efferent targets of the posterior nucleus: the Nucleus of Bellonci (NB) and the ventrolateral thalamic nucleus (dorsal (VLd) and ventral (VLv) divisions). We found that responses to tone combinations (350 and 1,700 Hz) were larger (by 10 – 210%) than the sum of responses to individual tones. These findings were consistent with those reported for the posterior nucleus indicating an ordinal, functional relationship between these structures. This was supported by results of HRP tracing studies which revealed reciprocal connections between the posterior, but not central, thalamic nucleus and the NB, VLd, and VLv. This further supports the concept of parallel processing in the thalamus regarding time and frequency cues; the processing of spectral components is distributed widely (i.e., NB, VLd, VLv and the posterior nucleus) whereas temporal processing occurs principally within the central nucleus. In conclusion, our data suggest that the call detection system within the thalamus is represented by a distributed neural network rather than a localized population of neurons.

201.11

PROJECTIONS OF THE CAT MEDIAL GENICULATE BODY TO THE PRIMARY AUDITORY CORTEX (AI). B.A. Peterson and J.A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

The laminar distribution of axons from the cat medial geniculate body in primary auditory cortex (AI) was examined as part of a study of the connections of AI. Adult, pentobarbital-anesthetized cats received stereotaxic injections of .10-.20 µl of HRP (30%) or HRP/WGA (5%) aimed at the ventral division. The animal was reanesthetized and perfused 48 h later, and sections were cut from the inferior colliculus through the somatic sensory cortex. Laminar cortical subdivisions and architectonic borders were identified in DAB-reacted, Nissl counterstained sections. Many inferior colliculus neurons were retrogradely labeled, especially in the central nucleus.

The main results are (i) that the ventral division projects heavily to layers IV and IIIb in AI; (ii) that the projection is a continuous band throughout the rostro-caudal axis of AI; (iii) that thalamocortical input may involve retrogradely labeled corticothalamic layer V pyramidal cell apical dendrites ascending into layer IV (see J.A. Winer and B.A. Peterson, this volume); a similar pattern occurs among layer V cells projecting to the inferior colliculus (K.D. Games and J.A. Winer, *Hearing Res.* [in press]); and (iv) that a smaller projection to layer IIIa may reflect anterograde transneuronal transport.

The high density of thalamocortical input to, and diverse neuronal composition of, layers IIIb and IV is consistent with the idea that, in AI as well as the primary visual cortex, a variety of neurons might be postsynaptic to thalamic input.

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201.12

ORIGINS OF AUDITORY CORTICOTHALAMIC PROJECTIONS ONTO THE CAT MEDIAL GENICULATE BODY. J.A. Winer and B.A. Peterson. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

We identified the layers from which auditory corticothalamic projections arise, characterized the spatial distribution of these neurons, and reconciled the main types of projection neurons with the cells seen in studies of neuronal architecture from Golgi preparations, plastic-embedded material, or immunostained sections.

Corticothalamic cells arise (in decreasing numbers) from layers VI, Vb, and Va. The layer VI projection is particularly heavy and continuous through the dorsal-ventral extent of AI, and it may extend into adjoining, non-primary fields. While far fewer layer Vb neurons are labeled, this projection is still substantial. These cells are larger and more obviously pyramidal than layer VI neurons. Finally, in the upper part of layer Va, solitary, labeled pyramidal cells were often separated by 100-200 µm intervals devoid of such somata or of anterogradely transported material. The thick, HRP-filled apical dendrites of these cells project into layer IV. A gap of up to 150 µm devoid of labeling separated the retrogradely labeled profiles in layers Va and Vb.

The primary conclusions are (i) that the auditory corticothalamic projection involves all of layer VI, and the lower and upper quarters (but not the middle half) of layer V; (ii) that the projection is continuous in layers VI and Vb and discontinuous in layer Va; (iii) that more than one type of pyramidal neuron projects to the medial geniculate body; and (iv) that robustly labeled pyramidal neurons, whose apical dendrites ascend into layer IV, may be a thalamic recipient (see B.A. Peterson and J.A. Winer, this volume).

This research was supported by United States Public Health Service Grant RO1 NS16832-08. We thank J.M. Popowits for technical assistance.

201.13

INTRINSIC CONNECTIONS OF CAT PRIMARY AUDITORY CORTEX , M.N. Wallace, H.D. Schwark and E.G. Jones. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Small injections of horseradish peroxidase (HRP) and the anterogradely transported lectin extracted from Phaseolus vulgaris (PHA-L) were made at different depths of the AI cortex. The HRP was injected iontophoretically using currents of 1-2 uA for 1-2 hours and after a survival time of 3-5 days activity was demonstrated using cobalt intensified diaminobenzidine. The PHA-L was injected using a current of 5 uA for 15-20 min. and pipette tips of less than 20 um. Larger tips mainly produced retrograde labelling.

The organization of intrinsic axons was demonstrated with PHA-L and labelled fibers were mainly found in two or three short bands. One band was centered on the injection site and contained clusters of denser terminal labelling at intervals of about 500 um. The other band or bands lay at a distance of 1-1.5 mm. and were roughly parallel to the principal band. The bands of labelling extended through all six layers of the cortex and were oriented obliquely to the coronal plane. Clusters of retrogradely labelled cells were observed at distances of 1-4mm. Most pyramidal cells were densely covered in spines and some cells also appeared to have spines on the axon hillock. However in the case of a few intensely labelled pyramidal cells there was no evidence of any spines. The proximal dendrites were completely smooth and the fine distal branches had a beaded appearance. Supported by NIH Grant NS 25674.

201.14

DESCENDING CORTICAL AUDITORY PATHWAYS IN A PRIMATE. R.E. Hunter,* A.M. Thompson and G.C. Thompson. Dept. of Otorhinolaryngology & Communicative Sciences and Program in Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The anterograde tracers HRP and PHA-L were utilized to elucidate descending pathways from auditory cortex of the lesser bush baby (Galago senegalensis). To determine the maximum extent of the descending projections, large pressure injections of HRP were made into auditory cortex of one animal. Subsequently, to demonstrate the distinct components of those projections and to label their terminal endings, PHA-L was iontophoretically injected into auditory cortex of 3 animals.

Upon light microscopic examination of brain sections from the HRP case (detected with TMB and BDHC), retrogradely labeled cell bodies were observed in the ipsilateral medial geniculate body (MG) and nucleus limitans and in contralateral auditory cortex (layers I and II). Anterogradely labeled terminals were observed in ipsilateral MG and the external, pericentral, and dorsomedial central nucleus of the inferior colliculus (IC).

Examination of the PHA-L cases (detected using immunohistochemical techniques) revealed labeled fibers and terminal-like varicosities in nucleus limitans, ventral division of MG, and the external, pericentral, and dorsomedial regions of IC. Localized regions of labeled terminals were uniquely related to the location of the injection site indicating a specific and finely organized projection system descending to auditory targets.

201.15

RESPONSES OF PERIPHERAL AMPHIBIAN NERVE (*XENOPUS LAEVIS*) TO ELECTRICAL STIMULATION. P.M. Backoff*, B.M. Clopton* and S.C. Bledsoe.(SPON: D. Anderson). Kresge Hearing Research Inst., U. of Mich., Ann Arbor, MI 48109

Responses to direct electrical stimulation were recorded from nerve fibers in the lateral line of the amphibian *Xenopus laevis*. Spontaneous activity had been eliminated by severing the nerve at the stitch, allowing an unambiguous determination of the response threshold to continuous electrical sinusoids. Threshold and suprathreshold spike counts were obtained, and spike time patterns(PSTH) collected to determine the temporal limits for the responses as a function of frequency. The frequency response (threshold tuning curve) of the fibers was very similar to that reported for mammalian auditory nerve fibers, with maximum sensitivity between 73 and 171 Hz for the sinusoids. Dynamic ranges, measured from spike count as a function of stimulus intensity at any particular frequency, varied between fibers, but were generally small, reaching saturation within 1 to 4 dB above threshold. Neuronal responses were strongly phaselocked to all the stimulating frequencies from 49-1221 Hz at very low stimulus levels. (Supported by NIH Grant NS21440)

202.1

EFFERENT PROJECTIONS FROM THE OCULOMOTOR REGION OF THE MACAQUE FASTIGIAL NUCLEUS, AS STUDIED BY ANTEROGRADE TRANSPORT OF HRP. S. Sugita, H.Noda, Y. Ikeda* and J. Paallysaho*. Dept. Visual Sci. Indiana Univ. Bloomington, IN 47405.

Efferent pathways from the tail of fastigial nucleus, which receive projections from vermal lobules VIc and VII, were studied. HRP injection site was determined by distribution of retrogradely labeled Purkinje cells. In one of 3 monkeys, in which effective sites were confined to caudal fastigial nucleus, labeled Purkinje cells were found only in the ipsilateral oculomotor vermis.

The majority of fibers from the fastigial oculomotor region crossed the midline and changed the direction to ventrolateral and entered the uncinate fasciculus. After reaching the brachium conjunctivum, most fibers crossed over it and entered the brainstem nuclei; such as LVN, IVN, MVN, PPRF in an area anterior to the abducens N, and RF immediately ventral to IVN. Some fibers advanced also to the lateral part of RF ventral to LVN. Clear terminals were found in the medial portion of the NRTP. Some crossed fibers terminated also in the superior colliculus and thalamic VPL nucleus. Uncrossed fastigial fibers (30 %) were traced to the four ipsilateral VN via the juxtarestiform body, and clear terminals were found only in LVN. There were retrogradely labeled neurons in the N. prepositus hypoglossi but no labeled terminals were identified in the nucleus.

202.2

CONVERGENCE OF TERMINAL ZONES OF THE CEREBELLOPONTINE SYSTEM WITH OTHER AFFERENT PROJECTIONS TO THE BASILAR PONTINE NUCLEI OF THE RAT. H.S. Lee, S.A. Azizi, and G.A. Mihailoff, Dept. of Cell Biology and Anatomy, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235

The role of the basilar pontine nuclei (BPN) as an important connecting link between the cerebral cortex and the cerebellum has been widely accepted. Moreover the cerebellum, via projections from the deep nuclei to the BPN, has the opportunity to influence the activity of pontine neurons and thus modify in some way a portion of the mossy fiber input to the cerebellar cortex. The present anatomical studies were undertaken to analyse the overlap between cerebellopontine (CBP) terminal zones with other pontine afferents in the rat BPN. Although previous reports identified some degree of overlap between terminal zones of CBP and corticopontine projections in the opossum, the rat, and the cat by comparing the right and left sides of a rostro-caudal series of BPN sections, the present study employed two different anterograde tracers, *i.e.*, PHA-L injection into the deep nuclei and WGA-HRP injection into other pontine afferent sources such as the sensorimotor, visual, or auditory cortex, or the superior colliculus, thus permitting a more precise visualisation of the overlap between CBP terminals and other pontine afferents in the same BPN section. Moreover, overlap between the terminal zones of CBP afferents and the projections of various sensorimotor regions was localised within discrete portions of the medial, ventral, and lateral BPN. On the other hand, in the lateral BPN, inputs from visual cortex, auditory cortex, or the superior colliculus exhibited the same terminal areas as those from the deep cerebellar nuclei. Preliminary single-unit recording indicates that some basilar pontine neurons receive convergent cerebral cortical and deep nuclear input, while ultrastructural studies are in progress to confirm that single BPN neurons are contacted by synaptic boutons from both of these afferent systems. Supported by USPHS grant NS 12644.

202.3

IS THERE A RECIPROCAL CONNECTION BETWEEN RED NUCLEUS AND INTERPOSED CEREBELLAR NUCLEI IN RABBIT? M.E. Rosenfield and J.W. Moore. Dept. of Psychology, Univ. of Mass., Amherst, MA 01003.

Dry WGA-HRP (Sigma L3892, formerly L9008) was implanted by pipette into the right cerebellar interpositus (IP) nucleus in six albino rabbits (method of Mori, J, et al, *Brain Res Bull*, 6:19, 1981) and left *in situ* for 43-52 hours before sacrifice by pentobarbital overdose. Animals were perfused transcardially (descending aorta clamped) with approximately 2 L of .9% saline followed by equal volumes of 10% formalin and 12% sucrose solution at 4 degrees C. Brains were blocked immediately on extraction (saving only the brain stem and cerebellum), placed in 30% sucrose in .1 M phosphate buffer (pH=7.2), and stored for 24 hours at 4 degrees C for 24 h. Brain stem and cerebellum were embedded in gelatin; frozen sections were cut transversely at 60 μ, mounted on subbed slides, and reacted with tetramethylbenzidine (TMB). Brightfield and darkfield microscopic examination of sections revealed fiber and terminal labeling of contralateral red nucleus (RN) but virtually no retrograde cell labeling. Retrograde cell labeling was observed in cerebellar cortex (Purkinje cells) and/or inferior olivary nuclei in all cases. With identical methods, two animals had WGA-HRP implanted into the left RN. These cases showed substantial retrograde labeling of cells in right IP nucleus but no evidence of terminal labeling (as distinct from cut fibers of passage). These findings are in accord with a study of reciprocal IP-RN connections in cat by Walberg and Dietrichs (*Brain Res*, 397:73, 1986) which also used the Mori et al procedure of implanting WGA-HRP. The evident absence of a RN-IP projection is relevant for studies of neural circuits underlying classical conditioning of the rabbit nictitating response (Yeo, C H, et al, *Exp Brain Res*, 60:114, 1985).

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202.4

ANATOMICAL ANALYSIS OF CEREBELLAR-OLIVARY PROJECTIONS IN THE RABBIT. C. Weiss, G. Tocco *, J. K. Thompson and R.F. Thompson. Dept. Psychol. Univ. Southern California., Los Angeles, CA. 90089-1061.

The cerebellum is critical for eyeblink conditioning and feedback to the inferior olive (IO) is likely to be important in shaping an appropriate conditioned response. Reciprocating pathways from the cerebellum to the IO have been demonstrated in both the cat and rat. One is an indirect projection via the red nucleus (RN) and dorsal column nuclei, another is a direct projection between the dentate/interpositus region and the IO. We are currently examining these two pathways in the rabbit which is a widely used subject in classical conditioning paradigms.

We injected the dentate/interpositus region with either 1% wheat germ agglutinated horseradish peroxidase (WGA-HRP, 100 nl) or 2.5% Phaseolous Leucoagglutinin (PHA-L , +5 uA, 10 min, 7 sec duty cycle). After a two day survival period rabbits injected with WGA-HRP were prepared and processed for visualization of the HRP using tetramethylbenzidine (TMB) as a chromogen. Following a two week survival period rabbits injected with PHA-L were prepared and processed for immunohistochemistry using a biotinylated secondary antibody, a peroxidase labelled avidin-biotin complex, and diaminobenzidine (DAB) as a chromogen. In other rabbits we injected either 3% Fluorogold (FG) or 1% WGA-HRP into the RN or IO to retrogradely label the cells which provide these two pathways.

Anterogradely labelled fibers from the dentate/interpositus region were observed to provide a massive input to the contralateral RN and also to return caudally and provide input to the IO. In addition, retrogradely labelled cells were found throughout the dentate/interpositus region following injections of either the RN or IO with either FG or WGA-HRP.

Details of these projections as well as results of double labelling experiments following injections of both the RN and IO on the same side, but with different labels, will be presented.

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202.5

CEREBELLAR PROJECTION FIELDS OF SPINAL CORD SEGMENTS IN THE RAT. P. S. Klinkachorn and J. L. Culberson, Department of Anatomy, WVU Health Sci. Center, Morgantown, WV 26506.

Despite the fact that both deep and cutaneous peripheral receptors influence activity of spatially defined cerebellar regions, the structural substrates mediating this somatotopy remain unclear. By injecting specific spinal segments with HRP-WGA solution, it is possible to label axons arising in these segments and to chart their cerebellar targets. Spinocerebellar tracts from all spinal segments studied reach anterior lobe and lobule VIII of posterior lobe; they generate a series of parasagittal bands across the mediolateral width of the lobules. Thoracic segments generate the densest projections to anterior lobe; these are heaviest to medial bands in lobules II-III and to lateral areas in IV-V. Less dense but well defined bands are labeled by lumbar and sacral injections. Projections from cervical segments are more diffuse and less dense than those from other levels. While differences in segmental projection patterns are seen, it is difficult to see how these differences correlate with somatotopic maps of the cerebellar cortex. (Supported by grants from WVU Med. Corp. and NIH RR05433).

202.6

THE LATERAL RETICULAR NUCLEUS OF THE RAT: PROJECTION TO THE CEREBELLUM. A COMPUTER RECONSTRUCTION STUDY. S.A. Azizi, A.J. Painchaud*, S. Askari*, D.J. Woodward. Dept. of Cell Biology, U. T. Southwestern Medical Ctr., Dallas, TX 75235

Ongoing investigation in our laboratory has involved the organization of the brainstem nuclear projections to the cerebellum. This study was undertaken to determine the detailed projections of the lateral reticular nucleus [LRN] to the cerebellar cortex in the rat. Small quantities of (.01 to .2μl) of HRP-WGA were pressure-injected into discrete areas of the cerebellar cortex in 130 rats. The sensitive chromogen, tetramethyl benzidine, was used to visualize labelled neurons.

Results of this investigation demonstrated that the point-to-point, nucleotopic projection system observed in the olivocerebellar and pontocerebellar systems does not exist in the reticulocerebellar system [RCS]. However, the RCS does exhibit discrete patterns in the density of labelling. Neurons in the parvicellular and subtrigeminal regions of the LRN project heavily to the anterior lobe and intermediate zones of the posterior lobe of the cerebellum [CB]. CB posterior midvermal lobules VI & VII receive sparse projections. Neurons in the magnocellular region of the LRN project mainly to the lateral hemispheres of CB, although a contribution to vermal anterior and posterior lobes was noted. These projections are bilateral.

Thus, in comparison to olivary and pontine projections, which originate and distribute in distinct patterns in the cerebellar cortex, axons of LRN seem to influence broader areas of cerebellar cortical circuitry. *Supported by NIDA 2338 and the Biological Humanics Foundation*

202.7

SOMATIC AFFERENT TERMINATION ON SPINES IN THE CAT MEDIAL ACCESSORY OLIVE. H.H. Molinari and K.A. Starr*. Department of Anatomy, Albany Medical College, Albany, N.Y. 12208.

In the caudal half of the medial accessory olive (cMAO), spines constitute the primary target of afferents from the gracile nucleus (Molinari and Sluyters, 1987). The present study examined the other major source for cMAO of ascending somatic information from the hindlimb, the lumbosacral spinal cord, and found a similar preferential termination on dendritic spines. The somatic terminals in cMAO were labeled, as in Molinari (Exp. Brain Res., 1987), by anterograde transport of wheat germ agglutinin-horseradish peroxidase and visualized for EM with tetramethylbenzidine.

Spines in cMAO vary widely in size and shape. Although somatic afferents sometimes synapsed on the stalks of these spines, the vast majority synapsed on spine heads. The spine heads participated in either spine clusters or glomeruli but to date none have been shown to form gap junctions (gap junctions between other elements have been observed). Thus, these data raise the possibility that in cMAO the spines influenced by somatic afferents may not be involved in electrotonic coupling. The alternative explanation that the site of coupling is some distance from the somatic synapse is presently under investigation.

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202.8

GABAERGIC COMPONENTS OF THE MAMMALIAN IO. B.J. Nelson, E. Mugnaini, J.C. Adams, N.H. Barmack. Lab of Neuromorph, U Connecticut, Storrs, CT 06268; Med U South Carolina, Charleston, SC 29425; NSI, 1120 NW 20th, Portland, OR 97209

The cytoarchitecture and climbing fiber topography of the IO is generally conserved across mammalian species, but the homology of afferent projections is less clear. Immunocytochemical detection of GAD, a marker for GABAergic neurons, was used to compare the distribution of GABAergic nerve terminals and cell bodies in the IO of rat, rabbit, cat, rhesus monkey and human. Frozen sections obtained from zinc-aldehyde fixed brainstems were immunoreacted with the GAD antiserum of Oertel et al. (1981) by the PAP method. The IO of each species contained an extremely high density of GABAergic boutons, but regional variations of intensity were apparent, and may indicate different afferent sources. The heterogeneous pattern of GAD immunostaining was strikingly similar for all species. Staining differences between some regions, such as the beta nucleus and the rostral MAO, were due to differences in GABAergic bouton size. The distribution of GABAergic somata within the IO was determined for each species, since they are a potential source of GABAergic boutons. A low density of small GAD-positive neurons was present in the IO of all species, and no distinctive phylogenetic progression in their number was observed. Therefore, the vast majority of GABAergic boutons in the IO of all the species investigated must have extraolivary origins. [Supported by PHS grant NS-09904.]

202.9

TOPOGRAPHICAL ORGANIZATION OF INFERIOR OLIVARY PROJECTIONS TO DEEP CEREBELLAR NUCLEI IN MACAQUE. Y. Ikeda*, S. Sugita, H. Noda and Y. Paallysaho*. Dept. Visual Sci., Indiana Univ., Bloomington, IN 47405.

Origins of IO projections to caudal fastigial nucleus (FN) and posterior interposed nucleus (PIN) were studied by retrograde HRP. Effective site in each monkey was determined by mapping retrogradely labeled Purkinje cells (P-cells). When HRP was localized in the fastigial oculomotor region, as evidenced by labeled P-cells confined in lobules VIc and VII, retrogradely labeled IO neurons were found in subnuclei b and lateral part of c of MAO, but caudal end of MAO was free of labeled IO cells. Labeled IO neurons were located also in caudal DAO. When HRP was placed in the ventral part of caudal FN, with labeled P-cells distributed in lobules VI, VII, VIII and IX, labeled IO neurons were located more caudally in MAO, including a large portion of subnucleus c. When HRP covered caudal half of FN, indicated by labeled P-cells distributed in lobules V - IX, labeled IO neurons were found in the caudal half of MAO and a few neurons scattered also in caudal DAO. When HRP was in medial PIN, with positive P-cell distribution in vermal lobule IV - IX and paravermal lobule IV - VIII, labeled IO neurons were found in subnucleus c, central MAO and caudal DAO. When HRP was injected in lateral PIN, with P-cells in paravermal lobules II - IX, labeled IO neurons were found in rostral halves of MAO and DAO.

202.10

THE DISTRIBUTION AND ORIGIN OF SEROTONINERGIC AFFERENTS TO THE CAT'S CEREBELLUM. G.A. Bishop, S. Dibert* and R.H. Ho. Dept. of Anatomy and Neuroscience Program, The Ohio State University, Columbus, Ohio 43210

Serotonin (5HT) has been shown to have a heterogeneous lobular and laminar distribution in the cerebellum of the rat and opossum (Bishop & Ho, Brain Res.331:195, 1985; Bishop et al., JCN 235:301, 1985). In the present study the PAP technique was used to determine the distribution of 5HT in the cat; these data are compared to those found in other species. The origin of 5HT to the cat's cerebellum was determined using a double labeling technique. In the cat, 5HT afferents form a beaded plexus of fibers, morphologically distinct from mossy fibers, that is confined to the granule cell and Purkinje cell layers. This differs from the distribution of 5HT in the rat, in which it is present in the molecular layer, in some folia. However, this pattern of laminar distribution is identical to that seen in the opossum. As in other species, there is not a uniform lobular distribution. All lobules contain 5HT except lobule X in which only scant labeling is present. The densest distribution of 5HT is to the bases of most folia; fewer fibers are seen at their apices. The cells of origin of this beaded plexus of 5HT fibers are located in the reticular formation; few double labeled cells are present within any of the raphe nuclei. These data indicate that: 1) the laminar and lobular distribution of 5HT is unique from that described in other species; 2) there are neurons in the cat's reticular formation whose axons terminate within the cerebellum as a beaded plexus of fibers; these double labeled reticular cells intermingle with retrogradely labeled cells that are not serotoninergic; and 3) 5HT may not influence cerebellar circuits uniformly based on its differential lobular and laminar distribution. (Funded by NIH 18028 and the Roessler Foundation).

202.11

COEXISTENCE OF CORTICOTROPIN RELEASING FACTOR AND ENKEPHALIN IN CEREBELLAR AFFERENT SYSTEMS. Sharon Cummings and James S. King. Dept. of Anatomy and Neuroscience Program. Ohio State University, Columbus, OH

The distributions of corticotropin releasing factor (CRF)- and enkephalin (ENK)-containing climbing fibers and mossy fibers were found to be coextensive within several regions of the cerebellum of adult and pouch young opossums. In order to determine if CRF and ENK coexist within the overlapping populations, single, 15um sections of opossum cerebellum were incubated simultaneously with primary antisera to CRF and ENK generated in different species, and processed for fluorescence immunohistochemistry. Within the vermis and hemispheres of 70mm pouch young animals, CRF and ENK were found to coexist in climbing fibers in the mid stage of differentiation and in developing mossy fiber rosettes. Each peptide is also found alone in each developing fiber type. Within the vermis of adult animals, climbing fibers are immunoreactive for CRF, or demonstrate colocalization of CRF and ENK. Mossy fibers within the adult vermis are immunoreactive for CRF alone, for ENK alone, or demonstrate colocalization of CRF and ENK. CRF and ENK also coexist in beaded fibers within the deep cerebellar nuclei. It has been reported that CRF and ENK coexist within cell bodies of the inferior olivary complex, the origin of climbing fibers (Cummings and King, Anat. Rec. 220:27A, 1988). Examination of alternate 5um serial sections of brainstem tissue from opossums pretreated with colchicine and processed for CRF or ENK peroxidase immunohistochemistry also revealed that these peptides are coexistent within the lateral reticular nucleus, a source of mossy fibers. These findings support the concept of chemical heterogeneity within the two major afferent systems to the cerebellum. (NS-08798)

202.12

TEMPORAL EXPRESSION OF CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY IN THE ANTERIOR LOBE OF THE DEVELOPING CEREBELLUM. J. S. King, R. H. Ho and G. A. Bishop Department of Anatomy and Neuroscience Program, The Ohio State University, Columbus, Ohio 43210.

Cholecystokinin (CCK)-like immunoreactivity is present in mossy fiber rosettes throughout the adult cerebellum (King et al, '86, Neurosci. Abstr. 12:461). In the present study we employed the PAP method to localize CCK in the developing opossum cerebellum. Immunoreactive CCK fibers are present by postnatal day (PD) 7. By PD 11 transversely oriented fibers are evident primarily in medial areas of the cerebellar plate. At PD 25 four sagittal bands of punctate immunoreactivity are present in the anterior lobe vermis. By PD 60 only two sagittal bands of CCK positive elements remain in lobules IV and V. These bands contain immature mossy fiber rosettes and focal areas of punctate immunoreactivity which circumscribe the somata of Purkinje cells. In lobules II and III four bands of immature mossy fiber rosettes are present. By PD 70 the punctate staining in sagittal bands seen at PD 60 is diminished. Purkinje cells in lobules IV and V are circumscribed by CCK fibers that resemble the capuchin stage of climbing fiber development. Mossy fiber rosettes are found throughout the anterior lobe at this age. By PD 80 CCK climbing fibers are rare in the anterior lobe, and the adult pattern of mossy fiber distribution is present. These data indicate that: 1) CCK is expressed in cerebellar afferents later in development than serotonin (Bishop et al, '85, Anat. & Embryol. 171:545) or enkephalin (Walker et al, '87, Neurosci. Abstr. 13:1262); 2) the time course for the development of mossy fiber rosettes is not uniform in the anterior lobe; and 3) there are transient climbing fibers in the anterior lobe that do not mature beyond the capuchin stage of development. (Supported by NS-08798).

202.13

IONTOPHORETIC EFFECTS OF SIGMA LIGANDS ON RUBRAL NEURONS. R.R. Matsumoto and J.M. Walker. Department of Psychology, Brown University, Providence, RI 02912.

High concentrations of sigma receptors are found in the red nucleus (RN), an area involved in posture and movement. Microinjection of sigma ligands into the RN of rats has been shown to produce abnormal postural changes (dystonia). DTG has marked effects while (+)-pentazocine has similar, but weaker actions. (+)-3PPP produces mixed effects. In this study, the responses of rubral neurons to iontophoretic applications of DTG, (+)-pentazocine and (+)-3PPP were investigated in the rat.

DTG had consistent inhibitory effects on rubral neurons. Dose-dependent inhibitions were observed in cells with constant spike amplitudes. Similar effects were seen with (+)-pentazocine although a smaller percentage of cells responded with inhibitions. (+)-3PPP produced mixed effects: both inhibitions and slight excitations were observed.

These data suggest that sigma receptors may be associated with abnormal postural changes resulting from inhibition of RN activity. The data also suggest that the sigma ligand, (+)-3PPP, may have complex actions, a finding that is consistent with the reports of other investigators.

202.14

MULTIPLE INNERVATION OF PURKINJE CELLS BY CLIMBING FIBERS AND OLIVOCEREBELLAR INTERLOBAR BRANCHING. A. Rosina*, L. Provini* and S.Morara* (SPON: European Neuroscience Association)Ist. Fisiol. Centri Nervosi CNR and Ist. Fisiol. Gen. Chim.Biol., Fac.Farmacia, 20131 Milano,Italy.

A neonatal stage of multiple innervation of Purkinje (Pk) cells by climbing fibers (Crepel et al.,J.Neurobiol.7,567, 1976) precedes the one-to-one relationship typical of the adult mammal. The regression of the redundant synaptic contacts is not due to cell death but to retraction of axon collaterals by the inferior olive (IO) neurons. A likely candidate for the redundant neonatal connectivity is an increased rostro-caudal interlobar collateralization which is known to be related to the functional somatotopy (Rosina and Provini, Brain Res. 289, 45, 1983).

The interlobar branching pattern of the olivocerebellar fibers was analyzed in neonatal kittens by applying retrograde fluorescent techniques. It was found that interlobar branches are indeed more numerous - by a factor of about 2 in terms of doubly projecting neurons - in the first postnatal week. Moreover, the somatotopic distribution of IO axon collaterals present in the adult is not yet settled:it is proposed that the retraction of the redundant IO interlobar collaterals contributes to the somatotopic refinement.

202.15

VISUAL INPUT TO THE CEREBELLUM OF MACAQUES. M. Glickstein, B. Mercier, and J. Baizer. Dept of Anatomy, University College London, Gower Street, London WC1 England.

The cerebellum plays a major role in the visual guidance of movement. What are the anatomical pathways by which visual information is relayed to the cerebellum? The pontine nuclei are known to receive an input from extrastriate visual areas and the superior colliculus, and axons of pontine cells relay visual information to the cerebellar cortex as mossy fibres. In the present experiments we studied the distribution of efferent projections to the pons following small injections of wheatgerm agglutinated horseradish peroxidase (WGA HRP) or tritiated amino acids into extra striate cortex or the superior colliculus. Each of the injections resulted in multiple patchy terminal fields, largely non-overlapping, principally within the dorsolateral quadrant of the pontine nuclei. Injection of WGA HRP into the flocculus/paraflocculus resulted in retrogradely labelled cells in the same region of the pontine nuclei. The evidence suggests that visual information is distributed widely on the cerebellar cortex, but as in rats and cats, the dorsal paraflocculus is a major target of pontine visual cells in monkeys.

202.16

NUCLEOCORTICAL CLIMBING FIBER DEMONSTRATED BY PHA-L METHOD IN THE CAT. F. Murakami and W.-J. Song*, Dept. of Biophysical Eng., Fac. of Eng. Sci., Osaka University, Toyonaka 560, JAPAN

It has been generally accepted that cerebellar nuclei neurons send mossy fibers to the cerebellar cortex. This is mainly based upon the observations that injection of tritiated amino acids into the cerebellar nuclei failed to label fibers in the molecular layer. We injected PHA-L into the interpositus nucleus of the cat and found labelled climbing fibers in the cerebeller cortex. PHA-L was injected iontophoretically into the interpositus nucleus of the kitten and adult cat using glass micropipettes with diameters of 15 μm - 35 μm. Survival times ranged from 5 - 14 days, the cerebellum was cut at 70 μm and processed for immunohistochemistry. Labelled axons were seen to course the white matter towards the cortex. Terminal-like axons were seen both in the granular and the molecular layer. The former often formed rosette-like structure and the latter exhibited tree-like structure with extensive varicosities. Injection of PHA-L into the white matter did not result in labelling of axons in the cerebellar cortex. These results suggest that interposed nucleus is the second source of climbing fiber.

202.17

ZEBRIN II: A NOVEL ANTIGENIC MARKER OF CEREBELLAR COMPARTMENTATION IN FISH AND RAT. Gino Brochu (a), Len Maler (b), and Richard Hawkes (a): a) Department of Biochemistry and Laboratory of Neurobiology, Laval University, Québec, P.Q. b) Department of Anatomy, University of Ottawa, Ottawa, Ont.

Monoclonal antibodies (mabs) have revealed an elaborate topographical organization in the mammalian cerebellar cortex. To extend these studies to lower vertebrates, adult Balb/c mice were immunized with an homogenate from the cerebellum of the weakly electric fish *Apteronotus leptorhynchus*, lymphocyte hybridomas were constructed by conventional methods, and secreted mabs were screened by peroxidase immunocytochemistry on frozen sections of rat cerebellum. One mab recognized a novel polypeptide, zebrin II. On Western blots of rat cerebellar proteins mab zebrin II recognizes a single band at apparent M.W. 36 Kd. Immunocytochemistry of adult cerebellar cortex reveals that zebrin II distinguishes between two subsets of Purkinje cells that are arranged in parasagittal bands reminiscent of the organization of cerebellar afferent axons. The zebrin II bands are co-extensive with antigenic bands of Purkinje cells stained by using mabQ113 (anti-zebrin I).

Selective staining of a subset of Purkinje cells is also seen in *Apteronotus*. Immnunoreactivity was confined exclusively to a subset of Purkinje cells located in the corpus cerebelli, the eminentia granularis (EGa) and the ventromedial part of the valvula. Purkinje cells in other regions are unreactive. Western blotting shows a single antigenic polypeptide apparently identical to that in the rat. Purkinje cells are completely labelled, including their dendrites and axons. As previously suspected Purkinje cells of the corpus and valvula cerebelli project locally to nearby eurydendroid cells. Purkinje cells of EGa project down to the acousticolateral region terminating primarily in the dorsal nucleus.

Given that it is confined to Purkinje cell subsets both in fish and rat, zebrin II may prove a valuable marker of cerebellar compartmentation throughout the vertebrates, and a useful probe for developmental and comparative studies.

202.18

TRANSIENT EXPRESSION OF ENKEPHALIN-LIKE IMMUNOREACTIVITY IN CLIMBING FIBERS OF THE DEVELOPING CEREBELLUM. J. J. Walker, R. H. Ho and J. S. King. Department of Anatomy and Neuroscience Program, The Ohio State University, Columbus, Ohio 43210

In the adult opossum's cerebellum, enkephalin (ENK) immunoreactive climbing fibers are confined primarily to vermal folia and the flocculus (King et al., '86, J. Neurocytol. 15:545). However, during development, ENK climbing fibers have a more widespread distribution in the cerebellar cortex (Walker et al., '87, Neurosci. Abstr. 13:1262). In the present report we used the PAP technique to study the transient expression of ENK in developing climbing fibers of the lateral cerebellar folia between postnatal day (PD) 18 and 83. Between PD 18 and PD 68 immunoreactive climbing fibers have reached the mid stage of development and distribute extensively in the paramedian lobule, parafloccullus, hemispheric parts of lobule IV and V, lobus simplex and crus I and II. By PD 77 climbing fibers have reached the molecular layer and show a marked decrease in the intensity of immunostaining. They are evident only in restricted foci of Crus I, the parafloccullus and the medial part of the paramedian lobule although they appear less mature when compared to those in vermal regions. By PD 83, when the adult pattern of distribution is reached, only those fibers in the paramedian lobule and parafloccullus persist while the remainder of the lateral folia are populated by fine beaded axons which distribute along the granule cell-Purkinje cell border. These data indicate that ENK is expressed in developing climbing fibers but not mossy fibers during a prolonged period of development coincident with the major stages of Purkinje cell maturation. We hypothesize that this transient expression suggests a developmental role for this peptide in early stages of cerebellar ontogeny. (Supported by NS-08798)

203.1

ACTH PEPTIDES INCREASE ENDPLATE PARAMETERS DURING PERIPHERAL NERVE REGENERATION. L.A. Zuccarelli and F.L.Strand. Center for Neural Science, New York University, Washington Square,New York 1003.

We have shown that ACTH and its fragments accelerate peroneal nerve regeneration, improving qualitative and quantitative aspects of reinnervation of rat extensor digitorum longus (EDL) muscle 1,[2].These peptides also increase terminal branching and endplate area of the developing neuromuscular junction (nmj) [3,4]. Here we investigate the morphological response of the regenerating nmj to ACTH 4-10 and MSH (ACTH1-13):length of terminal branching, perimeter and area as determined by light microscopy and digitizing morphometry. Crush denervation is performed under 8% chloral hydrate (0.6ml/0.1kg IP) with a Dumont #5 forceps producing a 1mm wide lesion 10mm from nerve exit from the spinal cord. Peptide treatment (10 ug/rat/48 h IP) is from time of denervation to day of sacrifice (15 or 21 days after crush). The EDL is fixed in situ and prepared for light microscopy with a combined silver-cholinesterase stain. Both peptides cause significant increases in terminal branching, while MSH also increases area and perimeter as compared to saline controls.Thus both the regenerating and the developing nmj show remarkable plasticity in terms of their response to ACTH peptides. 1.Strand,F.L.& T.T.Kung(1980)Peptides 1:135-138; 2.Saint-Come C. & F.L. Strand(1985)Peptides 6(Suppl.1)77-83; 3.Rose et al.(1988) Peptides 9:151-156; 4.Frischer,R. and F.L.Strand (1988) Exp. Neurol. 100:531-541.

203.2

IMMUNOCYTOCHEMICAL CHANGES IN RAT SPINAL CORD LESIONS. C.P. Barrett*, L. Guth*, and E. Roberts (SPON. T.H. Oh) Dept. Anat., U. MD Sch. Med., Balt., MD and City of Hope Med. Ctr. Duarte, CA

After compression-induced axotomy of the rat spinal cord, regenerating axons enter the lesion along regrowing capillaries, ependymal cells, and astrocytes. This model thus allows us to test drugs that might enhance spinal cord regeneration. We crushed the cords of rats for 1 sec and inserted a subdural polyethylene tube through which we infused isotonic solutions of triethanolamine (TREA, 0.2 ml, 0.1 M, pH 7.2) alone or TREA with dehydroepiandosterone (DHEA, 0.2 ml, 0.1 M, pH 7.2) or vehicle (physiological saline, 0.2 ml, pH 7.2) at four hour intervals for two days. Two weeks postoperatively, the spinal cord was examined histologically and immunocytochemically. At two weeks histological staining revealed no discernible differences between the responses of animals treated with TREA alone and those treated with TREA/DHEA. When compared with saline controls, both treatments revealed decreased cellular infiltration and a more uniformly arranged ingrowth of ependymal cells, astrocytes and capillaries. The drug treatments also increased the phosphorylated neurofilament (PNF) reactivity of neurites and of the laminin around blood vessels. The PNF-stained neurites were especially prominent along the basal lamina of the newly formed blood vessels. These findings further support previous observations (i) that regenerating CNS neurons grow along reconstituted vascular, ependymal, and astrocytic elements and (ii) that the ingrowth of neurites and synthesis of laminin can be enhanced by treatment with simple chemical agents. Supported by NIH grant NS-21460.

203.3

NEURITE OUTGROWTH FROM RAT CORTICAL NEURONS ON ONE MICRON DIAMETER COLLAGEN FIBERS. E. Wong[1], A. Rizvi[1]*, D. Christiansen[1]*, D. Dickerman[3]*, R. Hahn[1]*, G. Zazanis[2]* H. Geller[3] and F. Silver[1]*. [1]Biomaterials Ctr., [2]Dept. of Neurosurg., [3]Dept. of Pharm., UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

Cortical neurons from fetal rats were grown on 1 um wide collagen fibers in an effort to develop an in vitro model for the study of CNS nerve regeneration. Fifty um thick collagen fibers were placed onto cover glasses. These fibers were cross-linked and then etched using collagenase. Some of the cover glasses containing the cross-linked and etched collagen fibers were subsequently stained with Sirius Red dye. On these cover glasses, one um wide collagen fibers were detected by their birefringence under polarized light. Cortical tissues from fetal rats were dissected aseptically from an etherized Sprague-Dawley rat at day 15 of gestation. These tissues were enzymatically dissociated into individual cells. These cells were allowed to grow on the 1 um wide collagen fibers in Dulbecco's Modified Eagles Medium with antibiotics at 37°C with 5% CO_2. Neurons were found to preferentially attach and send out processes along the long axes of these fibers. This result suggests the possibility of transecting neuronal axons on collagen fibers using microsurgical techniques.

Support was provided by a fellowship from the New Jersey Center for Advanced Biotechnology and Medicine.

203.4

EXTENSIVE NEURITE REGENERATION ON RAT AND HUMAN CNS TISSUE SECTIONS IN VITRO K.A. Crutcher. Department of Neurosurgery, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Embryonic sympathetic or sensory neurons have been reported to exhibit little or no regeneration when grown on mature CNS tissue sections but extensive outgrowth on PNS or embryonic CNS tissue (Carbonetto et al., '87; Sandrock and Matthew, '87). However, the CNS areas that were studied (optic nerve, spinal cord and cerebellum) contain predominantly myelinated fiber tracts. Since mature CNS white matter is a non-permissive substrate for neurite elongation (Caroni and Schwab, '88), CNS regions lacking myelin were tested for neurite growth-promoting ability. Fresh frozen coronal sections of adult rat brain or human temporal cortex were thaw-mounted directly onto 35 mm tissue culture plates. Chick sympathetic or dorsal root ganglia (E9) were then placed on the tissue sections and grown in F-12 with 5% horse serum. In some cases, NGF was added to the medium. Neurite outgrowth was assessed 2-3 days later. Explants consistently attached to tissue sections but did not attach to the untreated tissue culture plastic unless NGF was present. Extensive neurite outgrowth (enhanced by NGF) was observed on gray matter, e.g., hippocampal formation, cortex, amygdala, and hypothalamus whereas no growth was observed on white matter e.g., corpus callosum, stria medullaris, fimbria and internal capsule. In fact, explants which attached in areas where both white and gray matter were encountered avoided the white matter tracts but extended neurites well otherwise. These results support the hypothesis that CNS white matter is a non-permissive substrate for axonal growth but also show that mature CNS gray matter is a suitable substrate, at least in vitro. (Supported by NIH # NS-17131.)

203.5

APPLIED D.C. ELECTRIC FIELDS IN THE DAMAGED RODENT OPTIC NERVE B.J. Albala*, B.H. Hallas and M.F. Zanakis. American BioInterface Corporation, New York, NY and New York College of Osteopathic Medicine, Old Westbury, NY.

Earlier studies have suggested that damaged optic axons of the rat can regenerate in the presence of applied D.C. electrical fields (Soc. Neurosci. abstract 383.4, 1987). In order to more fully characterize the response to such treatment, the visual system was studied using HRP. In blinded experiments, adult rats received a unilateral crush of the optic nerve, 1mm behind the orbit. The nerves were then implanted with a galvanic nerve cuff (TRAXON®) delivering 2-14μA to the tissue. The cathode was oriented 3mm distal to the lesion site, while the anode was oriented 0.5mm rostral. Control animals received similar devices, but they were rendered inactive (not delivering current). After 4 weeks, all animals were intraocularly injected with HRP, and then sacrificed 2 days later for histological examination of the visual system. The results showed that in all but one of the electric field-treated animals, HRP was present in the optic nerve distal to the lesion as well as in the contralateral optic tract. Often, the lateral geniculate nucleus and the tectum also demonstrated some, although slight HRP transport. In contrast, none of the control animals demonstrated presence of HRP distal to the lesion site. These and other ongoing experiments support the use of electric fields to promote repair of the damaged CNS.

203.6

PARTIAL RECOVERY FROM SPINAL CORD INJURY FOLLOWING APPLICATION OF D.C. ELECTRIC FIELDS IN THE RAT. M.F. Zanakis and M.J. Politis. American BioInterface Corporation, New York, NY and Spinal Cord Research Laboratory, Shaugnessy Hospital Research Center, University of British Columbia, Vancouver, BC.

Previous studies have shown that partial recovery of function can occur following the application of weak D.C. electrical fields to the damaged spinal cord. The present study examined short-term behavioral and histological alterations following contusion lesions of the spinal cord. In blinded procedures, adult rat spinal cords were contused using the weight drop method. DC stimulators (TRAXON®) were applied to the dorsal portion of the cord, orienting the cathode rostral to the lesion. Control animals received either a stimulator with the cathode oriented caudally, or an inactive one. All animals were tested weekly on an inclined plane for sensori-motor function. After 3 weeks, all animals were sacrificed and the dorsal funiculi rostral to the lesion were assessed for numbers of axons. The results showed that the cathode rostral treated animals performed statistically significantly better on the inclined plane at 3 weeks than controls. These animals also exhibited a statistically significantly greater number of axons rostral to the lesion site. Whether such treatment has an effect on growth or regeneration in the spinal cord is now being studied. Other experiments are presently assessing the effect of a delay in the electric field treatment. Investigations of increased vascular perfusion to the damaged region are currently being pursued.

203.7

STIMULATION OF NERVE REGENERATION IN VITRO AND IN VIVO USING PULSED ELECTROMAGNETIC FIELDS. BF Sisken, M Kanje*, G Lundborg*, E Herbst*, R Zienowicz*, M Orgel* and W Kurtz*. Center Biomed. Engineer., Dept. of Anatomy & Neurobiol., University of Kentucky, Lexington, KY 40506; Dept. of Zoophysiology and Dept. Orthop. Surg., Univ. of Lund, S-22362 Lund, Sweden, and Univ. of Mass./ Hershire Med. Ctr., Pittsfield, MA 01201 and Dietic Res., NY 10033.

Cultures of dorsal root ganglia from chick embryos were exposed to a 20 ms magnetic pulse, repeating at 2 Hz with an amplitude of 0.4 G. Cultures exposed for 2 hrs/day for 2 days exhibited neuritic growth scores 30-35% higher than controls. Reducing the pulse duration to 5 ms while maintaining the same electrical parameters eliminated the growth enhancement. In a crushed sciatic nerve model, rats were placed in a pulsed magnetic field (20 ms, amplitude of 3.0 G, repetition rate of 2 Hz) for 6 days, 4 hrs/day produced a 22% increase in the rate of axonal sprouting relative to controls. In a transected sciatic nerve model, nerves repaired immediately after transection and treated for 4 hours per day for 5 days gave the clinical impression of reduced neuroma formation. Gait analyses of animals with transected and repaired nerves were conducted at 35, 75, 105 and 140 per days. Animals treated immediately after transection and repair did not show a long-term gain relative to controls. However, animals whose repair was delayed for 5 days and then treated showed a sustained improvement in gait 15-20% above controls. Our results demonstrate that pulsed magnetic field treatment with the appropriate clinical regimen could become a treatment modality for various nerve injuries.

203.8

LAMININ EXPRESSION IN KAINATE LESIONED LATERAL GENICULATE NUCLEUS SUGGESTS POTENTIAL FOR REGENERATION. G. Nilaver, C.K. Meshul, L.M. Lund* and W.R. Woodward. Depts. of Neurology and Biochemistry, Oregon Health Sciences Univ. and VA Medical Center, Portland, OR 97201.

Kainate lesions in rat dorsal lateral geniculate nucleus (dLGN) eliminate geniculate neurons but spare retinal and cortical axon terminals. Light microscopic immunohistochemistry with a monoclonal anti-human laminin exhibited specific punctate staining arranged in a laminar array in the lesioned dLGN. Capillary endothelium and olfactory bulb glia were the only other CNS structures showing specific labelling. Electron microscopic examination of immunostained lesioned dLGN demonstrated specific reaction product over plasma membranes and rough endoplasmic reticulum of astrocytes, consistent with reports of laminin expression within reactive astrocytes following kainate lesions (Liesi, EMBO J: 1984, 1985). Laminin reactivity was also noted in basal lamina of endothelial cells. Plasma membranes of unmyelinated axon terminal profiles and microtubules in unmyelinated, but not myelinated, axons were also labelled. While the precise origin of laminin immunoreactivity in unmyelinated axons of lesioned dLGN is unclear, its presence suggests these terminals may be capable of forming synaptic contacts if provided with appropriate targets.

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203.9

CELL SURFACE INHIBITORS OF NEURITE GROWTH ON DIFFERENTIATED OLIGODENDROCYTES: APPEARANCE AFTER NERVE FIBER GROWTH AND BEFORE MYELINATION. P. Caroni and M.E. Schwab. Brain Research Institute, University of Zurich, Switzerland.

Differentiated oligodendrocytes and myelin in vitro are non-permissive substrates for neurite growth and fibroblast spreading, due to 35kD and to 250kD membrane-bound inhibitory proteins. Inhibitory activity is specifically blocked by monoclonal antibody IN-1, which binds to both inhibitors and to the surface of differentiated oligodendrocytes (Caroni and Schwab, Neuron 1, 85-96, 1988). We have determined the time course of appearance of IN-1-sensitive inhibitory activity and of inhibitory oligodendrocytes in the rat. In the spinal cord, inhibitory activity was absent at E16, was substantial at birth, and reached near to adult levels at P10. An analogous schedule was found in the optic nerve and no inhibitory activity was found outside the CNS or in CNS areas devoided of oligodendrocytes, e.g. in the retina. Our data are consistent with the following model: 1.) inhibitory activity in the CNS is due to differentiating (and to differentiated) oligodendrocytes, 2.) inhibitory oligodendrocytes appear when a fiber tract has completed its growth phase and before the formation of compact myelin.

203.10

MONOCLONAL ANTIBODY ENHANCES PROCESS REGENERATION OF ADULT PRIMATE RETINA IN VITRO. *M.R. Burrous and P.R. MacLeish* Laboratory of Neurobiology, Rockefeller University, New York, New York 10021

We have produced a monoclonal antibody, MR-1, directed against monkey retinal membranes which binds fixed, frozen sections of retina, and the membranes of living, dissociated retinal cells. Papain-dissociated retina from adult macaque monkey was cultured on coverslips treated with MR-1 or other substrates and maintained in L-15 medium supplemented with 10% FCS and 0.7% methocel. Immediately after plating, a variety of retinal cell types could be distinguished including photoreceptor cells with outer segments and pedicles, Muller cells, and a variety of neurons with truncated processes. Within hours, cells retracted their processes and began to show differential adhesion to substrates. After 48 hours, cells plated on conventional substrates (e.g., polylysine, laminin, collagen) showed moderate adhesion at best and rare process outgrowth. However, cells plated on MR-1 showed improved adhesion and many more cells had extended processes several microns long. After 7 days, the difference in cultures was striking. Virtually no neuronal cells were seen growing directly on conventional substrates, although the proliferating subpopulation of flat, non-neuronal cells formed the substrate for the small number of remaining neurons. In contrast, MR-1 supported the marked enhancement of process outgrowth of a large proportion of the initially-plated retinal neurons. Several distinct neuronal cell types could be identified, the vast majority of which stained with neuron-specific enolase. A much smaller population of cells stained positively with an anti-GABA antiserum. Whole cell patch-clamp recordings showed the presence of voltage- and time-dependent conductances; fast inward currents or action potentials were observed in most cells, while a minority showed a slowly developing outward current. Given the variety of cell size and morphology, and of electrophysiological properties, it is likely that several of the major sub-types of retinal cells are present in our cultures. This system allows an exploration of the cellular and molecular biology of adult mammalian CNS regeneration. Supported by EY-05201, The Klingenstein Fund, and the Javits Center Award.

203.11

INO ANTIBODY INHIBITS RETINAL REGENERATION IN RANA TADPOLES. T. Nagy and T.A. Reh, Dept. Med. Physiology, Univ. Calgary, Calgary, Alberta, T2N 1N4.

Previously, in Rana tadpoles, we demonstrated a role for the retinal pigment epithelium (RPE) in retinal regeneration via transdifferentiation to retinal neurons. Further, we demonstrated a strong association of the RPE cells with the vascular membrane prior to the onset of transdifferentiation, suggesting a role for this membrane in transdifferentiations. We have subsequently demonstrated that the extracellular matrix (ECM) component laminin is capable of inducing RPE transdifferentiation in vitro. Therefore, the vascular membrane (VM) may potentially influence transdifferentiation via its ECM. To examine this possibility we injected the INO (inhibitor of neurite outgrowth) monoclonal antibody, which recognizes the laminin-heparin sulfate proteoglycan complex, into regenerating Rana tadpole eyes. While 31 out of 32 eyes injected with control antibodies exhibited normal regeneration, only 12 out of 22 eyes regenerated normally when injected with INO. Those eyes exhibiting abnormal regeneration appear stalled at stages at, or prior to, association with the VM. These results indicate that laminin is essential for retinal regeneration in vivo. Supported by: Retinitis Pigmentosa of Canada, MRC. T.A.R. is a Sloan Foundation Fellow and and AHFMR Scholar.

203.12

EXTRACELLULAR MATRIX AND RETINAL PIGMENT EPITHELIUM (RPE) DIFFERENTIATION IN RAT EYE DEVELOPMENT. E. Espinoza-Hoffer* and T.A. Reh (SPON: R. Gellman). Dept. Med. Physiology, Univ. Calgary, Calgary, Alberta T2N 4N1, Canada.

In amphibians, laminin can induce transdifferentiation of RPE cells into neurons (Reh and Nagy, Nature 330:68, 1987). We have tested whether the same observation applies to the mammalian retina, using two protocols. In an initial series (46 cultures) we isolated RPE cells from fetal (14-18 days of gestation) or postnatal rat (1-13 days old). Single-cell suspensions were subcultured in either matrigel, laminin, fibronectin or collagen for up to 21 days. In contrast to the amphibian, there was no evidence of RPE transdifferentiation into neurons on any of these substrates.

Since an essential factor needed for transdifferentiation could be missing from the single-cell cultures we also examined the role of matrigel in whole-eye cultures. Eyes were taken from rat embryos (13-15 days of gestation). The mesenchyme was removed and the eyes were placed in 3D matrigel culture. Two or four days later the eyes were processed and analyzed in plastic sections stained with toluidine blue.

In contrast to control eyes, RPE layer cells in treated eyes began to progressively resemble the neuroepithelial cells of the retina. This result was observed in all eight series of experiments where the mesenchyme was removed. Thus, if the extracellular matrix comes in contact with the RPE cell layer, it can influence the differentiation state of RPE cells in the whole developing mammalian eye.

(Funded by the Retinitis Pigmentosa Foundation)

203.13

EXOGENOUS NERVE GROWTH FACTOR ENHANCES THE REGENERATION OF RAT SCIATIC NERVE. **A Derby*, W. Engleman*, G. Neises*, S. Rapp* and D. Roufa. G.D.Searle/CNSDR, St. Louis,MO 63198 and **Dept. of Biology, Univ. of Mo.-St. Louis, MO 63121.
Functional recovery of injured nerves is influenced by both soluble factors and structural elements present in the neurons' environment. The study reported here was aimed at evaluating the effect of pharmacological doses of NGF on sciatic nerve regeneration. A small section of adult rat sciatic nerve was removed and the nerve endings are inserted into a 10mm section of silastic tubing. The tube was prefilled with either 60µg NGF or Cytochrome C and neuronal regeneration within the tube was followed histologically. In NGF treated animals, regenerates fixed 2-4 weeks after surgery exhibit greater numbers of myelinated and unmyelinated fibers as determined by quantitative analysis performed at the light and electron microscopic levels. During this period, there is a 60-80% increase in the number of myelinated fibers, and at two weeks there is an eleven fold increase in the number of unmyelinated axons in the regenerates. These effects on neuronal regeneration appear to part of a more general enhancement in cytoarchitecture that is observed in the regenerate. This observation suggests that in addition to its neurotrophic functions NGF may also affect non-neuronal cells (i.e., fibroblasts, schwann cells and macrophages) that are participating in this regenerative process. These cells are involved in reconstructing the environment that is needed for axonal regeneration and supply neurotrophic substances.

203.14

INVOLVEMENT OF LAMININ IN NERVE REGENERATION IN VIVO. B. Toyota*, S.Carbonetto and S.David, (SPON: D.G. Lawrence). Neurosci. Unit, Mont.Gen.Hosp.Res.Inst. and McGill Univ. 1650 Cedar Avenue, Montreal, Canada.
Laminin (LAM), a major glycoprotein of basement membranes, can promote neurite outgrowth in vitro. We used collagen (COL) tubes as regeneration chambers to study the influence of (i) LAM, and (ii) a monoclonal antibody (mAb) 3A3 (Turner et al. J. Cell Biol. 105:137a, 1987), against a LAM/COL receptor,on nerve regeneration.

COL tubes (2cm; American Biomaterials) with distal ends sealed, were sutured to the proximal stump of transected sciatic nerves in adult SD rats, which were divided into 3 groups. Group I: COL tubes soaked in LAM/PBS and filled with PBS (n=4). Group II: COL tubes soaked in LAM/PBS (370µg/ml) and filled with PBS (n=4). Group III: COL tubes soaked in LAM/PBS (370µg/ml) and filled with mAb 3A3 (2mg/ml) (n=4). At 8 day intervals the tubes were reinjected: groups I & II with PBS and group III with mAb 3A3 (2mg/ml). After 25 days, longitudinal sections were labeled with anti-neurofilament antibodies by immunofluorescence and the length of axon growth measured.

The average length of axonal regeneration from the proximal stump was: Group I = 3.6 mm ± 0.3; Group II = 4.4 mm ± 0.6; Group III = 2.9 mm ± 0.5. These results suggest: (i) that exogenous LAM enhances nerve regeneration in vivo by 20% over COL alone, and (ii) this effect is mediated, in part, by the LAM/COL receptor recognized by the mAb 3A3.

203.15

MECHANICALLY PRODUCED PARTIAL BLOCK OF AXONAL TRANSPORT IN REGENERATING AXONS. R.E. Snyder*, R.S. Smith, and H. Chan*. Department of Applied Sciences in Medicine and Department of Surgery, University of Alberta, Edmonton, Canada T6G 2G3.

We describe studies in which the method of removal of the perineural sheath from a regenerating sciatic nerve exerted a profound influence upon the axonal transport of protein and organelles at and distal to the junction between parent and daughter axons.

Sciatic nerves of *Xenopus laevis* were crushed at mid-thigh and allowed to regenerate for 21-27 d. The axonal transport of a pulse of ^{35}S-methionine labelled protein was examined in an *in vitro* dorsal root ganglion-sciatic nerve preparation using a position-sensitive detector and liquid scintillation analysis. The perineural sheath was removed from some preparations. The transport of vesicles and other organelles was detected in individual axons by computer-enhanced microscopy.

Nerves with intact perineural sheaths, or in which the sheath was slit longitudinally, or was removed following longitudinal slitting exhibited radiolabel transport characterized by a uniform 2-5 times increase in the deposition of label in the daughter axons and junctional region compared to the parent nerve. In contrast, removal of the sheath by eversion without pre-slitting resulted in deposition in the junctional region of 70 ± 7% (±SEM) of the label that entered the region and a monotonically decreasing deposition of label in the daughter axons. Computer-enhanced microscopy of similar preparations showed bidirectional transport of organelles in both the parent and daughter axons. Individual axons showed a mean loss of anterograde organelle traffic of 73.5 ± 8.1% (±SEM) across the parent-daughter axon junction and a gain in retrograde traffic of 299 ± 67%.

Ultrastructural features of the junction did not depend upon the method of removal of the sheath. No structural damage was detected in the axolemma or the cytoskeleton. Membrane-bounded organelles and 10 nm granules were concentrated in the junctional region. We conclude that a mechanically-produced deficit that partially blocks axonal transport in individual regenerating nerve fibers can occur at the junction of parent and daughter axons. (This work was supported by the MRC.)

REGENERATION: NERVE GUIDES

204.1

THE INFLUENCE OF ANGIOGENESIS ON RECONNECTION PRIOR TO AXONAL ELONGATION. K. Harman and J.C. de la Torre. Dept. Anatomy, Univ. Ottawa.

Before regenerating axons of transected rat sciatic nerve cross a 3mm gap, a tissue bridge (t.b.) reconnects the proximal and distal stumps. Using Long Evans Hooded rats, a polyethylene nerve guide model was used to study possible influences of: i) nerve cell bodies/target tissue, ii) extrinsic or iii) intrinsic factors on t.b. formation. Three groups, TRIPLE TRANSECTION, LIGATION and FILTER, were studied at three or nine days. Group TRIPLE TRANSECTION tested influences i) and ii). The sciatic nerve was cut in three places; the middle, proximally and distally. The nerve tissue was isolated from extrinsic factors with petroleum jelly or heat-sealed plastic discs. The petroleum jelly preparations produced a t.b. reconnection, but the heat-sealed ones did not. To test influence iii), nerve trunks in Group LIGATION were ligated proximally or distally and then transected. The nerve stumps reconnected and a network of blood vessels was observed in the t.b. In Group FILTER a 0.45 um nylon filter provided access to oxygen and other nutrients, but intrinsic cells did not produce a t.b. Nerve cell bodies, target tissue, intrinsic cells and diffusible extrinsic factors did not appear to influence t.b. formation. Our results indicate that revascularization of the nerve stumps was essential in forming the tissue bridge preceding axonal elongation.

204.2

EXTENSION OF CRITICAL AGE FOR RETINAL AXON REGENERATION BY POLYMER BRIDGES CONDITIONED IN NEONATAL CORTEX. L.S.Carman*, G.E.Schneider and I.V.Yannas*. Depts. Brain & Cognitive Sciences, and Material Sciences and Engineering, M.I.T., Cambridge, MA, 02139. (Spon.: C.M. Oman)

Axons of the hamster optic tract, transected rostral to the superior colliculus (SC), regenerate and reinnervate SC only if the lesion is made on or before postnatal day 3 (P3; P0=day of birth). Such transections after P3 result in a small amount of sprouting proximal to the lesion but axons fail to re-grow across the lesion site. We examined the effect of an artificial bridge, conditioned by temporary implantation in neonatal hamster neocortex, on regeneration of retinal axons transected after P3.

Conditioning of the bridge was based on a technique of Silver & Ogawa ('83) for harvesting astrocytes (GFAP+): a porous biodegradeable collagen-glycosaminoglycan polymer (Yannas et al.,'87) was implanted into the neocortex of P4 hamsters. In all cases the conditioned bridge was harvested on P6 or P7. Host animals received unilateral transection of the brachium of the SC at P6, P9, P14, or P21: A gap (0.5mm wide)was made in the tract by aspiration, and the conditioned implant was transplanted into it. After a 2-week survival, the contralateral eye was injected with HRP. Brains were cut and processed for visualization of the label.

Large bundles of regenerating axons grew on or through the polymer in animals with a transection on P6, and in several cases, reinnervated the SC. In control cases with non-conditioned implants, no regeneration was seen. For transections in older animals, we have not yet found SC reinnervation. Thus, conditioned polymer bridges can extend the age at which hamster retinofugal axons can regenerate. [Supported by NIH grants EY00126, EY02621. Procedures complied with NIH Publ. No. 85-23, 1985.]

204.3

INTRACEREBRAL IMPLANTATION OF SYNTHETIC POLYMER /BIOPOLYMER MATRICES. S. Woerly, R. Marchand, C. Lavallée* and R. Guidoin*. Lab. Neurobiol., Hôp. Enfant-Jésus, Centre de rech. Sci. & génie des macromol., Univ. Laval, Lab. Biomat., Hôp. St-François d'Assise, Québec.

In the past, we implanted collagen hydrogels into artificially made brain cavities as (1) tissue substitutes and (2) scaffolding substrates for neural grafts. In the present study, we designed semi-synthetic hydrogels that combine the adhesive surface properties of collagen with the mechanical stability of synthetic polymers. Micro- and macroporous hydrogels of poly (2- hydroxyethyl methacrylate) (P-HEMA) and poly (glyceryl methacrylate) (P-GMA) were prepared and coated with collagen. The porosity and surface homogeneity of hydrogels were analyzed by scanning electron microscopy (S.E.M.), and samples were implanted into the parietal cortex of adult rats. After 3 months, the implants and the surrounding tissue were taken out for histology and SEM. Macroscopically, all implants were accepted and well-tolerated. Microscopic investigations revealed that, macroporous P-GMA hydrogels with good surface homogeneity were intensely cellularized with the deposition of basal lamina (B.L.) revealed by the Jone's periodic acid methenamine silver method, S.E.M., and of collagen of type I and III by the picrosirius- polarization method. The deposition of B.L. followed the geometric pattern of the inner surfaces of the hydrogels. There was no or little encapsulation of implants, the interfaces showed a fusion between the hydrogels and the host tissue, and the beginning of tissue ingrowth. The tissue reactivity to P-HEMA hydrogels was very mild only. The use of biocompatible, porous, synthetic matrices could be the basis of a reconstructive brain surgery, involving (1) permanent, chemically inert tissue substitute (2) a lattice-surface network coated with B.L. components as potential guiding substrates for regeneration. (Supported by MRC and FRSQ).

204.4

REGENERATION CAPACITY OF THE PROXIMAL NERVE STUMP IN THE ABSENCE OF DISTAL NERVE STUMP EXAMINED IN AN IN VIVO WINDOW CHAMBER. **M.F. Humphrey, W.I. Levy, W.D. Dietrich, J. Mora. Departments of Neurological Surgery and Neurology. University of Miami School of Medicine, Miami, Florida 33136.**

We have recently described the growth characteristics of the peroneal branch of the rat sciatic nerve across a 5mm gap within a thin, glass walled, chamber which allows repeated visualization of the contents. In this study, only the proximal nerve stump was sutured into the chamber. The distal nerve stumpwas resected and the opening plugged. In each case blood vessels,axons and associated cellular elements grew into the chamber for several millimeters. The ingrowth structure was similar to that found when the distal nerve stump was also present. The mean growth rate of 0.30 ± 0.1mm/day was also similar to that in the presence of a distal stump. By 15-20 days myelination of axons occurred within the 2mm closest to the proximal entrance. Subsequently, however, the proximal growth progressively withdrew from the chamber leaving it empty by 25-30 days. This suggests that in some circumstances, the proximal nerve stump can actively regenerate for 2-3 weeks without the presence of a distal nerve stump or a ready made substrate for growth. However, maintenance of this growth does not occur.

204.5

A COMPARISON OF SALINE AND MATRIGEL AS SUBSTRATES FOR PERIPHERAL NERVE REGENERATION IN AN IN VIVO WINDOW CHAMBER. **Walter J. Levy, Martin F. Humphrey, Dalton Dietrich. Department of Neurological Surgery and Neurology, University of Miami School of Medicine, Miami, Florida 33136.**

The efficacy of Matrigel (a basal lamina extract) as a substrate for peripheral nerve growth was assessed in a chronic in vivo chamber implant. A comparison of the regeneration rate and morphology of peroneal nerve growth in Sprague Dawley rats (250g) was made between chambers prefilled with Matrigel or saline. The chamber consists of two coverslips 5mm square separated by a coverslip spacer, making a 70-120um space. It is sealed at its edges by silicone elastomer except for two 1mm ports on opposite sides, which have silicone sleeves attached. The chamber is implanted in the sectioned peroneal of the sciatic nerve, and the stump sewn in at each port. The nerve regenerates as a thin strand through the chamber, and the rate of regeneration was measured by reanesthetizing the animal, re-opening the wound, and examining the chamber under an operating microscope. Forty chambers were studied for up to 100 days. The growth rates were 0.32mm/day (saline) and 0.48mm/day (matrigel). These rates were not significantly different (p>0.05,t-test). Matrigel appears to be a suitable substrate for growth, however, this offers no clear advantage over a saline filled gap.

204.6

PERIPHERAL NERVE REGENERATION THROUGH SEMI-PERMEABLE BLIND-ENDED TUBES: EFFECT OF THE MOLECULAR WEIGHT CUT-OFF. C. Rampone *, V. Guénard *, R.F. Valentini *, P. Aebischer * (SPON: M. Epstein). Artificial Organ Laboratory, Brown University, Providence, RI 02912.

In contrast to impermeable tubes, the use of semi-permeable blind-ended tubes allow peripheral nerve regeneration in the absence of a distal nerve stump, suggesting that factors produced by the external wound environment can support regeneration (Aebischer et al. Brain Res., 456, 1988). By varying the molecular weight cut-off of the tubular membrane it may be possible to assess the relative contribution of wound healing molecules on peripheral nerve regeneration. Asymmetric polysulfone tubular membranes with a molecular weight cut-off of 10 and 100 kilodaltons (KD) were capped at one end with a compatible polymer glue. The proximal nerve stump of a transected hamster sciatic nerve was secured 2 mm within a 10 mm long tube; the distal nerve stump was fully resected and discarded. Cohorts of 6 animals were implanted for 4 weeks. Upon retrieval, all tubes contained tissue cables extending to the distal cap. The cables present at the midpoint of the 10 KD tubes were significantly smaller than those regenerated in the 100 KD tubes. The cables regenerated in the 10 KD tubes were composed of granulomatous tissue devoid of myelinated axons. In contrast, 5 out of 6 100 KD tubes showed nerve cables containing myelinated axons. These results suggest that humoral factors from the external wound healing environment with molecular weights greater than 10 KD are able to support peripheral nerve regeneration in the absence of a distal nerve stump.

204.7

FUNCTIONAL RECOVERY FOLLOWING RAT SCIATIC NERVE REGENERATION THROUGH COLLAGEN NERVE GUIDE TUBES: COMPARISON OF DIRECT ANASTOMOSIS, NERVE GRAFT, AND ENTUBULATION REPAIR. **S.J. Archibald* and R. Madison. Department of Neuropathology and Neuroscience, Harvard Medical School and Children's Hospital, Boston, MA. 02115.**

Although numerous natural and synthetic materials have been utilized to effect peripheral nerve regeneration, the preferred standard method to repair human peripheral nerves is either direct anastomosis or the use of autologous peripheral nerve grafts. The present study indicates that in terms of physiological recovery of muscle EMG activity, nerve guide tubes fabricated from Bovine Type I collagen (American Biomaterials Co., Plainsboro, N.J.) are as effective as the standard nerve graft or direct anastomosis to repair transected rat sciatic nerve.

Under deep anesthesia 40 adult male Long Evans rats (N=10 in each group) received transection of the sciatic nerve at mid-thigh level, and repair by: A) direct anastomosis, B) 4mm section of nerve reversed and rotated 180°, C) 6mm porous tube (4mm nerve gap) of standard Type I collagen, and D) 6mm porous tube (4mm nerve gap) of highly purified Type I collagen. At 4 and 12 weeks postoperatively (N=5 for each group at each time), the sciatic nerve was reexposed, stimulated, and averaged evoked EMG recordings (N=64) were made from the gastrocnemius muscle. At 4 weeks group A (575±340 uV, mean ±S.D.) was significantly different (p<.001) from groups B (68±45 uV), C (0.18±.4 uV), and D (12±10 uV). At 12 weeks there was no significant difference among the groups, with EMGs ranging from 6-10 mV. Supported by NS22404 to R.M.

204.8

PRIMATE PERIPHERAL NERVE REPAIR BY ENTUBULATION: AN ELECTROPHYSIOLOGICAL STUDY. **C. Krarup*, S.J. Archibald*, R. L. Sidman, A. Sabra*, and R. Madison (SPON: M. Moore). Harvard Medical School, Children's Hospital, Boston, MA.**

In recent years there has been increasing interest in the development of a prosthetic nerve guide as an alternative method to repair peripheral nerves. This study follows the time course of regeneration after implantation of a polylactate nerve guide tube (Allied-Signal, Inc.).

The median or ulnar nerve was sectioned at the wrist in two monkeys (Macaca Fasicularis), the proximal and distal stumps were inserted into each end of an empty nerve guide and were sutured in place with two 10/0 sutures leaving a gap of 5mm between the nerve stumps. Motor (MAP) and sensory (SAP) nerve conduction studies were performed over a period of 19 months. The MAP from the APB/ADQ muscles were first detected at 55-56 days after section with latencies of 18 and 28 msec (vs control side of 1.3-1.2 msec), and amplitudes of 0.1-0.2 mV (vs control side of 20-30 mV). Over the subsequent 17 months the latencies recovered to 130% and the amplitudes to 50% of the control.

The amplitude of the SAP recovered to 15-25% and the conduction velocity to 65-85% of contralateral control. The SAP remained dispersed with late components conducting at 6 m/sec. After regeneration SAP could be evoked in both animals by discrete tactile stimulation of the appropriate finger pad, suggesting functional reinnervation of sensory nerve receptors. The results demonstrate that following this type of entubulation repair, motor and sensory fibers reach significant levels of functional recovery following reinnervation of specific groups of appropriate target organs. Supported by NS22404 to RM.

204.9

ADSORBED HUMORAL FACTORS ALLOW PERIPHERAL NERVE REGENERATION THROUGH IMPERMEABLE BLIND-ENDED TUBES. V. Guénard *, M. Goddard *, M. Liu, P. Aebischer *. Artificial Organ Laboratory, Brown University, Providence, RI 02912.

Nerve guidance channels are useful tools to study the effect of humoral factors on nerve regeneration. We investigated the influence of acidic fibroblast growth factor (a-FGF) and ∂1-acid glycoprotein (∂1-GP), a wound healing protein, on the regeneration of rat sciatic nerves. Blind-ended impermeable silicone elastomer (SE) channels were used since they exclude from the regenerating environment humoral factors derived from the distal nerve stump and the surgical wound. The lumens of capped SE tubes were separated into 2 compartments by a 10 mm-long strip of nitrocellulose (NC) paper. The channels were soaked prior to implantation in sterile physiologic saline or DMEM with either a-FGF or ∂1-GP. The proximal nerve stump of a transected rat sciatic nerve was positioned 2 mm within a 12 mm long tube; the distal stump was fully resected and discarded. Cohorts of 6 animals were implanted for 3 weeks. Upon retrieval, all tubes contained tissue strips adhering to both sides of the NC paper and extending for an average of 5 mm. Saline filled tubes were composed mainly of blood cells, fibrin and circumferential cells, whereas a-FGF or ∂1-GP filled tubes were composed of nerve cables all containing myelinated and unmyelinated axons. Three mm distal to the proximal stump the number of myelinated axons present in the ∂1-GP channels was significantly higher than that in the a-FGF channels (2584±897 vs 790±225). These results indicate that adsorbed humoral factors are able to induce peripheral nerve regeneration in the absence of a distal nerve stump. We surmise that factors secreted by the healing wound play a significant role in peripheral nerve regeneration.

204.10

EFFECTS OF DIFFERENT TYPES OF SURGICAL INTERVENTION ON NERVE REGENERATION K.L. Gibson*, L. Remsen*, G. Strain, and J.K. Daniloff. Louisiana State University School of Veterinary Medicine, Baton Rouge, LA 70803

Functional restitution following peripheral nerve repair is far from optimal. Entubulization repair, or the implantation of nerve cuffs, has been used both experimentally and clinically. Nerve cuffs have been shown to be effective conduits for nerve regeneration across small gaps. These cuffs provide mechanical support, decrease the amount of implanted suture material required, decrease adhesions, and act as guidance channels for regenerating axons. However, no substance has yet emerged as the material of choice for this procedure. Therefore, the purpose of this investigation was to compare several different types of implants. Specifically, absorbable and non-absorbable materials were compared to delineate which substance is optimal for nerve entubulization.

Nerve cuffs composed of silicone, marlex, or vicryl were implanted to span 0.5 cm gaps in the ischiatic nerve of adult Sprague-Dawley rats. Nerve regeneration through these implants was compared to repair of the same size gap by homogenic nerve grafts. Evaluations of functional return after 10, 24, and 90 days included comparative analyses of walking tracks, electromyography, histopathology, morphometry, and a radiochemical assay of choline acetyltransferase activity. Supported by grant LSU-SVM 873 and NIH Grant NS25102.

CEREBRAL ISCHEMIA II

205.1

INFLUENCE OF GLUCOSE ON RECOVERY OF RAT HIPPOCAMPAL SLICES FROM ANOXIA. E.L. Roberts, Jr*. and T.J. Sick (SPON: D.M. Easton). Dept. of Neurology, Univ. of Miami School of Med., Miami, FL 33101.

Glucose improves recovery of synaptic transmission *in vitro* following anoxia in rat hippocampal slices (Schurr, A., *et al., Brain Research*, 421:135, 1987). The mechanisms of this recovery were studied in more detail in the CA1 region of the rat hippocampal slice. Extracellular K^+ activity (K^+o) and field potentials were recorded from stratum pyramidale stimulated orthodromically via Schaffer collaterals. Slices were exposed to normoxia (95% O_2 - 5% CO_2) and to physiological solutions containing 5-20 mM glucose, made anoxic (95% N_2 - 5% CO_2), and returned to normoxia. Increasing concentrations of glucose delayed the onset of anoxic depolarization (AD) (sudden loss of ion homeostasis) and improved recovery of synaptic transmission when anoxia duration was kept constant. However, when the duration of AD was kept constant (total duration of anoxia allowed to vary), apparent $Na^+ - K^+$ pump activity (K^+o undershoot) following anoxia was increased by elevation of glucose, but recovery of synaptic transmission was not improved. Thus, glucose improved recovery of both synaptic transmission and ion homeostasis by delaying the onset of AD. However, recovery of ion homeostasis and synaptic transmission following AD were not equally affected by glucose, suggesting that different recovery mechanisms are involved. (Supported by PHS grants NS05820, NS14325, and HL38657)

205.2

SYNERGISTIC EFFECTS OF ARACHIDONIC ACID AND GLUTAMATE ON THE INJURY OF ASTROCYTES IN CULTURE. A.C.H. Yu,* P.H. Chan (Sponsor: R.A. Fishman), Dept. of Neurology, University of California, San Francisco, CA 94143

Arachidonic acid (20:4) and extracellular glutamate (glu) contents increased significantly following cerebral ischemia. We have hypothesized that these factors play an important role in the pathogenesis of cellular edema, particularly the astrocytic swelling. One possible mechanism is that 20:4 inhibits the re-uptake of glu in cultured astrocytes and thereby exerts a synergistic effect in inducing astrocytic swelling (Yu et al, J Neurochem 47:1181, 1986). We now further study the effect of 20:4 and/or glu by measuring intracellular water space of cultured astrocytes (IWS; measured by 14C-0-methyl-glucose), lactate dehydrogenase (LDH) efflux and formation of malondialdehyde (MDA). After 4 hrs of exposure, 20:4 (0.2 mM) increased the IWS from 3.5 to 9 uL /mg protein, medium LDH from trace to 300 U/L and MDA content from 0.59 to 3 nmol/mg protein. These effects were time and dose related. Other polyunsaturated fatty acids like docosahexaenoic acid but not saturated fatty acids induced similar changes. Four hrs glu (1mM) incubation increased astrocytic IWS to 11-12 uL/mg protein and the effect was also dose and time dependent. However, 1mM glu alone did not induce release of LDH and formation of MDA in the culture. 20:4 (0.1mM) + glu (1mM) increased the IWS to 17 uL/mg protein in 4 hrs, hastened the LDH efflux, but did not further enhance the MDA formation induced by 20:4 alone. Results suggest that in the presence of glu, the injurious effects of 20:4 were enhanced. Our data indicated that 20:4 and glu injured astrocytes synergistically. (Supported by NS-14543, NS-25372)

205.3

THE EFFECT OF GABAERGIC TRANSMISSION ON ANOXIC DAMAGE TO THE HIPPOCAMPAL SLICE. I.S.Kass, A.E.Abramowicz*, G.Chambers*, J.E.Cottrell*. Anesthesiology Dept., State University of New York, Health Science Center, Brooklyn, N.Y. 11203

We have investigated the mechanism of anoxic damage using the in vitro hippocampus as a model system. Midazolam (1 uM) did not affect the size of the evoked response recorded from the CA1 pyramidal cells before anoxia and did not protect these cells against anoxic damage. A much higher dose of Midazolam (100 uM), which exceeds the amount needed to activate the benzodiazepine receptor, increased the size of the evoked response by 40 ± 8 % over its predrug level. The response in the CA1 region recovered to 27 ± 7 % of its preanoxic level after 5 minutes of anoxia with Midazolam (100 uM) pretreatment. This was significantly different from untreated anoxic slices which showed no recovery (0 ± 0 %). GABA (10 mM), an inhibitory transmitter in the hippocampus, blocked the evoked population spike in the CA1 region. GABA did not protect against anoxic damage (0 ± 0 %) in spite of its blockade of neuronal activity. We found that tissue treated with GABA has lower ATP levels at the end of 5 minutes of anoxia than untreated tissue (1.21 ± .07 vs 1.82 ± .13 nM ATP/mg dry wt). Our results indicate that blocking neuronal activity is not sufficient to protect against anoxic damage.

205.4

POSSIBLE DOPAMINERGIC ACTIONS OF MK-801. D. Corbett. Fac. of Medicine and Dept. of Psychology, Memorial Univ. of Newfoundland, St. John's, NF, CANADA A1B 3V6.

The NMDA antagonists, MK-801, ketamine and phencyclidine protect against cerebral ischemic damage. These latter two compounds are also drugs of abuse. Since many of the behavioral effects of MK-801 are similar to those of ketamine and phencyclidine the abuse potential of this compound was assessed in a brain stimulation reward (BSR) paradigm. Drugs that enhance DA synaptic transmission (e.g. amphetamine) facilitate BSR whereas drugs that impede DA synaptic transmission (e.g. neuroleptics) attenuate BSR.

MK-801 produced a significant facilitation at hypothalamic and cortical BSR electrode sites. This facilitation was blocked by concurrent administration of a DA antagonist. A rotational model was also used to investigate DA effects of MK-801. MK-801 induced an ipsilateral rotation similar to that produced by amphetamine.

These results suggest that MK-801 has DA actions and therefore abuse potential. Excessive DA activity may also contribute to ischemic damage in some brain areas. Such properties may limit the clinical usefulness of MK-801.

205.5

DOPAMINE D-1 BUT NOT D-2 RECEPTORS ARE SELECTIVELY VULNERABLE TO TRANSIENT ISCHEMIA IN THE RAT STRIATUM. M.Y.-T. Globus, D.C. Mash, W.D. Dietrich, R. Busto*, I. Valdes*, and M.D. Ginsberg. Cerebral Vascular Disease Research Center, Department of Neurology, University of Miami, School of Medicine, Miami, FL, 33101.

We have previously demonstrated that dopamine (DA) release during ischemia is involved in the development of ischemic damage in the striatum (Globus et al, Neurosci. Lett. 80:251-256, 1987). Striatal density and distribution of DA D-1 and D-2 receptors was measured autoradiographically 3 and 14 days after 20 min of transient ischemia produced by 4-vessel occlusion in the rat. Adjacent sections were processed for D-1 and D-2 receptors and stained with hematoxylin and eosin for ischemic cell evaluation. D-1 and D-2 receptors were labelled using a saturating concentration of [^{3}H]-SCH 23390, and [^{3}H]-spiroperidol respectively. In control animals the densities of D-1 and D-2 receptors in the dorsolateral striatum were 323 ± 67 and 212 ± 16 fmol/mg tissue, respectively. After 3 days of recirculation, in the presence of ischemic cell damage, no significant changes were detected in the receptor density (D-1 = 242 ± 73, D-2 = 182 ± 45). However, after 14 days of reperfusion the density of D-1 receptors was markedly reduced (40 ± 56) while D-2 receptors were not affected (210 ± 43). The distribution of the D-1 receptor drop-out correlated with the location of the ischemic area. In contrast to the dorsolateral region, no changes were detected in the mediovcntral striatum. These results indicate that striatal neuronal components with D-1 receptors are selectively vulnerable to transient ischemia.

205.6

REDUCTION OF EXTRACELLULAR GLUTAMATE RELEASE DURING ISCHEMIA DOES NOT AMELIORATE ISCHEMIC STRIATAL DAMAGE. R. Busto*, M.Y.-T. Globus, W.D. Dietrich, I. Valdés*, E. Martinez*, B.D. Watson*, and M.D. Ginsberg (SPON: W. J. Weiner). Cerebral Vascular Disease Research Center, Univ. of Miami Sch. of Med., Miami, FL, 33101.

Several lines of evidence suggest that glutamate (Glu) release is involved in the development of ischemic neuronal damage in the hippocampus. However, its involvement in striatal ischemic injury is uncertain. In the present study we examined the effect of a corticostriatal pathway lesion on extracellular release of Glu and on the evolution of histopathological changes in the striatum. Two weeks after photochemically induced unilateral decortication, rats were subjected to 20 min of global ischemia produced by 2-vessel occlusion combined with systemic hypotension (50 mmHg). In one group of animals, striatal extracellular sampling was performed by a microdialysis probe while a separate group was processed for histopathological evaluation after 3 days of recirculation. A 50% reduction in Glu release was observed, during ischemia and early recirculation, on the denervated striatum as compared to the nonlesioned side. However, the degree of ischemic cell damage was similar on both sides. The number of ischemic cells per high power field (mean ± S.D.) in the denervated and non-denervated dorsolateral striata were 45.5 ± 14.7 and 47.3 ± 19.5 respectively. These results suggest that a 50% reduction in striatal extracellular Glu release during ischemia does not alter the final histopathological outcome. It is possible that another injury mechanism may prevail.

205.7

THE EFFECT OF FOCAL CEREBRAL ISCHAEMIA ON GLUTAMATE AND DIHYDROPYRIDINE BINDING SITES IN THE RAT. M.C. Wallace and J. McCulloch, (Spon. S.E. Blackshaw). Wellcome Surgical Institute, University of Glasgow, Glasgow, G61 1QH, Scotland.

Excitatory amino acids and calcium entry into neurones have been implicated in the pathophysiology of cerebral ischaemia. The temporal profile of glutamate and calcium entry blocker binding has been examined in focal cerebral ischaemia in the rat.

Sprague-Dawley rats (n=20) underwent middle cerebral artery occlusion under anaesthesia. Animals were sacrificed at 6, 12 and 24 hours and in-vitro binding with ^{3}H-glutamate (200 nM) and ^{3}H-nitrendipine (0.4 nM) was assessed with quantitative autoradiography. There was an apparent decrease in ^{3}H-nitrendipine binding in the ischaemic cortex and caudate, with marked enhancement of dihydropyridine binding along the margin of the ischaemic area. However, these changes in binding were reflected in both total and non-specific binding. No significant changes in ^{3}H-glutamate binding were detected up to 24 hours after focal ischaemia.

Thus, recognition sites of glutamate and dihydropyridine binding sites are robust, and remain largely unchanged at 24 hours after ischaemia. The interpretation of changes in receptors must be made with consideration given to the effects of tissue quenching of tritiated labelled ligands. Supported by the Wellcome Trust, MCW is an MRC Fellow (Can).

205.8

OPIATE RECEPTOR SUBTYPE BINDING IN GERBIL HIPPOCAMPUS ALTERED BY FOREBRAIN ISCHEMIA. D.C. Perry[1] & L.P.Miller*[2]. [1]Dept. Pharmacology, George Washington Med. Center, Wash. D.C. 20037, & [2]Vet. Adm. Med. Ctr., Wash. D.C. 20422

Although present theories suggest involvement of excitatory amino acids in the specific cell pathology seen after transient forebrain ischemia, endogenous opioids may also play a role. To test for the involvement of opiate receptor subtypes in hippocampal ischemic damage, we used quantitative in vitro autoradiography to measure opiate binding in ischemic gerbils. Ischemia was induced in 10-14 week old female gerbils by bilateral clamping of the common carotid arteries for 10 min under halothane anesthesia. Seven days post-ischemia brains were removed and frozen coronal sections were cut through several hippocampal regions. Autoradiography was done for mu binding (3H-DHM and 3H-DAGO), kappa binding (3H-bremazocine) and lambda binding (3H-naloxone + 300 nM diprenorphine; Eur. J. Pharmacol. 129:147, 1986). Quantitation was done with a Loats/Amersham RAS1000. No changes in mu binding were detected in the hippocampus with either ligand. Kappa binding was significantly lower over the pyramidal layer in CA1 & CA3 (20.5% decrease, $p<0.001$), but not in dentate gyrus. Lambda binding over the mossy fibers was strikingly higher in ischemic animals (65.3% increase; $p<0.01$). These results suggest that dynorphin released from mossy fibers may play a role in ischemia-related neurological damage. Supported by DA04191.

205.9

PARALLEL PATTERNS OF HYPERSENSITIVITY TO NMA TOXICITY AND HYPOBARIC/ISCHEMIC DAMAGE IN DEVELOPING RAT BRAIN. C. Ikonomidou*, J. Mosinger, K. Shahid Salles, J. Labruyere* and J. W. Olney (SPON: M.S. Shahid Salles). Dept. of Psychiatry, Washington University Sch. of Med., St. Louis, MO 63110.

Accumulating evidence implicates the endogenous excitotoxins, glutamate (Glu) and aspartate, in hypoxic/ischemic brain damage. For example, we have found that hypobaric/ ischemic (H/I) conditions cause a form of acute cytopathology in the 10 day old rat brain which is indistinguishable from that induced by exogenous Glu and is blocked by MK-801, a potent and selective antagonist of the N-methyl aspartate (NMA) receptor (the most prevalent sub-type of Glu receptor in brain). Here we have studied, at various postnatal ages, the sensitivity of rat CNS neurons to H/I conditions or to the neurotoxic effects of NMA injected directly into brain.

In the early postnatal period (days 1 to 10), unilateral injection of small doses (6-15 nmol) of NMA into the striatum or neocortex caused a surprisingly severe and widespread excitotoxic reaction affecting many neuronal groups bilaterally. Sensitivity to this fulminating type of reaction increased in the very early period (days 2-6), reaching peak intensity at days 6-8. From days 10 to adulthood, it required progressively higher doses (e.g., 50 nmol at 60 days) to produce significant damage and the damage was confined to the injection site. We found that the period of peak sensitivity to H/I damage also occurred at days 6-8 with progressively diminishing sensitivity being evident with increasing age. The cell populations most sensitive to NMA toxicity in the 2 to 10 day period closely correlated with those most vulnerable to H/I degeneration in this period.

Since the kainic acid (KA) receptor is considered the second most prevalent Glu receptor in brain, its potential complicity in H/I brain damage warrants consideration. In the present study we have corroborated findings from other laboratories that KA is relatively non toxic when injected into the striatum of the 6 day old rat in doses which would produce severe widespread damage in adult rat brain.

Here we show that rat CNS neurons in the early neonatal period are exceedingly sensitive to either H/I degeneration or the direct toxic effects of NMA (but not KA) and that the period of peak sensitivity to the two toxic phenomena coincides. This suggests the interesting possibility that there may be a developmental phase in human ontogenesis when the NMA receptor-ionophore complex is peculiarly hypersensitive and CNS neurons are correspondingly hypervulnerable to hypoxic/ischemic damage. Supported by RSA MH 38894 (JWO), HD 24237, DA 05072. and ES 00875.

205.10

CYCLOHEXYLADENOSINE (CHA) REDUCES THE DOWN-REGULATION OF ADENOSINE A1 RECEPTORS, G-PROTEIN AND ADENYLATE CYCLASE (AC) FOLLOWING ISCHEMIA IN THE GERBIL HIPPOCAMPUS. J.L. Daval, P.J. Marangos, J. Deckert, D.J. Redmond*, S.L. Cohan*, and D.K.J.E. Von Lubitz*. Unit on Neurochemistry, BPB, NIMH, Bethesda, MD 20892.

Transient ischemia produces selective neuronal damage in specific brain areas such as hippocampus. In the CA1 subfield, the pyramidal cells undergo a "delayed neuronal death" within a few days after recirculation. It has been proposed that postanoxic hyperexcitability and synaptic release of glutamate are involved in this process. Adenosine is a neuromodulator in hippocampus formation. Adenosine A1 receptors are believed to mediate the action of adenosine via an inhibition of AC activity. Previous studies have shown a decrease in adenosine A1 receptors after transient brain ischemia in gerbils. In the present quantitative autoradiographic study, the effects of a single IP administration of CHA (2mg/kg) were investigated in gerbil hippocampus 1, 3 or 6 days following a 20 min episode of ischemia induced by occlusion of the carotid arteries. [^{3}H]CHA, [^{3}H]Gpp(NH)p and [^{3}H]forskolin were used to label adenosine A1 receptors, G-protein and AC respectively in brain sections. The data showed a down-regulation of adenosine A1 receptors associated with a decrease in G-protein and AC after ischemia, especially in the CA1 subfield, while CHA treatment considerably reduced the effects of ischemia. CHA might protect the brain by reinforcing the neuromodulatory action of adenosine

205.11

COOLING GREATLY REDUCES EFFECTS OF ANOXIA IN RAT HIPPOCAMPAL NEURONS. M.E. Morris*, K. Krnjević, J. Leblond. *Pharmacology Department, University of Ottawa; Anaesthesia Research Dept., McGill University, Montréal, Canada.

When compared with slices kept at 33.5°, slices at 23-24° show some sharp differences in membrane properties, recorded in the CA1 pyramidal layer: input resistances are higher, action potentials longer, and Ca currents more difficult to elicit. Of particular interest is a much greater resistance to anoxia. In contrast to the near 50% decrease in resistance, marked hyperpolarization and loss of excitability typically evoked by 2-4 min periods of anoxia at 33.5°, there is a much smaller resistance fall (by 15.6 ± 4.0% (n=12)) and no consistent hyperpolarization at 23°; moreover, the characteristic post-anoxic hyperpolarization is inconspicuous or even replaced by depolarization. Moreover, at 23° anoxia as a rule fails to generate either slow steady increases of $[K]_o$ or the dramatic rise - associated with spreading depression-like changes in potential and resistance - that are commonly seen at the higher temperatures.

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CARDIOVASCULAR REGULATION IV

206.1

MICROINJECTION OF ATRIAL NATRIURETIC FACTOR (ANF) INTO THE SOLITARY NUCLEUS (NTS) FACILITATES THE BARORECEPTOR REFLEX INDUCED BY STIMULATION OF THE AORTIC DEPRESSOR NERVE (ADN) IN THE ANESTHETIZED RAT D.J. McKitrick* and F.R. Calaresu Dept. of Physiology, University of Western Ontario, London, Ontario, Canada. N6A 5C1

As microinjection of ANF into the rat NTS is known to elicit a depressor response (Am. J. Physiol., in press), we tested the hypothesis that there may be a synergistic action on arterial pressure (AP) of subthreshold stimulation of the ADN and microinjection of subthreshold amounts of ANF into the NTS. In 19 Wistar rats under urethan anesthesia, electrical stimulation (0.5 ms duration, 40 Hz, 10-100 μA, for 10 s) which did not change AP or heart rate (HR) was applied to the ADN, and volumes (2-11 nL) of 10^{-7}M ANF, which did not influence AP or HR, were microinjected into the middle third of the rostrocaudal extent of the ipsilateral NTS. When subthreshold intensities of electrical stimulation and subthreshold amounts of ANF were delivered simultaneously, decreases in AP (-13.4 ± 2.9 mmHg, n=15) and HR (-29.4 ± 7.0 bpm, n=15) were observed. These results indicate that ANF and baroreceptor fibers affect the function of a common pool of neurons involved in the baroreceptor reflex. (Supported by the Medical Research Council of Canada)

206.2

D2 RECEPTORS IN THE POSTERIOR REGION OF THE NUCLEUS TRACTUS SOLITARIUS MEDIATE THE CENTRAL PRESSOR ACTION OF QUINPIROLE (LY171555). R.H. Yang*, Y.F. Chen, J. M. Wyss, S. Oparil (SPON: S. King). Hypertension Program, University of Alabama at Birmingham, Birmingham, AL.

We have previously shown that injections of quinpirole into the 4th ventricle produces a greater pressor response of a more rapid onset than similar injections into the lateral ventricle in conscious Sprague-Dawley (S-D) rats, suggesting a site of action in brainstem. The present study was designed to determine the neurons in the brainstem which mediates this pressor response. Microinjections of quinpirole (5 μg) into the posterior region of nucleus tractus solitarius (P-NTS) caused a consistent increase in BP (Max Δ=12.4±1.1mmHg) with a rapid onset (26 sec) in decerebrate S-D rats, while microinjections into the anterior region of NTS, area postrema, C_1/A_1 regions, raphe obscurus nucleus, locus coeruleus or the regions which are 0.5mm lateral, superior or inferior to P-NTS produced little response. The pressor response induced by bilateral microinjections of quinpirole into P-NTS was not different from that of unilateral microinjection. The pressor response to quinpirole in P-NTS was abolished by pretreatment with metaclopramide (5 mg/kg, i.v. 10 min before), a DA D2 antagonist that crosses the blood brain barrier. Taken together, these data demonstrate that D2 receptors in the P-NTS mediate the central pressor action to quinpirole.

206.3

PICROTOXIN BLOCKS TIME-DEPENDENT INHIBITION OF PERIPHERAL AFFERENT INPUT TO NUCLEUS TRACTUS SOLITARIUS (NTS) NEURONS. B.D. Miller*, R.B. Felder, Dept. of Int. Med. and CV Ctr, Univ. of Iowa, Iowa City, IA 52242.

Recent studies have shown that the responsiveness of NTS neurons to peripheral afferent input is markedly diminished following an appropriately timed conditioning stimulus (CS) to the same or another afferent source. We examined the influence of the γ-aminobutyric acid (GABA) antagonist picrotoxin (PIC) on these time-dependent inhibitory interactions. In an *in vitro* rat brain slice, we made extracellular recordings from 5 medial NTS neurons activated by electrical stimulation of ipsilateral solitary tract (ST). For each unit, we determined the number of action potential responses to a test stimulus (2-8 V; .3 ms;.3-1 hz; 50 stimuli) and the manner in which these responses were modified by a prior CS (2-8 V,.3 ms) applied to the same ST at 50-350 ms intervals. The conditioning paradigm was repeated after adding PIC (10-25 μM) to the perfusate. With perfusate alone, the CS reduced the number of test-evoked responses (expressed as % control, mean±SE) to 77±11% at 350 ms, 60±9% at 250 ms, 33±9% at 150 ms, 26±10% at 100 ms, and 24±7% at 50 ms. With PIC, these values were 96±5% at 350 ms, 91±9% at 250 ms, 88±10% at 150 ms, 75±17% at 100 ms, and 71±17% at 50 ms. PIC significantly ($p<0.05$) blocked the inhibitory influence of the CS at intervals ranging from 50-250 ms. The data suggest that time-dependent inhibitory interactions in NTS are mediated at least in part by GABA receptors.

206.4

PRESSOR RESPONSES TO STIMULATION OF THE NTS IN ANESTHETIZED CAT. S.C. Robertson*, R.B. Felder and W.T. Talman (SPON: R. Lim). Lab of Neurobiology, VAMC and Univ. of Iowa, Iowa City, IA 52242.

Classic studies in the decerebrate cat demonstrated a predominantly depressor effect with electrical stimulation of the nucleus tractus solitarius (NTS). We sought to determine the effects on arterial pressure (AP), heart rate (HR), and renal sympathetic nerve activity (RSA) of electrical stimulation of the NTS and to compare the effects with those elicited by microinjections of glutamate (GLU-20 mM) or glycine (GLY-400 mM) into the NTS of fourteen paralyzed, ventilated anesthetized (chloralose) cats. In 36 of 45 sites in the NTS, electrical stimulation elicited pressor responses with variable changes in HR. At 43 of the 45 sites GLU also increased AP and usually (31/45) HR. At 22 sites GLU and GLY each increased AP. Independent of the AP response GLU tended to increase HR when injected medially and decrease HR when injected laterally. The direction of changes in RSA correlated with the direction of change in HR. In 3 unanesthetized, decerebrate cats electrical stimulation of dorsal NTS consistently decreased AP while more ventral stimulation increased AP. These data suggest that chloralose anesthesia modifies the cardiovascular response to stimulation of the NTS. The NTS pressor response is mediated by local neurons. Support: HL32205, HL14388, and NS24621.

206.5

NMDA RECEPTORS AND THE BARORECEPTOR REFLEX. N. Kogo*, J. Graff*, P.A. Grieve*, and W.T. Talman. Lab of Neurobiology, VAMC and Univ. of Iowa, Iowa City, Iowa 52242.

We have previously shown that blockade of the response to N-methyl-D-aspartate (NMDA) and kainate (KA) in the nucleus tractus solitarius (NTS) also blocks the baroreceptor reflex. We have now sought to study the role of NMDA receptors in the baroreceptor reflex. Twenty-one anesthetized rats were instrumented for recording arterial pressure (AP) and heart rate (HR) and in some for stimulating the aortic depressor nerve (ADN) and recording single units in the NTS. Microinjections of 2-amino--5-phosphonovalerate (APV) or vehicle were made into the NTS unilaterally before the injection of NMDA or KA or bilaterally before testing the reflex bradycardic responses to pressor doses of phenylephrine. The decrease in AP (41.5 ± 3.7 mmHg, mean ± SEM) elicited by NMDA (3 pmol) was reduced to an insignificant change (4.7 ± 2.1) by the microinjection of 10 mM APV at the same site. In contrast, APV did not affect the depressor response elicited by KA. The bilateral injection of APV led to an 85% reduction in the bradycardic response to equivalent increases of AP before and after APV. APV transiently blocked the orthodromic single unit responses to electrical stimulation of the ADN. These data suggest that the NMDA receptor is integral to the baroreceptor reflex in the NTS and that NMDA blockade abolishes the baroreceptor reflex. Supported by HL32205, HL14388, and VA Merit Review Tab 18.

206.6

DEPRESSOR RESPONSES TO CHOLINERGIC AGONISTS IN THE NUCLEUS TRACTUS SOLITARIUS (NTS) ARE MEDIATED BY M_2 MUSCARINIC RECEPTORS. J. Murugaian*, K. Sundaram* and H.N. Sapru (SPON: J. McArdle). Section of Neurosurgery, UMDNJ - New Jersey Medical School, Newark, NJ 07103.

Microinjections (0.2-2 nmol/site) of an M_2 muscarinic receptor agonist (cis-methyldioxolane; CD) into the intermediate portion of NTS decreased BP (23-52 mmHg) and HR (16-50 beats/min). These effects were produced by the reduction of sympathetic nerve activity. AFDX-116 (a specific blocker for M_2 receptors) when microinjected (0.8 nmol/site) into the same sites prevented the depressor and bradycardic responses induced by CD. Pirenzepine (a specific blocker for M_1 receptors), when microinjected (1.5 nmol/site) into the NTS failed to block the effects of CD. The M_1 receptor agonist (McN-A343; 3 nmol/site) failed to evoke any response from the NTS. These results indicate that muscarinic receptors of M_2 type are present in the intermediate portion of NTS and activation of these receptors by cholinergic agonists results in depressor and bradycardic responses. Support: NIH (HL 24347) and AHA (NJ).

206.7

SYSTEMIC GLUTAMATE DESTROYS SOME TYROSINE HYDROXYLASE AND SEROTONIN IMMUNOREACTIVE NEURONS IN AREA POSTREMA OF SHR. D.K. Hartle and C.F. Phelix*. Cardiovascular Laboratories, Univ. of Ga., Coll. of Pharmacy, Athens, GA 30602.

Neurons within the area postrema (AP) are outside the blood-brain barrier. High doses of systemic glutamate (G) (9 mg/ g, s.c.) produce neuroexcitotoxicity in a significant fraction of total AP neurons in adult rats. Of the circumventricular organs, AP is uniquely susceptible to toxicity at this dose of G. Immunocytochemical localization of tyrosine hydroxylase (TH) and serotonin (5HT) in 20 µm brain sections throughout the extent of the AP was done separately in groups of SHR and WKY rats treated with G, isosmotic saline or glycinate (pH 7.4). Degenerating neurons were consistently observed 4, 6, and 8 hr after G administration. Degenerating elements were eliminated by 24 hr after G. Light microscopic examination revealed that a significant fraction of neurons killed by systemic G were TH or 5HT immunoreactive (IR). Quantitative localization of these populations was performed by video image analyses. Both density and total number of these neurons decreased after G treatment. EM examination verified degenerating TH and 5HT-IR neurons and dendrites in AP, but not within NTS, of G-treated rats. Isosmotic saline and glycinate treatments produced no AP somal or dendritic necrosis. The potential role of susceptible AP neurons in the antihypertensive effect of G treatment in SHR vs. WKY will be discussed. NIH 1 RO1-HL37705-02 (DKH).

206.8

CARDIOVASCULAR ROLES OF TACHYKININ PEPTIDES IN THE NUCLEUS TRACTUS SOLITARIUS OF RATS

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The primary afferent fibers of the glossopharyngeal and vagal nerves transmit baro- and chemoreceptor signals to the nucleus tractus solitarius(NTS). In addition, our previous studies have demonstrated high levels of substance P like immunoreactivity(SP-LI) and neurokinin A like immunoreactivity(NKA-LI) in the NTS (Brain Res.,369,1986). In the present study, we have examined the cardiovascular responses of NKA, SP and related peptides microinjected into the NTS of rats.

Male Wistar rats were anesthetized with urethane (1.0g/kg,i.p.), and artificially ventilated through a tracheal cannula with a respirator. Since saline-vehicle (0.1µl) injected into the NTS caused hypotension and bradycardia (Brain Res.,422,1987), tachykinin peptides were dissolved in artificial cerebrospinal fluid (ACSF) in this study. Microinjection of NKA (10ng and 100ng/0.1µl) into the caudal area of the NTS caused rapidly hypotension and bradycardia. The lower dose of SP (1ng/0.1µl) caused rapidly hypotension and bradycardia, whereas the higher dose of SP (100ng/0.1µl) resulted in increase in heart rate and blood pressure. These results indicate that NKA as well as SP may be involved in the baroreceptor reflex in the NTS.

206.9

CAPILLARY DENSITY AND GLUCOSE METABOLISM IN BARORECEPTOR SUBNUCLEI OF RAT NTS. P.M. Gross, J.J. Pang* and S. Shaver. Neurosurg. Res. Unit, Queen's Univ., Kingston, Ont. K7L 3N6

Caudal to obex, the nucleus of the solitary tract (NTS) is composed of several discrete subnuclei forming synaptic sites for afferent fibers from peripheral chemoreceptors and baroreceptors. Relationships intrinsic to reflex activity in NTS have been identified previously, e.g., subnuclear cytoarchitecture, neural connections, histochemistry, and impulse interactions. We describe other features supporting these processes within caudal NTS (obex -0.2 to -0.4 mm) of adult albino rats by analyses of capillary density (CD) and tissue glucose metabolism (GM), measures closely interrelated. Image processing was applied both to micrographic prints (x240) of coronal NTS sections to assess CD and [^{14}C]deoxyglucose film autoradiographs to measure GM. CDs in n. commissuralis, n. medialis and dorsal strip varied between 297 and 440/mm^2. Values for GM were between .55±.03 and .73±.04 umol/g/min. Mapping correlations between CD, GM and distribution of peptides and catecholamines in NTS subnuclei gives further insight to factors supporting autonomic regulation within the dorsal medulla oblongata.

206.10

METABOLIC ACTIVATION OF EFFERENT PATHWAYS FROM THE RAT AREA POSTREMA. D.S. Wainman*, S.W. Shaver, A.V. Ferguson and P.M. Gross (SPON: M. Caudarella) Neurosurg. Res. Unit and Dept. Physiol., Queen's Univ., Kingston, Ont. K7L 3N6

Specific medullary and pontine sites receive innervation from area postrema (AP) neurons. We examined the metabolic responsiveness of these projections by applying the [^{14}C]deoxyglucose method in anesthetized, ventilated (N_2O-O_2-halothane) albino rats during electrical stimulation (ES, 200 uA, 15 Hz) of the dorsomedial AP. ES provoked an abrupt 20% decrease in arterial pressure and bradycardia, both of which were sustained over time. Rates of glucose metabolism (umol/g/min; control values in parentheses) were doubled by ES in rostral or caudal subregions of AP more than 0.1 mm from the electrode (0.61) and in several subnuclei of NTS (mean = 0.61). DMN of the vagus nerve (0.56), n. ambiguus/A1 noradrenergic region (0.63), locus coeruleus (0.88), and lateral parabrachial nuclei (0.56) were also activated (+27-71%). The results indicate that AP neurons can influence the function of numerous brainstem structures within their efferent trajectories and implicate roles for the AP in regulation of autonomic reflexes.

206.11

CARDIOVASCULAR RESPONSES ELICITED FOLLOWING OPIOID RECEPTOR ACTIVATION IN THE AREA POSTREMA OF ANESTHETIZED RAT.

A.H. Hassen, E.P. Broudy* and J. Nemitz. West Virginia School of Osteopathic Medicine, Lewisburg, WV 24901.

Previous studies have described the cardiovascular responses elicited following selective activation of different opioid receptor types in the nucleus of Tractus Solitarius and the Dorsal Motor nucleus of the Vagus,two components of the dorsal vagal complex, in the anesthetized rat. Mu, kappa or delta receptor activation evoked qualitatively different patterns of cardiac and pressor responses. We have now examined the responses elicited following opioid receptor activation in the third component of the dorsal vagal complex, the circumventricular Area Postrema.

Male Sprague-Dawley rats were anesthetized with urethane (1.6 gm, ip), and prepared for recording of blood pressure and heart rate. The hindbrain was exposed in order to permit the stereotaxic placement of a pulled glass capillary (40-50 um diameter) into the Area Postrema. Mean arterial pressure and heart rate were monitored prior to a single injection (10 nl) of either the saline vehicle, glutamate, a mu-agonist (DAGO), a kappa-agonist (U50,488), or a delta-agonist (D-Pen). Tachycardia and pressor responses were elicited by the mu-agonist, whereas the delta- and kappa-agonists produced transient decreases in heart rate and blood pressure. Glutamate elicited small, transient biphasic changes in blood pressure and heart rate. These data suggest that different opioid receptor types in the area postrema can selectively modulate cardiovascular function. Supported by WVSOM.

206.12

DIFFUSION OF ^{3}H-OPIOID AND LATEX MICROSPHERES IN RAT AREA POSTREMA. J.W. Nemitz and A.H. Hassen.

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Male Sprague-Dawley rats were anesthetized with urethane, paralyzed, ventilated, placed in a stereotaxic frame, and the dorsal hindbrain was exposed. Single microinjections (300 pmol/10 nl) of either ^{3}H-D-Ala^{2}, MePhe^{4}, Gly-ol^{5} enkephalin solution (^{3}H-DAGO, a mu-opioid agonist) or cold DAGO in a 5% suspension of latex microspheres, were delivered to the area postrema (AP) or gracile nucleus (Gn). Blood pressure, heart rate and temp. were monitored throughout the experiment. Animals were sacrificed with pentobarbital, brains were removed and frozen on dry ice. Serial sections were cut in a cryostat and mounted on slides. The ^{3}H-labelled material was processed using standard autoradiography. All slides were stained and analyzed for distribution of the injected labels. Injections of ^{3}H-DAGO into the AP resulted in a bilateral distribution into the dorsal vagal complex; this was in contrast to the microsphere injections into the AP which remained within the AP. Injections into the AP of either ^{3}H-DAGO or DAGO mixed with microspheres resulted in cardiovascular responses (see abstract by Hassen et al.). Labelling of the AP with ^{3}H-DAGO also occurred after injection into the adjacent Gn, but no marking of the AP resulted from latex microsphere injections into the Gn. The injections into the Gn did not result in observed cardiovascular responses. These data provide evidence for selective diffusion of solutions into and out of the AP after microinjection. Supported by WVSOM.

206.13

THE EFFECTS OF CHEMICAL LESION OF THE AREA POSTREMA ON ARTERIAL BAROREFLEX RESPONSE TO PHENYLEPHRINE AND VASOPRESSIN. M. Hay*, B.F. Cox* and V.S. Bishop. Dept. of Pharm., The Univ. of TX Hlth. Sci. Ctr., San Antonio, Texas 78284-7764.

Previous studies have shown that electrolytic lesions of the area postrema abolishes the enhanced baroreflex inhibition of renal sympathetic nerve activity and heart rate (HR). The purpose of this study was to determine if similar effects could be produced by chemical lesions of the area postrema with kainic acid. The area postrema was lesioned with micropressure applications of kainic acid (1 ng/nl) in a series of 5, 15 nl injections given over 20 minutes. In control animals, infusion of arginine-vasopressin (AVP) resulted in a much greater fall in HR for any given change in mean arterial pressure (MAP) as compared to phenylephrine (PE) infusions. A 10 mmHg rise in MAP with PE produced an average 26 beat/min fall in HR whereas a 10 mmHg rise in MAP with AVP produced an average 56 beat/min fall. Lesions of the area postrema with kainic acid shifted the MAP - HR relationship obtained with AVP infusions to approximate the relationship obtained with PE infusions. These data suggest that discreet chemical lesions of the area postrema normalizes MAP - HR responses evoked with AVP and PE, thus further supporting the role of the area postrema in vasopressin modulation of cardiovascular regulation. (Supported by NIH Grants 12415 and 36080).

206.14

EVIDENCE FOR THE INVOLVEMENT OF SEROTONIN IN CENTRAL CONTROL OF CARDIAC VAGAL MOTONEURONES IN THE CAT.

D. Jordan, P.N. Izzo* and A.G. Ramage*. Departments of Physiology and Pharmacology, Royal Free Hospital Medical School, Rowland Hill Street, LONDON NW3 2PF, England.

5-HT$_{1A}$ agonists (e.g. 8-OH-DPAT) given i.v. cause vagally mediated bradycardia (Ramage, A.G. & Fozard, J.R. Eur. J. Pharmac., 138:179, 1987). The present experiments were carried out to investigate the possibility that 5-HT-containing nerve terminals can directly affect cardiac vagal motoneurones (CVM's) located in the nucleus ambiguus (NA). Horseradish peroxidase (HRP) was applied to identified cardiac branches of the vagus nerve and after 48-72 hours recovery the animals were killed, the brain fixed and the medulla sectioned (70μm). The sections were processed to reveal both retrogradely transported HRP and for 5-HT immunoreactivity using the PAP method. Electron microscopic examination of the material revealed 5-HT-immunoreactive boutons forming synaptic contacts with retrogradely labelled CVM's in the NA. In parallel experiments, microinjections of 5-HT or 8-OH-DPAT into this region evoked falls in heart rate of up to 37 beats/min in addition to reducing both renal and phrenic nerve activities. These results support our hypothesis that central 5HT-containing pathways are involved in vagal control of the heart.

This work was supported by The Wellcome Trust and M.R.C.

206.15

BARORECEPTOR MODULATION OF HYPOGLOSSAL MOTONEURONES.

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Investigation of the physiological characteristics, morphology and precise localization of neurones in the dorsal medulla involved in cardiovascular control have been carried out in the cat using intracellular recording and labelling techniques. One clearly distinguishable group of neurones exhibiting baroreceptor modulation have been identified as a sub-population of hypoglossal motoneurones. Nine hypoglossal neurones, characterized on the basis of antidromic activation and/or intracellular labelling with HRP have been examined. All of these neurones were spontaneously active: the activity in three of them showing respiratory rhythm. Further analysis of their ongoing activity revealed pulse related activity in six of them, three of which were activated by carotid sinus nerve stimulation. One of these neurones, which showed an epsp-ipsp sequence of response to the carotid sinus nerve stimulation, was further characterized by natural stimulation of the carotid sinus baroreceptors, which inhibited the ongoing activity in this neurone.

These studies have identified an influence of the baroreceptor reflex on the activity of hypoglossal motoneurones.

This work was supported by the M.R.C.

206.16

FRONTAL-PARIETAL (F-P) CORTEX RECEIVES AN EFFERENT COPY OF SYMPATHETIC NERVE DISCHARGE (SND). Z.-S. Huang*, K.J. Varner, S.M. Barman and G.L. Gebber. (SPON: T.M. Brody). Dept. Pharmacol., Mich. State Univ., E. Lansing, MI 48824.

We studied the relationship between F-P cortical activity and inferior cardiac SND in chloralose-anesthetized cats. Cross-correlation analysis revealed that the 2- to 6-Hz rhythmic components in cortical activity and SND were temporally related in 10 of 17 baroreceptor-denervated cats. SND lagged cortical activity by 50±20 ms and the value of the crosscorrelation function at this lag was 0.4±0.1. In contrast to baroreceptor-denervated preparations, cortical activity and SND were not related in cats with intact baroreceptor nerves. Since electrical stimulation of F-P cortex increased SND (20-200 μA threshold current; response onset latency, 64±2 ms), the possibility arose that F-P cortex contributes to basal SND in baroreceptor-denervated cats. This possibility was tested by performing a F-P lobotomy bilaterally 1-2 mm behind the apex of the ansate sulcus. F-P lobotomy failed to significantly affect SND and blood pressure, or the reduction in SND and blood pressure produced by subsequent midbrain transection. Thus, the temporal relationship between cortical activity and SND does not reflect a tonic influence of the F-P cortex on SND. Rather, it appears that F-P cortex receives an efferent copy from the generator of 2- to 6-Hz SND. What the F-P cortex does with the efferent copy of SND remains to be determined. (Supported by NIH grant HL33266.)

206.17

PREFRONTAL STIMULUS-PRODUCED HYPOTENSION IS BLOCKED BY BRAINSTEM LIDOCAINE INJECTIONS. S.G.P. Hardy, D.E. Holmes*, and S.M. Mack*. Dept. of Anatomy, Univ. of Miss. Med. Ctr., Jackson, MS 39216.

Electrical stimulation of the prefrontal cortex (PFC) typically causes hypotension in the urethane-anesthetized rat. It has been suggested that PFC projections descending through the ipsilateral crus cerebri and terminating upon the contralateral nucleus tractus solitarius (NTS) may mediate the stimulus-produced hypotension (SPH) (van der Kooy, D., et al., J. Comp. Neurol., 224:1-24, 1984). To test this hypothesis, microinjections (0.5ul) of 4% lidocaine were made into various levels of this pathway in anesthetized rats. The area of functional blockade resulting from similar injections has been reported to typically be 0.9 mm in diameter (Sandkuhler, J., et al., Exp. Brain Res., 68:168-178, 1987). In our study it was observed that injections made into the ipsilateral crus cerebri or into the vicinity of the contralateral NTS resulted in the attenuation or complete blockade of SPH. Similar injections made into the superior colliculus, periaqueductal gray and various sites within the reticular formation failed to attenuate SPH. The results of this study support the concept that prefrontal SPH is mediated by prefrontal projections to the NTS.
(Supported by a grant from the Miss. Affiliate of The American Heart Association).

206.18

CENTRAL H1-BLOCKADE ABOLISHES PRESSOR RESPONSE TO INTRAVENOUS HYPERTONIC SALINE INFUSION. V. Schaumloffel* and S.L. Bealer (SPON: L. Share). Department of Physiology and Biophys., Univ. Tenn., Memphis, TN 38163.

Peripheral infusion of hypertonic saline results in increased mean arterial pressure (MAP), mediated partially by release of vasopressin. Central administration of histamine (HA) results in increased MAP, bradycardia, and vasopressin secretion in the conscious rat. Therefore, we observed the effect of central histaminergic blockade on the cardiovascular response to hypertonic saline infusion. Rats were implanted with lateral ventricular cannulae and catheters in the femoral vein and artery. After recovery, conscious animals received an intracerebroventricular injection of saline (VEH), H1-antagonist promethazine (PMZ), or H2-antagonist cimetidine (CMT), followed by a 30-minute intravenous infusion of 2.5 M or 0.17 M NaCl (10 μl/100 g/min). MAP was continuously recorded.

Isotonic saline infusion had no effect on MAP in any group. However, after a 30-minute hypertonic saline infusion, MAP was significantly elevated in VEH- (20.5 ± 3.8 mm Hg, n=6) and CMT- (15 ± 3.5 mm Hg, n=6) treated animals but not in animals pretreated with PMZ (0 ± 2.2 mm Hg, n=6). These data support the hypothesis that H1 receptors mediate pressor responses to hyperosmotic stimulus.

206.19

CIRCULATORY INSTABILITY MODIFIES BRAINSTEM CATECHOL METABOLIC BASELINE ACTIVITY. JY. GILLON*, L. QUINTIN, B. MILNE*, M. GHIGNONE, G. CHOUVET* and B. RENAUD. Neuropharmacologie, Univ. C BERNARD and CNRS, 69008, LYON, FRANCE.

The cardiovascular, circulatory and metabolic conditions of anaesthetized rats (S-D, 400g) were assessed for 12h, when managed A) conventionnally (CM, n= 9, chloral, spontaneous ventilation, no correction of acid-base balance, no volume infusion) B) under optimized management (OM, n= 7, chloralose, mechanical ventilation, O2= 40%, PetCO2= 40 mmHg, control of acid-base equilibrium, 7 ml/kg/h of a dextran-saline-bicarbonate solution). Voltammetric recordings of a catechol oxidation current (CA.OC) in the caudal ventrolateral medulla (VLM) allowed monitoring of the variations of the catecholamine metabolism under the CM and OM conditions. The CM group experienced i) a decline in mean arterial pressure (MAP), central venous pressure (CVP) and cardiac output 3 to 6 h after induction of anaesthesia, ii) severe metabolic acidosis, iii) progressive increase of CA.OC and iv) death before the end of the study in 6 rats. Deterioration of a rodent preparation was previously reported (Andrade et al., Brain Res., 1982, 242, 125; Guyenet et al., Brain Res., 1984, 303, 31). In the OM group, MAP, CVP, and blood gases remained stable for 12 h and CA.OC was recorded as a stable signal (n= 6). Similar results were obtained in OM rats, anaesthetized with halothane and infused with Ringer's lactate solution. It is concluded that baseline activity of VLM cardiovascular structures may be easier to assess in a thoroughly stable circulatory and metabolic preparation.

206.20

PERIPHERALLY ADMINISTERED γ-MSH CAN HAVE A CENTRAL SITE OF ACTION FOR ITS CARDIOVASCULAR EFFECTS. M.F. Callahan, K.A. Gruber* and S.L. Eskridge-Sloop*. Wake Forest University Medical Center, Winston-Salem, NC 27103.

I.V. administration of γ-MSH produces a pressor response which is partially dependent on the forebrain AV3V region and central vasopressin pathways. We previously showed that a pressor response could be elicited by lateral (icv) or fourth ventricular (ivt) injection of γ-MSH. The current studies examined whether icv injections produce pressor responses similar to iv injections and whether injection into the carotid circulation would produce greater pressor responses than iv administration.

ICV administration of 10 ug of the peptide produces a peak pressor response of 53±10 mmHg at 91±12 sec, while ivt administration results in a 65±6 mmHg pressor response at 54±16 sec. The pressor response following icv doses is associated with a tachycardia response while that following ivt administration results in no change in heart rate. In preliminary experiments, we observed that the icv pressor response is attenuated by ivt vasopressin antagonist.

Finally, 0.5 and 1.0 ug of γ-MSH elicited a pressor response (35±2 and 46±4 mmHg) when infused intra-carotid. Jugular infusions of the peptide produced a significant pressor response (40±4 mmHg) only at a 5.0 ug dose.

These studies suggest that γ-MSH produces greater pressor and tachycardia responses when given either directly into the CNS or into the cerebral circulation.
(Supported by AHA/NC Affiliate grant 1985-1986-A42)

PHARMACOLOGY OF SYNAPTIC TRANSMISSION I

207.1

THE EFFECTS OF IRREVERSIBLE ACETYLCHOLINESTERASE INHIBITORS ON SYNAPTIC TRANSMISSION IN THE SYMPATHETIC GANGLIA OF THE BULLFROG, Rana catesbieana. J.F. Fiekers and T.J. Heppner*. Dept. Anat. and Neurobiol., Univ. of Vermont Coll. Med., Burlington, VT 05405.

The effects of soman, sarin, and VX were examined on synaptic transmission through the isolated 9th and 10th sympathetic ganglia of the bullfrog using extracellular and intracellular recording techniques. Each agent produced a frequency-dependent reduction in the amplitude of the compound action potential (CAP). At 30 Hz, the CAP amplitude was reduced to 28% of control. This effect was use-dependent with the amplitude of the second train reduced to >15% of control values with each agent. Agent application induced an initial depression in CAP amplitude which corresponded with the amplitude and frequency of spontaneous depolarizing afterpotentials (APs). These extracellular APs (1) occurred ~18msec after the initial CAP component (2) had an amplitude ~35% of the fast component, (3) were not the result of C fiber activation and (4) were irreversible with washing. Intracellular action potentials elicited by orthodromic stimulation demonstrated a frequency-dependent sustained membrane depolarization (1) that failed to return to resting levels within 5 minutes and (2) frequently abolished action potential generation. This work was supported in part by the US Army Medical Research and Development Command, Contract No. DAMD17-86-C-6031.

207.2

SOMAN INCREASES EXCITABILITY OF SYMPATHETIC GANGLION NEURONS OF THE BULLFROG. T.J. Heppner* and J.F. Fiekers (SPON: J. Held). Dept. Anat. and Neurobiol., Univ. of Vermont Coll. Med., Burlington, VT 05405.

The effects of soman were examined on B cell neurons in the 9th and 10th ganglion isolated from the paravertebral sympathetic chains of the bullfrog Rana catesbeiana. Application of soman produced an increase in spontaneous spiking and in the number of spikes during depolarizing step pulses. Intracellular recordings following soman exposure demonstrated (1) a depolarization of ~6.0mV (2) a 49.3% decrease in membrane resistance and (3) a 33% decrease in the duration of the hyperpolarizing afterpotential (HAP). The soman-induced depolarization and the change in membrane resistance were partially reversed by nicotinic and muscarinic receptor blockade and also by current clamping the membrane potential. Receptor blockade prevented the soman-induced shortening of the HAP. Single electrode voltage clamp measurements demonstrated that soman reduced the HAP current amplitude ~50%. These studies demonstrate that soman has multiple actions on ganglion cells which lead to an increase in ganglion cell excitability due to a combination of membrane potential depolarization and a decrease in the duration of the HAP.
This work was supported in part by the US Army Medical Research and Development Command, Contract No. DAMD17-86-C-6031.

207.3

EFFECT OF M_2 MUSCARINIC RECEPTOR ANTAGONISTS, AF-DX 116 AND GALLAMINE, ON MEMBRANE PROPERTIES OF SYMPATHETIC NEURONS. C. A. Yarosh, A. C. Olito* and J. H. Ashe. University of California, Riverside, CA 92521.

The effect of AF-DX 116 and gallamine on membrane electrical properties of sympathetic neurons was examined in rabbit superior cervical ganglion (SCG). AF-DX 116 and gallamine preferentially bind to the M_2(cardiac) muscarinic subtype(Hammer et al., Life Sci.,38,1986), and selectively antagonize the s-IPSP but not the s-EPSP recorded from SCG (Ashe and Yarosh, Neuropharmacol., 23, 1984; Yarosh et al., JPET, 245, 1988). Using intracellular recording techniques, it was demonstrated, in the present study, that superfusion of ganglia with AF-DX 116 (300-1000nM) was without effect on membrane potential (V_m) or membrane input resistance (R_i). In contrast, gallamine (5-35 μM) produced a dose-dependent depolarization that was sustained in the presence of gallamine. Peak depolarization was about 19.0 ± 0.8mV ($\bar{X}$±S.E.; n=34). Furthermore, superfusion with gallamine resulted in an increase in R_i observed with manual voltage clamp. The effects of gallamine were reversible when ganglia were superfused in gallamine-free Krebs-Ringer solution. The effects of gallamine were not diminished by superfusion of ganglia with d-Tubocurarine(35-70 μM) or quiniclinidyl benzilate (100 nM). These observations suggest that gallamine, but not AF-DX 116, may also produce non-cholinergic receptor mediated action(s) on ganglion neurons.

207.4

BINDING OF THE HIGHLY POTENT NICOTINIC AGONIST, ISOARECOLONE METHIODIDE, AND 19 OF ITS ANALOGUES AT NICOTINIC AND MUSCARINIC RECEPTORS. C.E. Spivak, J.A. Waters* and R.S. Aronstam. NIDA, Addiction Res. Ctr., Baltimore, MD 21224, NIDDKD, Bethesda, MD 20205, and Dept. Pharmacol. Toxicol., Med. Coll. Georgia, Augusta, GA 30912.

Isoarecolone methiodide (ISO) was previously shown to be among the most potent agonists tested at the frog neuromuscular junction (Spivak et al., Eur. J. Pharmacol. 120:127, 1986). Since nicotinic receptors from different sources vary in their selectivity, ISO as well as 19 additional congeners were studied in binding assays.

Competition studies on electric organ membranes from Torpedo nobiliana using [^{125}I]-alpha bungarotoxin yielded K_i values that correlated strongly (Spearman's correlation coefficient -0.91) with potency of the agonists at the frog neuromuscular junction. Activation of the ion channel by the agonists, as indicated by the enhanced binding of [^{3}H]-perhydrohistrionicotoxin, confirmed the preeminent activity of ISO. Competition studies at muscarinic receptors from rat brains, using [^{3}H]N-methylscopolamine, revealed that ISO was nearly as potent as carbamylcholine. This agent, therefore, appears to be a general, cholinergic agonist. By contrast, 1-methyl-4-acetylpiperazine methiodide, while nearly as active as ISO at the nicotinic receptor, was very weak at muscarinic receptors.

207.5

CHANGE IN ANTICHOLINESTERASE ACTIVITY OF PHYSOSTIGMINE AND PYRIDOSTIGMINE BY STRUCTURAL MODIFICATION. R. Ray and O.E. Clark*. US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

Several synthetic physostigmine and pyridostigmine derivatives were studied for their structure-activity relationship in inhibiting acetylcholinesterase (AChE) in electric eel (purified) and in rat brain homogenate. These compounds were (-)-physostigmine salicylate (1), and (+)-physostigmine salicylate (2); decarbamylated physostigmine derivatives, (-)-eseroline sulfate (3), and (+)-eseroline sulfate (4); and pyridostigmine bromide (5), and its reduced tertiary derivative tetrahydropyridostigmine bromide (6). The concentration-response relationship of the AChE inhibition by these compounds was determined using an automated colorimetric microtiterplate AChE assay (Ray, R. and Clark, O.E., the FASEB J., 2(5): A1541, 1988). For both sources of AChE, the relative anti-AChE potencies of the carbamylating groups of compounds were in the order of 1 > 2; and 5 > 6; for the reversible inhibitors, 3 > 4. The (-)-optical isomers were more potent than the (+)- optical isomers, as expected, for all enantiomers tested. These results show that the anti-AChE potencies of the physostigmine and pyridostigmine derivatives used in this study depend on the structures of these derivatives, and that their relative potencies are similar for both eel and mammalian brain AChE.

207.6

EFFECT OF MAGNESIUM ON FREQUENCY-DEPENDENT CONTRACTION IN INDIRECTLY STIMULATED FROG SKELETAL MUSCLE. R.S. Manalis and B. Pickelheimer*. Dept. Biol. Sci., Indiana Univ.-Purdue Univ., Fort Wayne, IN 46805.

Sciatic nerve/sartorius muscle preparations from the frog (Rana pipiens) were used to study the effect of high-Mg/low-Ca Ringer on frequency-dependent contraction. Control measurements were obtained from preparations bathed in normal Ringer. A series of four trains of suprathreshold stimuli was applied every 5 min. The duration of each train was 4 sec; the interval between trains was 30 sec. The stimulus frequency within each train was constant and was increased sequentially from .5 Hz (first) to 15 Hz (fourth). Contraction ratios were determined from isometric tensions measured at a given frequency divided by those obtained at .5 Hz. At 15 Hz, the control ratio was 2.56± .33. In the presence of 1.25 mM Mg/1.0 mM Ca-Ringer, this ratio increased to 8.54± 2.70 (n=9). Mg did not alter the frequency-dependency of directly stimulated muscle. Therefore, inferences about transmitter release can be made from a population of neuromuscular synapses by measuring muscle tension from preparations bathed in high-Mg/low-Ca Ringer's solution. (Supported by NSF grant BNS-8609047.)

207.7

Alterations of endplate currents by Tetram (an irreversible anticholinesterase agent). E.G. Henderson, D.J. Post*, M.R. Burkard*, and C.A. Pappas*. Dept. of Pharmacology, University of Connecticut Health Center, Farmington, CT. 06032

We have previously shown that 217AO (the tertiary methylamine analog of echothiophate) an irreversible organophosphate acetylcholinesterase inhibitor decreases endplate current (e.p.c.) decay and interrupts the voltage dependence of decay in transected frog cutaneous pectoris muscles (Henderson et al. Fed. Proc. 42, 991, 1983). In contrast Tetram (a tertiary ethylamine analog of echothiophate) slows e.p.c. decay and increases e.p.c. amplitude without significantly altering the voltage dependence of e.p.c. decay. After Tetram is removed from the preparation e.p.c. decay remains normal but e.p.c. amplitude is less than control. At the concentrations used (10^{-8} - 10^{-5} M) these drugs have no affect on either [^{125}I]-α-Bungarotoxin or [^{3}H] phencylidine binding to torpedo electric organ membranes. Thus they are not acting on the receptor recognition site or on the traditional acetylcholine receptor channel blocking site. Further evidence for this is obtained from single channel currents in chick myotubes where they do not act as agonists and in cutaneous pectoris muscle where, at much higher concentrations, they do not depolarize endplates previously treated with MSF. Supported by ES04189.

207.8

COMPARATIVE EFFECTS OF ACUTE IN VIVO AND IN VITRO EXPOSURE TO THE PARALYTIC AGENT DITHIOBIURET. J.M. Spitsbergen* and W.D. Atchison. Dept. Pharm./Tox., Mich. State Univ., E. Lansing, MI 48824.

Chronic treatment of rats with dithiobiuret (DTB) causes a delayed onset muscle weakness associated with decreased quantal release of ACh. Acute administration of DTB at doses far in excess of those needed to cause paralysis when given chronically does not cause muscle weakness. The objective of this study was to determine to what extent acute in vivo and in vitro administration of DTB mimicked effects of chronic DTB treatment on neuromuscular transmission. EPPs and MEPPs were recorded from rat hemidiaphragms. In vivo experiments used diaphragms taken from rats 1 hr after acute treatment with 25 mg/kg DTB, ip. In vitro experiments used diaphragms from untreated rats exposed by bath application to a DTB concentration (1.85 mM) approximating that in rats after an acute dose. One hr after injection EPP and MEPP amplitude, and MEPP frequency were decreased, and MEPP and EPP rise and decay times were prolonged. Bath application of DTB initially increased MEPP amplitude and frequency and EPP amplitude. With continued treatment, all three variables were reduced. EPPs were abolished after approximately 10 min exposure to DTB. Rise and decay times for MEPPs and EPPs were also prolonged significantly compared to pretreatment values. These experiments suggest that acute in vivo and in vitro exposure to DTB causes both pre- and postsynaptic effects and that these effects are similar to those seen in rats paralyzed following chronic DTB treatment. (Supported by NIH grant NS20683.)

207.9

INTERACTION OF PREDNISOLONE, CHOLINE, AND VESAMICOL (AH5183) ON NEUROMUSCULAR TRANSMISSION. D.F.Wilson* and P.Zhong* (SPON: R.G.Sherman) Zoology Department, Miami Univ., Oxford, OH 45056.

The interactions of prednisolone, choline,and vesamicol on spontaneous transmitter release were examined in an attempt to identify the mechanism of action of prednisolone on neuromuscular transmission. Intracellular recording techniques were used to monitor miniature end-plate potentials (MEPPs) in the isolated rat diaphragm-phrenic nerve preparation. The preparation was bathed in saline containing 10 mM K^+ to enhance transmitter synthesis and vesicle turnover. It is known that prednisolone (10 μM) enhances the amplitude of the MEPPs, enhances ACh release, and increases the size of the synaptic vesicles. There are at least four mechanisms that could explain these actions a) enhances choline uptake b) enhances the supply of acetyl-CoA, c) enhances the ACh uptake mechanism of the vesicles d) directly enhances ACh synthesis. We examined two of these hypotheses. In choline free bathing medium, 10 μM prednisolone enhanced the amplitude of the MEPPs by 29% . Choline concentrations of 0.1, 1.0 and 30 μM had no effect on the MEPPs in the presence or absence of prednisolone. Vesamicol in low concentration (0.5 μM) depressed the MEPP amplitude by 24% and blocked the action of prednisolone. We conclude that prednisolone does not directly influence ACh uptake into the vesicles or choline uptake into the nerve terminal . We suggest that prednsiolone may stimulate ACh synthesis via stimulating choline acetyltransferase activity or enhance the supply of acetyl-CoA. (Supported by NIH grant NS-23195).

207.10

"L" TYPE OF CALCIUM CHANNEL IN NERVE TERMINAL IS ASSOCIATED WITH TRANSMITTER RELEASE. C.A. Lindgren & J.W. Moore & A.H. Sostman*. Dept. of Physiology, Duke University Medical Center, Durham, N.C. 27710

Fox, Nowycky & Tsien (1987, *J. Physiol. 394*, 149) demonstrated the *coexistence* of three types of calcium channel, called L, N, and T, in neurons from chick dorsal root ganglia (DRG) and showed that it was possible to distinguish between them on the basis of their sensitivity to various pharmacological agents (see Table). This raised the issue of which (if any) of these types of calcium channels is responsible for transmitter release at a neuromuscular junction (NMJ).

We asked this question in presynaptic motor nerve terminals on the ceratomandibularis muscle in the lizard, *Anolis carolinensis*, and have found a single calcium channel which is best characterized as an "L" type. Using focal, extracellular electrodes we measured both end-plate current (EPC) and presynaptic calcium current (I_{Ca}) while applying the inorganic and organic calcium channel blockers and activators used by Fox *et. al.* (1987). The results are summarized on the right below:

	DRG NEURONS			LIZARD NMJ	
	L	T	N	I_{Ca}	EPC
BLOCKERS:					
Cadmium	Yes	No	Yes	Yes	Yes
Nickel	No	Yes	No	No	No
Omega Conus Toxin	Yes	No (weak)	Yes	Yes	Yes
Nifedipine	Yes	No	No	Yes	Yes
ACTIVATOR:					
Bay K 8644	Yes	No	No	—	Yes

207.11

INCREASED EXPRESSION OF TYPE II CALCIUM/CALMODULIN-DEPENDENT KINASE AND VOLTAGE-SENSITIVE CALCIUM CHANNELS DURING DIFFERENTIATION OF NEUROBLASTOMA/GLIOMA CELLS. C.M. Beaman-Hall*, R.D. Veenstra* and M.L. Vallano. Dept. of Pharmacology, SUNY/Health Sci. Ctr., Syracuse, NY 13210.

A type II calcium/calmodulin-dependent protein kinase (CaM kinase) was characterized in a neuroblastoma/glioma cell line (108CC15/NG108-15) using biochemical and immunochemical techniques. Under specific culture conditions the cells "differentiate", a process characterized by elaboration of long neurites, ability to form synapses and secrete ACh, and development of electrical excitability. Since CaM kinase utilizes Ca^{2+} as a second messenger and has been implicated in a variety of depolarization-dependent synaptic events, we compared the expression of CaM kinase and voltage-sensitive Ca^{2+} channels in NG108 cells in the undifferentiated and differentiated states. A 2-5 fold increase in the amount and specific activity of CaM kinase in differentiated cells was observed compared to control cells. Whole-cell voltage-clamp recordings revealed a small (200-300pA), rapidly inactivating N-type Ca^{2+} current in undifferentiated cells which increased 8-10 fold during the first 48 hours of differentiation. Expression of a second inward current with properties similar to the L-type Ca^{2+} channel also emerged during differentiation. Depolarization-dependent, Ca^{2+}-dependent phosphorylation of several potentially important substrate proteins for CaM kinase was also observed in differentiated cells. (Supported by NSF Grant 8602617).

207.12

A ROLE FOR PROTEIN KINASE C ACTIVATION IN THE RELEASE OF EXCITATORY AND INHIBITORY TRANSMITTERS FROM THE ISOLATED RODENT OESOPHAGUS. C.R. Triggle*, S. Will* and D. Bieger (SPON: P. Redfern) Division of Basic Medical Sciences, Memorial University of Newfoundland, St. John's, NF, Canada, A1B 3V6

High concentration of protein kinase C (PKC) in neuronal tissue is suggestive of a function in regulating neuronal activity and several studies have indeed linked activation of PKC to enhanced neurotransmitter release. In this investigation, we have studied the effects of phorbol esters, the diterpine mezerein and the synthetic diacylglycerol, 1-oleoyl-2-acetylglycerol (OAG), on tetrodotoxin-sensitive contractions and relaxations elicited by field stimulation of the isolated inner smooth muscle component of the rat oesophagus. Our results show that low concentrations (20-200 nM) of the active phorbol ester, phorbol 12, 13 dibutyrate (TPA), and the non phorbol ester mezerein enhance both contractions and, in the presence of active tone, relaxations elicited by field stimulation. The inactive phorbol ester, phorbol 12-myristate-13-acetate 4-O-methyl ether, even at 10 μM, was without action on these parameters, whereas OAG, at 5 μM, mimicked the actions of TPA and mezerein. The effects elicited by the assumed activation of PKC could not entirely be explained by post-synaptic effects thus suggesting PKC activation leads to an enhanced release of both excitatory and inhibitory neurotransmitters. Supported by the MRC (MA 9141).

207.13

THE EFFECT OF LITHIUM ION ON PROTEIN KINASE C (PKC) TRANSLOCATION AND ITS RELATION TO SEROTONIN (5-HT) RELEASE IN CORTICAL BRAIN SLICES H-Y Wang and E. Friedman Medical College of Pennsylvania, Philadelphia, PA 19129

Activation of Ca^{++}-, phospholipid-dependent PKC by phorbol esters induces its translocation from cytosol to membrane. PKC stimulation also potentiates K^+-induced serotonin release. Li, especially following long-term administration was found to (1) affect 5-HT release, (2) interfere with PI hydrolysis, and (3) alter membrane lipid metabolism. The effect of Li on PKC activity was, therefore, investigated.

PMA (Phorbol 12-myristate, 13-acetate) or PDBu but not 4 α-PDD increases K^+-induced 5-HT release and to a smaller extent spontaneous [^{3}H]5-HT efflux in a dose-dependent manner (Wang and Friedman, Eur. J. Pharmacol., 141, 15-21, 1987). This effect was obviated by 2-4 mM LiCl in vitro. Furthermore, 3 but not 1 week of Li treatment suppressed PMA-induced facilitation of 5-HT release. In vitro Li affected neither PKC distribution nor PKC activity at concentrations of 1-4 mM. However, Li blocked PMA-induced PKC translocation at therapeutic concentrations both in vitro and in vivo. This Li-evoked inhibition of PKC translocation may be responsible for the reduction in PMA-induced potentiation of 5-HT release and its therapeutic effectiveness.

207.14

EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON PROTEIN PHOSPHORYLATION IN RAT CEREBRAL CORTEX. N. Brunello, D. Tinelli*, J. Perez* and G. Racagni. Center of Neuropharmacology, Univ. of Milan, Italy.

Chronic antidepressant administration is associated with a number of adaptative changes in monoaminergic system. In the present study the pattern of protein phosphorylation in the soluble and microtubule fraction of rat cerebral cortex was investigated after chronic desipramine administration. This experiment demonstrated a higher incorporation of ^{32}P in the protein band of apparent M.W. 280 KD in basal conditions. ^{32}P incorporation, was determined in the presence of cAMP thus demonstrating that the 280 KD protein is effectively phosphorylated by an endogenous cAMP-PK. The high molecular weight and the cAMP dependent protein kinase phosphorylation could suggest that this protein may represent a microtubule associated protein. Since one important step in the phosphorylation system is the regulation of PK, we have studied the soluble cAMP-PK after chronic DMI treatment. The enzyme was partially purified on DEAE cellulose column. After elution with a linear gradient of NaCl the peaks I and II were subjected to a SDS-PAGE electrophoresis (10%). In these conditions an increase in the protein band of apparent M.W. 56 KD following DMI administration was observed. The elution profiles and the molecular weight could suggest that this protein band is the regulatory subunit of cAMP-PK. An acute administration of DMI did not induce any modification indicating that changes in the phosphorylation pattern could be related to the biochemical modifications induced by a chronic treatment with tricyclic antidepressants.

207.15

INHIBITORY SYNAPTIC POTENTIALS IN DORSAL RAPHE: ACTION OF COCAINE Z.Z. Pan*, W.F. Colmers & J.T. Williams. Vollum Institute, Oregon Health Science University, Portland, OR 97201.

Intracellular recordings were made from rat dorsal raphe neurons in slice preparations. Focal electrical stimulation was used to evoke synaptic potentials which consisted of a depolarization (DSP) that was made up of two components (GABA and excitatory amino acid) followed by a hyperpolarizing potential (IPSP). The IPSPs were blocked by spiperone and were increased in amplitude and duration by cocaine (300 nM). With higher concentrations of cocaine (1-10 μM), the duration was further prolonged but the amplitude declined progressively. The IPSP was also studied following blockade of the DSP with antagonists at $GABA_A$ and excitatory amino acid receptors. In these experiments, cocaine depressed the amplitude of the IPSP, even at 300 nM. The cocaine induced increase in IPSP duration was not different from control. These results suggest that the time course of 5-HT mediated synaptic potentials is dependent on a cocaine sensitive reuptake mechanism. Cocaine also seems to cause presynaptic inhibition of 5-HT release in this preparation; an effect which is also sensitive to excitatory input onto the 5-HT neurons.
Supported by USDHHS grant DA04523.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CORTEX III

208.1

MOTOR CORTICAL UNIT ACTIVITY PRE AND POST LATERAL COLUMN LESION IN THE BEHAVING MONKEY. S.A. Sahrmann, M.H. Clare, E.B. Montgomery, W.M. Landau. Dept of Neurology, Washington Univ Sch of Medicine, St. Louis, MO 63110

A macaque was trained to perform 4 mirror image isometric ankle tasks triggered and guided by light signals: (1) from rest to large (L) plantarflexion (PL), hold for a random period, rapidly reverse into large dorsiflexion (DO) and return to rest; (2) the mirror image task; (3) small (S) PL with a random hold and relax to rest; and (4) its mirror image S DO. Units were recorded from hindlimb area 4 (M1). The mean of 250 msec intervals of the 4 rest periods was used for resting frequency (RF). A 50% change from RF in the mean of a 100 msec period was considered significant. Preliminary analysis of 99 of 284 related units indicated the RF was increased after complete unilateral section of the lateral spinal column at T10 (47 units, pre 15.8; 52 units, post 23.6). Forty-six percent of pre and 33% of post-lesion units were tonic, maintained activity during hold. Unidirectional (UD) units, those with increased activity with only PL or DO action, were found in equal numbers as bidirectional (BD) units. BD units had a greater peak frequency (PF) for one direction but also increased with movement in the opposite direction. Two times more PL UD pre and post lesion and BD pre units were recorded than DO units but BD PL were less than BD DO units post-lesion. Eighty-four percent DO units participated in all 3 DO task opportunities, both pre and post-lesion. Only 26% pre and 46% post PL units responded to the 3 PL opportunities. The PF for L and for S tasks was the same for DO pre and post lesion and for PL post. The PF for pre lesion PL units was 2 times greater for L than for S tasks.

208.2

INTERHEMISPHERIC ASYMMETRY IN THE AREA 4 REPRESENTATION OF MOVEMENTS IS CORRELATED WITH HAND PREFERENCE. R.J. Nudo, W.M. Jenkins, and M.M. Merzenich, Department of Neurobiology & Anatomy, University of Texas Health Science Center, Houston, TX 77225 and Coleman Laboratory, University of California at San Francisco, San Francisco, CA 94143.

We have found large interhemispheric differences in the representations of distal forelimb movements in primary motor cortex of the squirrel monkey. These differences appear to be related to hand preference in a reaching task. Hand preference was determined using a modified Klüver board. Each animal's hand preference became stable after only limited exposure to the task and remained so when retested over four months later. In each animal, standard intracortical microstimulation techniques were used to map the primary motor cortex of both hemispheres. At each site, the movement obtained with just-suprathreshold stimulation was determined. For brevity, only major classes of movement are described here (e.g., "digit" includes thumb extension, digit 2-5 flexion, etc.). Representations of movements of the digits, wrist, elbow and shoulder were studied in parallel rows of penetrations, about 200 µm apart, then the borders separating these movements were defined. In the primary motor cortex contralateral to the preferred hand, the total digit and wrist area was larger, the total boundary length between digit and wrist regions was longer, and the number of discrete digit and wrist regions was greater compared with the ipsilateral cortex. Indices of complexity ,which normalized absolute areas, also were greater contralateral to the preferred hand. Other features of representational maps, such as relative proportion of digit and wrist area, were approximately the same in the two hemispheres. Large interhemispheric differences are present in the representation of movements in primary motor cortex. These map differences may underlie behavioral asymmetries.
Supported by NS-10414 & NS-07553.

208.3

CHARACTERISTICS OF THE FUNCTIONAL ORGANIZATION OF MOTOR CORTEX (MI) HAND REGION IN THE ALERT MONKEY T.M.J.Wannier*, M.A.Maier* and M.-C. Hepp-Reymond Brain Research Institute,8029 Zurich, Switzerland

To investigate the properties and topographical distribution of neurons related to isometric force, 4 monkeys were trained to generate in the precision grip 2 successive step-and-hold force increases. Single cell activity was recorded in MI hand region during the task and in response to somatic and occasionally visual stimulation. The neurons related to force were classified according to their firing patterns (phasic, phasic-tonic, tonic, decreasing, mixed). Two-dimensional reconstructions of the recording sites were made. The convexity contained a greater proportion of decreasing neurons, and some neurons active during ipsilateral finger movements. Most neurons responded to stimulation of deep rather than cutaneous receptors, without clear gradient towards the convexity. A few cells were also activated by stimuli moving across the monkey's visual field. Microstimulation often elicited more complex movements in the convexity than in the bank.

In conclusion, somatic and visual inputs to MI neurons suggest complex sensorimotor integrations. Further, dissimilar properties have been disclosed for the cell populations located in the bank and convexity of the precentral gyrus.

208.4

PINNA MOVEMENTS ELICITED BY MICROSTIMULATION IN THE PREFRONTAL CORTEX OF MONKEYS. D. Burman, C.J. Bruce, and G.S. Russo. Sect. Neuroanatomy, Yale Univ. School Medicine, New Haven, CT 06510.

The position of the pinnae (external ears) were studied in conjunction with microstimulation of cortex in and near the frontal eye fields (FEF) region of the rhesus monkey's prefrontal cortex. Pinnae movements were observed visually and measured via search coils acutely mounted on both the left and right ears. Eye movements were simultaneously observed via a search coil in one eye.

Movements of one or both ears were consistently elicited by microstimulation (thresholds 10-50µA) within a discrete cortical region. At most sites the elicited movement served to direct one or both ears contralaterally, with the contralateral ear usually moving back and the ipsilateral ear either stationary or moving foward to a lesser degree. Ear movement latencies ranged from 40-70 ms. The movement usually continued for the duration of stimulation (25-250 ms). Ear movements of 2-5° were common at threshold and increasing the stimulation current could increase the amplitude to 10° or more.

The cortical zone from which ear movements were obtained at low current thresholds lay between the superior limb of the arcuate sulcus and the posterior portion of the principal sulcus. This is very near the the FEF zone where large amplitude saccadic eye movements are obtained; however, the ear and eye fields appear to be separate as we did not observe eye and ear movements together at a single site. Ear movements were elicited from superficial cortex of the prearcuate gyrus or lip of the arcuate sulcus whereas saccadic eye movements were elicited deeper (in the anterior bank of the arcuate sulcus) and posterior to the ear movement area.

These data suggest that this frontal ear movement area serves primarily to position the pinnae to better monitor sounds from contralateral space, just as FEF stimulation contralaterally directs the eyes. Moreover, we recently discovered that monkeys consistently direct their pinnae towards their direction of gaze. Together, these studies indicate that the primate's FEF, together with this neighboring pinna movement area, constitute a cortical system for orienting both the eyes and ears.

208.5

DISTRIBUTION OF THRESHOLD CURRENTS FOR MOVEMENT IN BABOON MOTOR CORTEX: FACE AND FORELIMB REPRESENTATION. D.D. Samulack, C. Chau*, R.W. Dykes, [1]R.S. Waters and P.A. McKinley. Depts. of Physiology, Physical and Occupational Therapy, Neurology and Neurosurgery, McGill Univ., Montreal, PQ, H3G 1Y5 and [1]Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, TN 38163.

Thresholds (TH) for motor responses of the digits, wrist, face and tongue were analyzed in the motor cortex of 4 female baboons using intracortical microstimulation. Distribution of TH currents for eliciting movement was analyzed with respect to body part, cortical layers and white matter. Results indicated that while all body parts had TH values <5μA, the overall distribution was different. 16% of THs were <5μA for face and tongue. However, 55% of tongue THs were evenly distributed between 5-25μA, while only 44% of face THs were <20μA; primarily <5μA. Only 6% of THs were <5μA in wrist and digits. 41% of the digit THs were <20μA and these were evenly distributed. In contrast, 50% of wrist THs were <20μA; distribution was bimodal with peaks at 20μA(16%) and 50μA(15%). Overall TH averages were lowest in layers V and VI (14μA), and highest in layer I and the white matter (34μA). However, THs <5μA were consistently recorded in the white matter.

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208.6

EFFECTS ON MUSCLE ACTIVITY FROM MICROSTIMULI APPLIED TO PRIMARY SOMATOSENSORY CORTEX (SI) DURING VOLUNTARY MOVEMENT IN THE MONKEY. G.L. Widener and P.D. Cheney. Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66103

Projections from SI cortical areas, particularly 3a and 2, to motor cortex suggest that SI may exert a direct and potentially powerful control over corticospinal output to motoneurons. To test the strength of the linkage from SI to motoneurons, we applied microstimuli to areas 3a, 3b, 1 and 2 in three monkeys trained to make wrist movements alternating between flexion and extension position zones. Microstimuli were delivered as individual pulses (S-ICMS) and as short trains 330 Hz (R-ICMS). Individual stimuli or stimulus trains were used as triggers for computing averages of on-going emg activity from 12 forearm flexor and extensor muscles. Effects from stimulus sites in areas 3a, 3b, 1 and 2 were compared to those from sites of corticomotoneuronal (CM) cells in area 4. All CM cell sites yielded effects on muscle activity with S-ICMS at 20 μA. In contrast, the same stimulation applied to areas 3a, 3b, 1 and 2 yielded effects at only 11%, 0%, 4% and 3% of the sites respectively. Effects from area 3a were predominantly inhibitory, whereas effects from other SI areas were more equally mixed. These results suggest that the linkage between SI cortical areas and motoneurons is weak and unlikely to be directly involved in the generation of muscle activity. Supported by NSF grant BNS-8216608.

208.7

RECEPTIVE FIELD AND DISCHARGE PROPERTIES OF PRIMARY SOMATOSENSORY CORTICAL UNITS DURING ACTIVE TACTILE DISCRIMINATION IN THE MONKEY. S.A. Ageranioti and C.E. Chapman, Université de Montréal, Montréal, CANADA

This study examined the receptive field (RF) and discharge properties of neurones in primary somatosensory cortex of 2 monkeys trained to perform an active scanning movement of the digits over either a smooth or a textured surface. Recordings were made from 92 neurones in the cortical hand area which were classified as receiving input from the glabrous skin of either a single digit (sd, n=16) or multiple digits (md, n=76).

Overall, 37% of the units were unmodulated in the task (26 md, 8 sd), with 18 of these being spontaneously active. Sixty three percent of the cells were modulated in the task, with 48 showing an increase (41 md, 7 sd) and 10 a decrease (9 md, 1 sd). Sd and md units had similar discharge patterns and the relative magnitude of their responses during the task were largely similar. Thus, units with apparently similar RF properties failed to show the same discharge in this task. The absence of any marked difference between units with md and sd RFs would argue against the former units having a special integrative role in the processing of tactile inputs from the glabrous skin of the hand. Supported by the Canadian MRC and the FRSQ.

208.8

THE TOPOGRAPHICAL MODULATION OF SOMATOSENSORY TRANSMISSION TO SI CORTEX PRODUCED BY MICROSTIMULATION OF MI CORTEX IN THE MONKEY. W. Jiang, C.E. Chapman and Y. Lamarre, Université de Montréal, Montréal, Québec, Canada H3C 3J7

Sensory transmission to primary somatosensory cortex (SI) is modulated during voluntary movement. The purpose of this study was to determine the topography, if any, of the modulation of the somatosensory inputs to SI cortex produced by intracortical microstimulation (ICMS) in primary motor cortex (MI) of the awake monkey.

ICMS (train of 11 pulses at 330 Hz, 0.2 ms pulse width) was applied to low threshold ($<14\ \mu A$) sites in MI and cutaneous evoked potentials (20 ms airpuff) were recorded in the adjacent SI arm/trunk representation.

The data of 31 experiments (2 macaque monkeys) showed that ICMS (1-1.5 x threshold) either inhibited the evoked potential in SI or had no effect. Inhibitory effects were observed when the SI receptive field was distal to, or overlapped, the muscle activated by ICMS. Cutaneous inputs from receptive fields proximal to the target muscle were not modulated by ICMS. The results indicate that motor cortex modulates sensory inputs in a topographical manner so as to suppress inputs from the limb segments distal to the moving part. Supported by the MRC of Canada and the FRSQ.

208.9

SINGLE UNIT ACTIVITY IN THE FRONTAL CORTEX OF MONKEYS PERFORMING A DELAYED LOCALIZATION TASK. E. Vaadia*, H. Bergman* E. Margalit* and M. Abeles* (SPON: A. Mitz). The Hebrew University, Hadassah Medical School, P.O.B. 1172, Jerusalem, 91010, ISRAEL.

In a previous study it was demonstrated that the activity of some neurons in the frontal cortex is related to active localization behavior. The present study was designed to distinguish neuronal activities related to sensory-perceptual processing from processing related to the preparation of movements. Monkeys were trained to perform two paradigms of a delayed response task: In a "localizing" paradigm two brief stimuli (auditory and visual "spatial cues") were presented successively from two different locations. After delay, a non-spatial cue instructed the monkey to select one of the two locations and touch it. In the second paradigm, the same stimuli were presented. However, the reinforced response was a movement to a predetermined, fixed location. Task related neurons were activated in relation to the spatial cues (88/201), between the onset of the non-spatial cue and the initiation of the movement (68/201) and during the movement (73/201). Some neurons had two or more components of activity. Many neurons showed some degree of spatial tuning (65/88) and paradigm dependency (109/201). The findings indicate that neuronal activities in premotor and prefrontal areas are involved in both stimulus-related perceptual processing and in the preparation of movements. Further, a single unit may be involved in both aspects of task performance.

208.10

SIMULTANEOUS RECORDING OF SINGLE UNITS IN THE FRONTAL CORTEX OF BEHAVING MONKEYS. H. Bergman*, M. Abeles*, E. Ahissar*, Y. Lavner* and E. Vaadia* (SPON:M.H. Goldstein).The Hebrew university, Hadassah medical school, P.O.B 1172, Jerusalem, 91010 Israel.

The activity of several (up to 11) neurons was recorded simultaneously by 6 microelectrodes. Recordings were made in the frontal cortex of Rhesus monkeys during performance of a behavioral task. The electrodes were inserted into the cortex in a straight line (65 penetrations) or a circular arrangement (40 penetrations). The functional properties among "adjacent" neurons" (detected by the same electrode) and "non adjacent neurons" (detected by different electrodes) were analyzed during several phases of task performance. The coactivation of neuron-pairs was examined by crosscorrelograms. Modulations of single unit activity were examined by Peri-Event Time Histograms (PETH) and by time-interval histograms.

The crosscorrelogram analysis indicated that many of the neurons pairs were co-activated even when the neurons were located 2mm apart (60% of the adjacent neuron-pairs and 30% of the non-adjacent pairs). However, the PETH analysis indicated that adjacent neurons could maintain distinctly different task related activity. These data support the view that while neurons in a small cortical area may be coactivated as a functional group to perform specific task, different groups may coexist within each small cortical area.

208.11

EEG AMPLIFIER AND FILTERS FOR DETECTING ALPHA AND BETA WAVES IN MONKEYS AND HUMANS. P.E. Volpe*, R.S. Remmel, (Spon:S. Roy), Biomedical Engineering, Boston Univ., and S. Schein, Massachesetts Eye and Ear Infirmary, Boston, M..

Our study with paralyzed monkeys requires an EEG instrument to determine states of alertness and anesthesia. A paralyzed monkey shows no reflexes for checking anesthesia. Alpha waves (approx. 5-10 Hz) indicate adequate anesthesia; beta waves (approx. 10-20 Hz) indicate stress and the need for more anesthetic.

The EEG from two scalp electrodes are input to a differential preamplifier having a common mode rejection of 100 dB. Motorola JFET LF356H operational amplifiers have very low noise for these 10-50 uV signals. Gain is adjustable between 3,000 and 300,000. Fourth order Butterworth filters have cutoff frequencies for alpha (4.97-10.6 Hz) and beta (10.6-22.7 Hz) waves. The lowpass filter with cutoff frequency f_c = 22.7 Hz is 6th order to attenuate 60 Hz noise; this Butterworth filter has gain

$$(1 + (f/f_c)^{12})^{-\frac{1}{2}}$$

This drops off as f^{-6} at high frequencies. Signals can be displayed on two channels of an oscilloscope or polygraph.

Noise and signals outside of a filter's bandwidth were sharply attenuated due to the multiple order of the filters. The instrument cost $300 for parts. Results with monkeys and humans will be reported.

208.12

AN IMPLANTED MICROWIRE TECHNIQUE FOR CHRONIC RECORDING IN THE CAT PERICRUCIATE CORTEX. C.L.Palmer, Institute of Physiology, Fribourg University, Switzerland.

One aspect of developing a reliable technique of recording with chronically implanted microelectrodes is that it can provide new insights into the functional micro-organization of the cortex. A technique is described in which lengths of 25ud. teflon insulated Pt/Ir wire are inserted into the pericruciate cortex for chronic single unit recording. The method was developed using 7 cats. In the last 2 cats from a total of 19 electrodes, 13 recorded single unit activity. The yield declined with time due to electrode movement, not electrode deterioration as their impedance measurements were constant.

The longest time a unit could be recorded from was two days, the unit was identified from the latency and threshold at which it was antidromically invaded from pyramidal tract (PT) stimulation. This occurred within the first 30 days after implantation and after 80 days of implantation suggesting that the electrodes were moving in the brain at a con constant rate.

Field potential recordings from PT and cutaneous stimulation were used to monitor electrode movement. The initial negative component of the cutaneous field potential had a longer latency and inverted to a positive potential as the electrode tip moved to superficial regions of the grey matter. The time course and sequence at which this occurred suggested that the electrode tip did not always move in a predictable manner which could be reconstructed from a histological examination of the electrode tract it left at the time of postmortem.

The power of the technique ie the nonselectivity of its recordings, the high yield, its recording stability, and its ability to obtain simultaneous recordings from different cortical regions.

208.13

CHARACTERISTICS OF RHYTHMICAL JAW MOVEMENTS (RJMS) ELICITED FROM CORTICAL MASTICATORY AREA (CMA) IN AWAKE PRIMATES. C.-S. Huang[+], H. Hiraba*, G.M. Murray and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Toronto, Canada M5G 1G6; [+]Chang Gung Mem. Hosp., Taipei, Taiwan.

We have recently described the topographical organization of three patterns of RJMs that we could induce by intracortical microstimulation (ICMS) within the lateral precentral cerebral cortex (CMA). This study describes the temporal features of the RJMs and associated EMG activity evoked by ICMS (0.2 ms pulses, 50 Hz, 3 s train, ≤ 60 μA) of CMA in 3 awake monkeys (Macaca fascicularis). We confirmed that the 3 patterns of RJMs were vertical RJMs, contralaterally directed RJMs, and ipsilaterally directed RJMs (RJMi). The mean (±SD) cycle times of the 3 RJM patterns ranged from 0.94±0.2 to 1.24±0.19 s, and the amplitude of opening ranged from 3-9 mm. Digastric and genioglossus EMG activity occurred in all 3 patterns, but masseter activity was prominent only in RJMi in which it often partly overlapped digastric and genioglossus activity. Digastric showed the first increase in activity (mean onset latency 0.39±0.08 s);this was often preceded by a period of inhibition. These features showed many similarities with natural chewing parameters recorded in the same animals and support the view that CMA is involved in the production and control of primate masticatory movements. Supported by Canadian MRC.

208.14

DISCHARGE PROPERTIES OF SINGLE NEURONES WITHIN PRIMATE TONGUE MOTOR CORTEX DURING TRAINED MOTOR TASKS. G.M. Murray, L.-D. Lin, C.-S. Huang and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Toronto, Canada M5G 1G6.

The tongue motor cortex (MI) has been implicated in the control of tongue movements. This study's aim was to see if tongue MI neurones increase their activity in advance of tongue movement during a trained tongue protrusion task and to compare this activity with that during a trained biting task. Extracellular recordings were made in an awake monkey from tongue MI (defined by intracortical microstimulation; ICMS ≤ 20 μA) in which 120 neurones were found to alter their activity during tongue movements. We studied 37 of them during the monkey's trained tongue task, and also tested them for an orofacial mechanosensitive afferent input; some were also studied during the biting task. ICMS at the recording sites of the 37 neurones evoked tongue movements, and an orofacial input was identified in 28 of which 23 had a mechanoreceptive field localized to the tongue. 34 of the neurones increased their activity during the tongue task, and 9 of these showed a marked increase in activity up to 100 ms in advance of EMG evidence of the commencement of tongue movement. 4 of the 9 were also studied during the biting task but showed little change in activity during this task. These data suggest that some tongue MI neurones may preferentially drive tongue movements and support the view that tongue MI is a major component of the control system for tongue movements. Supported by Canadian MRC.

208.15

NEURONAL ACTIVITY IN FACIAL PRIMARY SOMATOSENSORY CORTEX (SI) DURING INGESTION AND MASTICATION IN AWAKE CATS. H.Hiraba*,T.Yoshida* and R.Sumino* (SPON:A.Mori). Dept. of Physiol., Sch. of Dent., Nihon Univ., 1-8-13 Kanda-surugadai, Chiyoda-ku, Tokyo 101, Japan

We investigated the function of the facial SI neurons in relation to spontaneous ingestion and mastication in awake cats. Cats rested comfortably in a box and the head was rigidly mounted by means of two metal bolts and embedded in a dental acrylic crown. The cats were trained to accept oro-facial natural stimulation, and ingest and masticate food (milk, fish flake, salami) without struggling. EMG electrodes were surgically implanted into the masseteric and the digastric muscles. Jaw movement was monitored in the vertical and horizontal axis by a photodiode transducer system. Single neuronal activities were recorded in left prefrontal cortex using a glass-coated Pt-Ir microelectrode. Receptive fields and submodality preferences of neurons were examined with hand-held probes, brushes and other tools.

Approximately one third of the neurons in the oro-facial area of SI were related to ingestion and mastication. Almost all were found in areas 3a and 3b, and few in areas 1 and 2. Area 3b which responded to simple light mechanical stimuli to the perioral hair showed the rhythmical firing when the lower lip touched the upper one during mastication. Neurons with their receptive field in intraoral organs fired in sustained bursts during mastication. Some neurons in area 3b showed increased activity prior to ingestion (about 400ms), which were not related to mastication. Area 3a neurons responded to pressure or tapping of muscles or periodontium. Most neurons in area 3a showed decreased firing at the time of ingestion and sustained bursts during mastication. (The Sato Scholarship of Nihon University School of Dentistry)

209.1

ANDROGEN RECEPTORS IN HUMAN TEMPORAL CORTEX. A. Sarrieau, J. Mitchell, and M.J. Meaney. Douglas Hospital Research Ctr., Dept. Psychiatry, McGill Univ., H4H 1R3 Canada.

This work describes the presence of androgen receptor sites in human temporal cortex obtained from 5 adult epileptic patients. Using an in vitro, exchange assay with soluble fractions, with ^{3}H 5a-dihydrotestosterone (5a-DHT) as radioligand with cold 5a-DHT as competitor, we found an apparent K_d of 1.5±0.3 nM with a B_{max} of 50.9±9.1 femtomoles/mg protein. The order of potency of various unlabeled steroids to compete for ^{3}H 5aDHT binding was as follows: 5a-DHT=testosterone>estradiol= androstanedione>progesterone≈5BDHT. This pharmacological profile for the androgen receptor in human brain is virtually identical to that reported in monkey and rat. These data provide evidence for an intracellular mechanism by which circulating androgens might regulate neuronal function in the human cortex. (Supported by grants from the Natural Sciences and Engineering Research Council to MJM).

209.2

PROLONGED SUPPRESSION OF ANDROGENS AND ANDROGEN-DEPENDENT TISSUES WITH A BRAIN-ENHANCED DELIVERY SYSTEM FOR ESTRADIOL. W.R. Anderson, J.W. Simpkins, M.E. Brewster* and N. Bodor*. Center for Drug Design and Delivery, Univ. of Florida, Gainesville, FL 32610.

We have developed a brain-enhanced delivery system for estradiol based upon an interconvertible dihydropyridine ⇌ pyridinium salt carrier. The aim of this study was to evaluate the effects of a single 1.0 mg/kg BW dose of estradiol chemical delivery system (E2-CDS) versus an equimolar dose of estradiol benzoate (E2-BZ) on weights of ventral prostate, seminal vesicles and serum hormones in gonadally-intact Sprague-Dawley rats. The single injection of E2-CDS reduced prostate weight by 52% on day 7 and weights remained suppressed to day 21 while E2-BZ was ineffective. Seminal vesicle weights were suppressed on day 1, were maximally reduced by 40% on day 7 and were less than controls for 21 days after E2-CDS treatment. Serum testosterone levels were suppressed with E2-CDS for 1 to 21 days and DHEA concentrations were reduced with both forms of E2 at 1 week only. Serum LH levels were diminished at 24 h with E2-CDS and E2-BZ. Serum estradiol was elevated for 1 week by E2-CDS and for 24 h by E2-BZ while prolactin levels and anterior pituitary weights were increased for 2 weeks with E2-CDS. These data suggest that the brain-enhanced delivery of estradiol causes chronic suppression of serum androgens and reduction in weights of androgen-dependent tissues. (Supported by grants HD 22540, GM 27167 and Pharmatec, Inc.)

209.3

BETHANECHOL-INDUCED INCREASE IN HYPOTHALAMIC ESTROGEN RECEPTOR BINDING IS RELATED TO CAPACITY FOR LORDOSIS BEHAVIOR. A.H. Lauber and R.E. Whalen, Dept. of Psychology, Univ. of Calif., Riverside, Ca 92521

Previously, we demonstrated that bethanechol, a muscarinic cholinergic agonist, increased the concentration of soluble estrogen binding sites by 30-35% in female rat hypothalamus but had no effect on males (Brain Res. 443). This study was designed to investigate the relationship between modulation of estrogen binding sites and a sexually differentiated function. Female rats were given high or low doses of testosterone neonatally. In adulthood, high androgen females were "male-like" in responsiveness to estrogen as they were anovulatory and failed to display lordosis. Low androgen females were anovulatory but showed high levels of lordosis. Bethanechol (30 mg/kg) or saline was given 30 min prior to in vitro receptor assays. Amount of estrogen binding by hypothalamic cytosols from drug-treated high androgen females did not differ from levels in saline-treated high androgen controls ($p > .05$). However, bethanechol induced a significant increase in estrogen binding by preparations from low androgen females compared to saline-treated low androgen controls ($p < .01$). These findings suggest that the capacity for drug-induced modulation of estrogen binding sites may be related to the ability to display estrogen-dependent lordosis behavior.

209.4

ESTRADIOL-INDUCED PROGESTIN RECEPTOR-IMMUNOREACTIVITY IS FOUND ONLY IN ESTROGEN RECEPTOR-IMMUNOREACTIVE CELLS IN GUINEA PIG BRAIN. J.D. Blaustein and J. Turcotte*. Neuroscience and Behavior Program and Psychology Department, Univ. of Mass., Amherst, MA 01003

A double-antibody, flourescent immunocytochemical technique was developed to determine if progestin receptors are induced in the same cells in the hypothalamus and preoptic area that contain estrogen receptors. As has been reported previously, priming ovariectomized guinea pigs with estradiol caused a large increase in the concentration of progestin receptor-immunoreactive cells in discrete regions of the hypothalamus and preoptic area, in particular, the arcuate nucleus, ventrolateral hypothalamus, periventricular preoptic area, and medial preoptic nucleus and area. Estrogen receptor-immunoreactive (ER-IR) cells were observed in high concentration in each of these areas, as well as in areas in which no PR-IR cells have been observed, such as the amygdala. PR-IR was never observed in the absence of ER-IR. In some areas such as the arcuate nucleus and ventrolateral hypothalamus, most highly-flourescent ER-IR cells also contained PR-IR, while in the amygdala no PR-IR was observed despite a high concentration of ER-IR cells. These results demonstrate that, in the brain as in other tissues, progestin receptors are induced in estrogen receptor-containing cells. (Supported by NS 19327 and RCDA NS 00970 from the NIH)

209.5

IN VIVO DOPAMINE (DA) RELEASE FROM THE CAUDATE NUCLEUS (CN) OF CONSCIOUS FEMALE RATS IN RESPONSE TO L-DOPA INFUSION IS INCREASED BY PROGESTERONE (P). D. Dluzen and V. Ramirez. Physiology & Biophysics, Univ. Illinois, Urbana, Il. 61801.

The in vivo release of DA following a direct infusion of L-DOPA into the CN of ovariectomized rats implanted with a push-pull cannula was examined under 3 hormonal conditions: 1)Estradiol Benzoate(EB)-4d sc EB(5µg/d); 2)EB+P+ 4-6h-EB+P(1.25 mg) at 4-6h pre-perfusion and 3)EB+P+24h-perfusion at 24h post-P. Perfusate samples(6-8 µl/min) were collected at 15 min intervals and assayed for DA using HPLC-EC. Following a one h(4 interval) basal collection period L-DOPA was directly infused through the push side of the cannula in 3 increasing doses(10^{-6},10^{-5} & 10^{-4} M) at intervals 5,9 and 14. For each of the 3 doses tested the EB+P+4-6h animals demonstrated a statistically significant increase in DA release following L-DOPA infusion compared to the EB and EB+P+24h groups. Areas under L-DOPA stimulated release curves(pg/75 min, $\bar{X}$+SEM, N=5) were:

	10^{-6}	10^{-5}	10^{-4}
EB -	337±67	4960±1460	12077±2214
EB+P+4-6h -	1296±397	10830±3585	22853±8733
EB+P+24h -	510±170	5253±1139	9986±374

While all 3 hormonal conditions show a dose-response release of DA to L-DOPA, overall release is double for EB+P+ 4-6h animals indicating that P initially exerts a positive modulatory influence upon DA release from the CN in response to L-DOPA infusions in freely behaving rats.

209.6

NORADRENERGIC TERMINALS MAY MEDIATE THE STIMULATORY EFFECT OF PREGNANOLONE ON LHRH RELEASE IN VITRO FROM RAT HYPOTHALAMI. O.K.Park and V.D.Ramirez, Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801

We have previously demonstrated that pregnanolone (0.01 ng/ml) when infused in a pulsatile mode stimulated LHRH release from rat hypothalami superfused in vitro. Since NE activates the LHRH neural apparatus, we examined in the present study whether noradrenergic neurons mediate the stimulatory effect of 5β,3β pregnanolone on LHRH release. Hypothalamic fragments from adult OVX+E_2 rats were superfused with a modified KRP medium in the absence or in the presence of 10^{-6}M TTX. Perfusate samples were collected at 10 min intervals and assayed either for LHRH (RIA) or monoamines-NE, Epi, DOPAC, DA and 5-HIAA (HPLC-EC). After 1 h control period of superfusion, 5β,3β pregnanolone (0.01 ng/ml) was administered in a pulsatile mode. Pregnanolone stimulated LHRH release in the absence of TTX, but not in the presence of TTX, indicating that pregnanolone does not directly affect LHRH nerve terminals. In addition, pregnanolone selectively ($p<0.005$) stimulated NE release from hypothalamic fragments superfused in vitro. This stimulation was also obtained even in the presence of TTX, indicating that pregnanolone directly affects noradrenergic nerve terminals. Taken together, these results suggest that pregnanolone stimulates LHRH release in vitro from hypothalamic fragments by activating noradrenergic neurotransmission.

209.7

PROGESTERONE RECEPTOR VIABILITY IN HYPOTHALAMIC SLICES CORRELATES TO INCUBATION OXYGENATION. RR Yeoman, LE White, JN Miller*, Ob/Gyn, Neurol, Anes; Univ South Alabama, Mobile, 36688

In vitro results may differ disturbingly from in vivo, and oxygenation may be a factor considering increased diffusional distances. In this study progesterone receptors and chamber media pO_2 were measured. Artificial CSF flowed (50 ul/min) from a reservoir through 3' of 1/8" vinyl tubing to superfuse the tissue. 400 um slices of estradiol primed rat hypothalami were incubated at 34° for 150 min. Control tissue assayed immediately after sacrifice had 54.7±15.9 fM/mg protein (mean ± SE). Neither air bubbled to tissue chamber nor 95%O_2/5%CO_2 bubbled to media reservoir maintained receptors (pO_2=162±6.4 and 178.8±3.9 Torr respectively). Alternately, 95%O_2/5%CO_2 bubbled to the chamber maintained receptors (68.9±13.1 fM/mg; pO_2=429.5± Torr). Additional oxygenation to the media reservoir and increased flow rate (500 ul/min) further elevated the chamber pO_2 (539±13.3 Torr) and sustained the receptors (41.9±4.5 fM/mg). In conclusion, adequate oxygenation appears necessary for maintaining progesterone receptors in brain slices and thus may be relevant to interpreting effects observed in vitro.

209.8

PROGESTERONE CONJUGATED TO BOVINE SERUM ALBUMIN BINDS BRAIN SYNAPTOSOMAL MEMBRANES. P.C.KE and V.D.RAMIREZ. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801

Neurophysiological studies have demonstrated a rapid action of progesterone (P_4) in the rat brain. Recently, we reported a LHRH releasing action from in vitro hypothalami of P_4 bound thru specific carbons to bovine serum albumin (BSA). The data suggested specific binding of the complex P_4-BSA to hypothalamic membranes. To investigate this possibility and extend our previous results, the binding to crude brain synaptosomal membranes of P_4 conjugated to BSA was studied. P_4-11-BSA (BSA conjugated at position 11 of the P_4 molecule) labeled with I^{125} to approximately 700 Ci/mmol specific activity was used as probe.

The binding reached saturation after 30 minutes of incubation. BSA conjugated at position 3 of the P_4 molecule (P_4-3-BSA), at position 11 (P_4-11-BSA), and BSA conjugated at position 21 of 11-deoxycorticosterone (DOC-BSA) were used for competition displacement studies. The order of the binding affinity in cerebullum was P_4-3-BSA (ED_{50} about 50 nM) > DOC-BSA (ED_{50} about 0.8 μM) > P_4-11-BSA (ED_{50} about 6 μM). Specific binding was showed in cerebral cortex, brain stem, corpus striatum and hypothalamus, but not in uterus. BSA did not have specific binding at all.

These results indicate that there is a plasma membrane binding site for P_4-BSA conjugate in rat brain. Because of the lack of specific binding for BSA, the binding site must be specific for progesterone. The order of the binding affinity among these three conjugated steroids demonstrated that the side chain and C ring of the P_4 moiety are important for binding. This agree well with our previous result demonstrating that only P_4-3-BSA had the capacity to stimulate LHRH release from rat hypothalami in vitro.

209.9

AN AUTORADIOGRAPHIC STUDY OF ESTRADIOL-INDUCED PROGESTIN RECEPTORS IN THE FEMALE SYRIAN HAMSTER BRAIN. Sarah W. Newman and Robert L. Meisel. Dept. of Anatomy and Cell Biology, Univ. of Michigan Medical School, Ann Arbor, MI 48109 and Dept. of Psychological Sciences, Purdue Univ., West Lafayette, IN 47907.

Estradiol treatment can stimulate the synthesis of progestin receptors in cells of the central nervous system, yet this receptor induction is evident only in a limited subset of the regions in which progestin receptors can be measured biochemically. In this study, we compared the autoradiographic pattern of progestin uptake (3H-ORG 2058) in the brains of estradiol-treated (10 ug estradiol benzoate/day for 2 days, N=4) or untreated (2 injections of oil, N=2) ovariectomized female Syrian hamsters.

In two females consistent labelling was found in cells in the medial preoptic area, particularly in the periventricular preoptic, arcuate nucleus, lateral edge of the ventromedial nucleus of the hypothalamus, and ventral premammillary nucleus. These findings are consistent with prior reports. In addition, we found a band of labelled cells along the base of the brain, lateral to the arcuate nucleus, perhaps corresponding to the caudal extension of the retrochiasmatic area. Of the remaining two estradiol-treated females, one had only a few labelled cells in these regions, and the other did not have any detectable label. No labelled cells were found in any region in females not treated with estradiol. Supported by NIH grants NS20269 (to SWN) and HD21478 (to RLM).

209.10

INTERMITTENT INFUSION OF PROGESTINS INTO THE HYPOTHALAMUS OF FEMALE RABBITS USING PUSH-PULL CANNULAE STIMULATES LHRH RELEASE IN VIVO. W. W. Lin and V. D. Ramirez, Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801.

Previously we have reported that intermittent infusion of progesterone (P_4, 10ng/ml), 5β-pregnane-3β-ol-20-one (0.001 ng/ml), and 20-α-hydroxypregn-4-en-3-one (20-α-OH-P, 10 ng/ml) into the hypothalamus of conscious unrestrained does stimulate LHRH release in vivo. In order to further investigate these phenomena, in the present studies, pulses of P_4 and 20-α-OH-P at various doses and another P_4 metabolite, 5α-pregnane-3α-ol-20-one (3α-5α-P), were infused into the hypothalamus of does using push-pull cannulae. Six does were perfused with modified KRP for 60-160 min followed by six pulses of a steroid (10 min on, 30 min off). P_4 was tested in one doe at 0.001, 0.01, 0.1, and 1 ng/ml while 20-α-OH-P was tested at 1 ng/ml (n=3) and 3α-5α-P was tested at 0.001 ng/ml (n=4). Pulses of P_4 were found to increase mean LHRH levels at 0.01, 0.1 and 1 ng/ml, but not at 0.001 ng/ml. 20-α-OH-P effectively increased mean LHRH levels in two does, but not in a third doe. 3α-5α-P was not effective in stimulating LHRH release at 0.001 ng/ml in three does although there was an increase in mean LHRH release in a fourth doe. Overall, these results indicate that P_4 is a very potent stimulator of LHRH release over a wide range of doses and P_4 metabolites are also effective at selective doses.

209.11

IN VIVO α_1 ADRENERGIC STIMULATION OF LHRH RELEASE FROM THE FEMALE RAT HYPOTHALAMUS USING PUSH-PULL CANNULAE. J.F. Gitzen, and V.D. Ramirez, Neural and Behavioral Biology Program and Dept. of Physiology and Biophysics, University of Illinois, Urbana, Il. 61801.

We have previously demonstrated that the in vitro pulsatile administration of the α_1 adrenergic agonist Methoxamine (MTX) stimulates the release of LHRH from superfused female rat medial basal hypothalami (MBH) (Endo. Soc.Abst., 1985, #945).

To establish that LHRH release is also stimulated in vivo by α_1 activation we utilized the push-pull cannula (PPC). PPC were implanted in the MBH of OVX rats. Experimental (N=5) and control (N=3) EB primed rats were initially perfused with modified KRP for two hours. KRP containing 10^{-4}M MTX or control KRP was substituted for the initial 20 minutes of the subsequent two hours. Effluent was collected every 10 min.

In both groups LHRH release showed moderate levels of pulsatility during the first two hrs. In all cases immediately following MTX infusion there was a robust spike of LHRH release. This was confirmed by PULSAR analysis. Total LHRH release during the pre MTX hour (.047±.007pg/min) increased significantly in the MTX treatment hour (.100± .019 pg/min). The pulsatility of control animals remained relatively constant; demonstrating a decrease rather than increase in total LHRH release over the corresponding two hours (.106±.013 v/s 0.070±.008pg/min). This in vivo observation confirms that the activation of rat MBH α_1 adrenergic receptors stimulates the release of LHRH.

209.12

LHRH PEPTIDE AND GENE EXPRESSION IN ORGANOTYPIC CULTURES GROWN IN THE PRESENCE OR ABSENCE OF SERUM: EFFECT OF TETRODOTOXIN. S. Wray and H. Gainer, LNC, NINCDS, Bethesda, MD 20892

Large numbers of luteinizing hormone releasing hormone (LHRH) neurons express their peptide hormone in slice explant roller cultures. These cultures are models for studying LHRH gene expression in a controlled environment. LHRH cells were characterized after 18 days *in vitro* by immunocytochemistry and *in situ* hybridization histochemistry (Wray et al., Soc. Neurosci. Abst., 13, 301.3, 1987). Large variations in steady state LHRH mRNA levels in neurons in the same culture slice were found. This could have been due either to different responses to biologically active agents in the serum containing media (SCM) and/or variations in spontaneous synaptic or electrical activity among LHRH cells in single culture slices. To address the influence of these factors on LHRH peptide and gene expression in culture, immunocytochemical and *in situ* hybridization histochemical studies were carried out in serum free media (SFM) or SCM in the presence or absence of tetrodotoxin (TTX).

400 micron brain slices from postnatal rats were plated on coverslips and grown in SCM (25% horse serum) for 12 days, and then divided into 2 groups: 1/2 were placed in SFM and the other 1/2 maintained in SCM. After 2 days, TTX (10^{-6}M) was added to 1/2 of the cultures in each group. On day 18, cultures were processed for either immunocytochemistry or *in situ* hybridization histochemistry. The number and distribution of immunopositive LHRH cells was maintained in SFM in the presence or absence of TTX. Although the addition of TTX did not change the intensity of LHRH immunopositive cells, secretion appeared to be inhibited since the intensity and extent of immunopositive fibers increased. We are currently examining whether the absence of serum and/or the presence of TTX differentially affected the mRNA level of LHRH cells in these slice explant roller cultures.

209.13

INVOLVEMENT OF ENDOGENOUS OPIOID PEPTIDES IN LHRH ULTRASHORT LOOP FEEDBACK IN THE SHEEP. A.M. Naylor, D.W.F. Porter* and D.W. Lincoln*. MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers St., Edinburgh, Scotland, U.K. EH3 9EW.

In the ovariectomized ewe, intracerebroventricular administration of LHRH causes a sustained and receptor-mediated inhibition of pulsatile LH release. Currently, the mechanism involved in this suppression is unknown but does not involve a desensitization of the pituitary gland to LHRH. Endogenous opioid peptides (EOP) have been implicated in the inhibitory control of LH secretion by modulating the hypothalamic release of LHRH. In addition, EOPs mediate the inhibitory effect of CRF on the LHRH pulse generator. The following studies were undertaken to determine whether opioid peptides are involved in LHRH ultrashort loop feedback using the opioid antagonist, naloxone.

At least 3 months prior to experimentation, Blackface ewes were ovariectomized and implanted (under halothane anesthesia) with cannulae directed towards the third ventricle. Plasma LH was measured in 10 min blood samples taken over an 11h period. Intracerebroventricular injections of LHRH were made twice at 4h and 5.5h whilst naloxone was injected intravenously (iv) 4h, 5.5h, 7h and 8.5h into the 11h sampling period.

Injection of LHRH (21pmoles in 50µl saline) into the third ventricle caused a marked inhibition of LH secretion. Pulse frequency and mean LH levels were reduced significantly when compared to the saline control (50µl). In contrast, iv naloxone (4 x 25mg, 1.5h apart) elevated mean LH levels. When LHRH was injected centrally along with iv naloxone, the inhibitory effect of LHRH persisted despite the ability of naloxone to stimulate LH release on its own.

In conclusion, these data suggest that EOPs are unlikely to mediate the LHRH-induced central suppression of LH release. Secondly, in the long term ovariectomized ewe, EOPs exert a tonic inhibitory influence on LH secretion.

209.14

CHANGES IN PRO-OPIOMELANOCORTIN GENE EXPRESSION IN THE ARCUATE NUCLEUS DURING PUBERTY IN THE MALE RAT. J.N. Wiemann*, D.K. Clifton* and R.A. Steiner (SPON: J.C. Slimp). Depts. of Ob-Gyn. and Physiol. & Biophys., Univ. of Wash., Seattle, WA 98195.

It has been proposed that during prepubertal life, high levels of endogenous opioid peptide (EOP) activity restrain gonadotropin secretion and that this activity diminishes over the course of puberty, permitting adult gonadotropin secretion to become fully manifest. The arcuate nucleus contains many POMC neurons, some of which could be involved in this process. We tested the hypothesis that POMC gene expression diminishes over pubertal development in the male rat by measuring and comparing POMC mRNA levels in prepubertal (25 day old) and adult (75 day old) animals. Twenty micron coronal slices were taken throughout the arcuate nucleus and prepared for *in situ* hybridization with an ^{35}S-labeled RNA probe complementary to mouse POMC mRNA. Sections (12/animal) were matched anatomically and read blindly. POMC mRNA content in the arcuate nucleus was estimated by counting silver grains over individual cells. These results show that over the course of pubertal development, neurons in the arcuate nucleus of the hypothalamus appear to increase, not decrease, their POMC message content.

Group	Grains/cell (Mean±S.E.M.)	n
Prepubertal	119±10	6
Adult	167±12	7

Conclusion: Diminishing EOP activity is unlikely to account for rising gonadotropin secretion during puberty; more likely, the higher level of POMC message observed in the adult brain is a consequence, rather than a cause, of pubertal maturation.

209.15

SEX DIFFERENCES IN BEHAVIORAL AND ENDOCRINOLOGICAL RESPONSES TO A CONTROLLABLE STRESSOR APPLIED REPEATEDLY. L.K. Takahashi, J.A. Vanden Burgt,* C.M. Barksdale,* and N.H. Kalin. Psychiatry Dept., Univ. Wisconsin Medical School, and VA Hospital, Madison, WI 53705.

We are examining differences between the sexes in response to controllable stressors applied acutely and repeatedly. Adult rats are able to control the termination of tail shocks by turning a wheel. Each session consists of 80 shocks (0.5 mA, 1 shock per min). Male and female rats rapidly learn to turn the wheel to control termination of tail shock in one shock session. Females, however, terminate shock more rapidly than males ($p < .01$) and have significantly higher plasma ACTH concentrations ($p < .01$). Both sexes display a significant increase in the latency to terminate shock ($p < .01$) over the course of six repeated shock sessions administered at 2- to 3-day intervals. This increase in latency to terminate shock is not reversed by naloxone and persists even after a 1-week recovery period. Because significant sex differences in response to the stressor were found, the role of gonadal hormones was investigated. Males gonadectomized during adulthood exhibit latencies to terminate shock similar to those of intact females, whereas gonadectomy increases the latency to terminate shock in females ($p < .05$). In summary, repeated exposure to a controllable stressor results in behavioral alterations that are influenced by gonadal hormones.

209.16

GENOTYPIC INFLUENCES ON REPRODUCTIVE CYCLES OF INBRED FEMALE MICE. S.P. Lerner*, R.B. Mitchell*, and C.E. Finch. (SPON: G. Miljanich). Andrus Gerontology Center and Department of Biological Sciences, USC, Los Angeles, CA 90089-0191.

Genetic influences on female reproductive cycles were found in histocompatibility-congenic strains of mice differing at the H-2 locus. Estrous cycle characteristics were specifically associated with individual H-2 haplotypes, regardless of the background strain into which these haplotypes have been incorporated. Estrous cycles of virgin mice of six strains were monitored between 4 and 7 months of age. Four congenic strains on the C57BL/10 background (C57BL/10SnJ H-2^b, "B10"; B10.BR/SgSnJ H-2^k, "B10.BR"; B10.D2/nSnJ H-2^d, "B10.D2"; B10.A(2R)/SgSnJ H-2^{h2}, B10.A(2R)), and two congenic strains on the C3H background were used (C3H/HeJ H-2^k, "C3H"; C3H.SW/SnJ H-2^b, "C3H.SW"). Estrous cycles were categorized by length: 4, 5, 6, or 7-14 days. As previously observed, B10 mice displayed predominantly 5-day cycles ($p<0.01$), while B10.BR mice displayed predominantly 4-day estrous cycles ($p<0.01$; Lerner, et al., Bioi Reprod, in press). C3H mice (H-2^k) also showed predominantly 4-day cycles ($p<0.01$), while their histocompatibility-congenic partner strain, C3H.SW (H-2^b) displayed a codominance of 4- and 5-day cycles; a pattern indistinguishable from C57BL/6J mice (H-2^b). B10.D2 mice showed mostly 4-day estrous cycles ($p<0.01$). Most interestingly, B10.A(2R) mice, which have either the k or d haplotype at all loci of the H-2 region, except the D locus, where there is the b haplotype, show a predominance of 5-day cycles. These findings both confirm and extend the observation of influences of genetic differences on estrous cycles of mice. The factor(s) responsible for the observed differences in estrous cycle lengths may be localized to one region of the H-2 complex. (Supported by grant AG-004419)

209.17

MAPPING OF PROLACTIN BINDING SITES IN RING DOVE BRAIN BY QUANTITATIVE AUTORADIOGRAPHY. J.D. Buntin and J.F. Fechner, Department of Biological Sciences, Univ. of Wisconsin-Milwaukee, Milwaukee, WI 53201.

Ring dove brain membranes contain specific binding sites for ovine prolactin (oPRL) which could conceivably mediate PRL-induced gonadal suppression and hyperphagia (Gen. Comp. Endocr. 65:243, 1987). In order to localize these sites, specific binding (SB) of [^{125}I]oPRL was examined in 34 ring dove brain regions in males (n=6) and females (n=6) by quantitative autoradiography. Slide-mounted sections (20µm) were incubated with [^{125}I]oPRL (75pM) plus unlabelled oPRL (85nM) or [^{125}I]oPRL alone. Sections were then exposed with ^{125}I standards (Amersham) to Kodak XAR-5 film for 7-10 days. Autoradiographs were analyzed by computerized video densitometry. A sex x region effect was observed ($p<.02$), with SB in the preoptic area (POA) being higher in males than in females ($p<.02$). Mean SB levels exceeding 2 standard deviations above zero were detected in the POA and choroid plexus in both sexes, and in several hypothalamic nuclei: periventricular (males), supraoptic and tuberal (females), and ventromedial (both sexes). Lower levels of SB were seen in several other brain regions. Densitometric results were corroborated by binding studies on tissue homogenates in three brain areas. These results suggest the existence of PRL-sensitive cells in brain regions regulating gonadal and ingestive activity. (Supported by NSF DCB 8303026)

210.1

INCREASES IN CGRP IMMUNOREACTIVITY IN LAMINAE I AND II FOLLOWING CHRONIC SPARED ROOT DEAFFERENTATION ARE DUE TO AN INCREASE IN TERMINAL NUMBER NOT TERMINAL SIZE. D.L. McNeill*, R.E. Coggeshall, S.M. Carlton and C.E. Hulsebosch. Marine Biomed. Inst. and Dept. of Anat. and Neurosci., Univ. of Texas Med. Br., Galveston, TX 77550.

At the light microscopic level, CGRP immunoreactivity is increased in laminae I and II of the rat dorsal horn following chronic versus acute spared root deafferentation. Preliminary results at the EM level, indicate that the number of CGRP immunoreactive terminals is increased by approximately 75% on the chronic versus acute spared root side. However, no significant difference was observed in the areas of CGRP immunoreactive terminals (chronic = 5.5 + 2.7 μm^2, acute = 5.4 + 2.0 μm^2). In addition, no significant difference was observed between the areas of laminae I and II on the acute and chronic sides. We therefore suggest that the increase in CGRP immunoreactivity in the chronically spared segment is due to terminal sprouting rather than an enlargement of terminal or laminar area. Studies are in progress to determine if there is a concomitant increase in the number of synapses per CGRP immunoreactive terminal. (Supported by NIH grants, NS11255 & NS25450, the Kent Waldrep National Paralysis Foundation, the Spinal Cord Research Foundation, the Hall Foundation and Bristol-Meyers.)

210.2

CHRONIC DEPOLARIZATION BY EXTERNAL POTASSIUM PROMOTES SYNAPSES BETWEEN RETINA AND MUSCLE IN CULTURE. J. M. Thompson and D. J. Tepper*. Department of Cell and Structural Biology, School of Life Sciences and College of Medicine, University of Illinois, Urbana, IL 61801.

In tissue culture, cholinergic retinal neurons will form synapses on myotubes as detected by intracellular electrophysiology (Puro et al., P.N.A.S., 74:4977, 1977; Ruffolo et al., P.N.A.S., 75:2281, 1978; Thompson et al., Int. J. Devel. Neurosci., 1:25, 1985). However, these retina-muscle synapses terminate within one week.

In the present experiment, cultures of 8-day chick embryo retinal neurons and rat myotubes were grown in medium containing 5.4 mM or 38 mM KCl (38 mM KCl produces a 20 mV depolarization in myotubes). Growth of retina-muscle cocultures in chronic depolarizing conditions prolongs the retina-muscle synaptic contacts. At 5 days in vitro, 42.5% muscles remained innervated when grown in 38 mM KCl, compared to 9% in controls. The increase in number of retina-muscle synapses in high KCl containing medium is not due to increased survival of myotubes or retinal neurons, or to increased retinal neurite extension. Chronic depolarization may be increasing retina-muscle synapses by blocking action potentials in both nerve and muscle through inactivation of sodium channels. Thus, inactivity reduces the elimination of normally transient retina-muscle synapses.
(JMT present address: Department of Biology, California State University, San Bernardino, CA 92407.)

210.3

EXPRESSION AND DISTRIBUTION OF SYNAPTIC VESICLE PROTEIN SVP65 IN CULTURED DORSAL ROOT GANGLION NEURONS. J.J. Lah* and R.W. Burry (SPON: J.M. Palmer). Dept. of Anatomy, The Ohio State Univ., Columbus, OH 43210.

Rat dorsal root ganglion (DRG) neurons do not form synapses on one another in culture, and thus present unique opportunities for experimental manipulation and analysis of synaptogenesis. In characterizing this culture system, we have studied the expression and distribution of a synapse-specific protein, svp65 (Matthew et al., J. Cell Biol., 91:257). This antigen appears to be ubiquitously present in neuronal cell types, and, while its function is unknown, its expression in regions of forming and mature synapses is well established.

When DRG cultures were probed for expression of this protein, it appeared to be present in some axons but not in others. Since DRG neurons do not form synapses, the observed expression of svp65 by these cells is somewhat paradoxical. Studies with cultured rat cerebellar neurons have shown that these neurons will form presynaptic specializations on polylysine coated surfaces. When DRG neurons are presented with polylysine coated beads, presynaptic terminals form at some of the bead-axon contacts. Furthermore, electron microscopic immunocytochemistry experiments indicate that the ability to form presynaptic specializations may be restricted to axons possessing the svp65 antigen.

Research support: USPHS, NIH NS-19961 (RWB), MSP fellowship from OSU-COM, and funds from the Dept. of Anatomy.

210.4

POLYLYSINE COATED CULTURE DISHES STIMULATE LONG TERM AXONAL OUTGROWTH AND SYNAPSE FORMATION WITHOUT INCREASED IN NEURONAL SURVIVAL Richard W. Burry and Diane M. Hayes*, Dept. of Anat., Neurosci. Res. Lab., College of Med., The Ohio State Univ., Columbus, OH.

Polylysine has been used in cell culture systems to enhance neuronal survival by a mechanism that is thought to involve increased cell adhesion. Polylysine coated beads have been used to stimulate development of presynaptic and postsynaptic specialization in cultured cells. We report here on experiments which attempt to differentiate between the two effects of polylysine on cultured neurons. Cell cultures of the rat cerebellum were plated in dishes pretreated with a solution of polylysine, or with no pretreatment. In the first several days, comparisons showed similar numbers of surviving neurons, similar onset of neuritic outgrowth and similar onset of synaptic vesicle antigen p65 expression. In cultures grown on polylysine, at 10 days or longer, there was a marked stimulation of neurite outgrowth and many large growth cones were seen. Electron microscopy showed numerous presynaptic terminals in contact with the polylysine coated culture dish. Immunocytochemistry of cultures on polylysine showed p65 at high levels in the axons and growth cones.

The long term effect of polylysine in neuronal cultures are the result of a metabolic change in the neurons as evidenced by increased axonal outgrowth, antigen accumulation and presynaptic terminal formation.
Research Support: USPHS, NIH, NS-19961.

210.5

Heparin and heparan sulphate inhibit nerve-induced ACh receptor accumulation. Y. Kidokoro and Y. Hirano.* Jerry Lewis Center, UCLA School of Medicine, Los Angeles, CA 90024

In Xenopus nerve-muscle cultures, it has been demonstrated that ACh receptors migrate in the membrane to the nerve contact area during junction formation and that "diffusion trapping" is the major mechanism for nerve-induced receptor accumulation (Kidokoro et al., 1986). A crucial remaining question is how the nerve induces the trap for randomly diffusing ACh receptors. In this study, we examined the effect of various glycosaminoglycans in the culture medium on the nerve-induced receptor accumulation and found that heparin and heparan sulphate partially inhibited nerve-induced receptor accumulation, but similar molecules, chondroitin sulphate type A and type C did not. By chemical modification of heparin we also showed the N-sulphate residues and a large molecular weight molecule are essential for this inhibitory effect. Heparin did not affect ACh receptor clustering (hot spot formation) in myocytes cultured without nerve. By changing the time and duration of heparin applicaton we found that heparin was effective in inhibiting nerve-induced receptor accumulation only when it was present in the culture medium during the period that neurites are actively forming contact with muscle membrane.
Kidokoro et al., (1986). J. Neuroscience 6:1941

210.6

ACCUMULATION OF EXTRACELLULAR MATRIX COMPONENTS AT AGRIN-INDUCED "SYNAPTIC" PATCHES ON MYOTUBES IN CULTURE. R.M. Nitkin and T.C. Rothschild*. Dept of Biol Sci, Rutgers Univ, Newark, NJ 07102

We have been examining the mechanism by which agrin, an extracellular matrix factor isolated from the *Torpedo* electric organ, induces dense accumulations of acetylcholine receptors (AChRs) and other synaptic components on chick myotubes in culture (J. Cell Biol. 99:615 and 105:2471). Agrin is related to molecules that are localized at neuromuscular junctions in vivo (Nature 315:571).

Here we report on the role of laminin, heparin sulfate proteoglycan (HSPG), fibronectin and type IV collagen in agrin-induced synaptic differentiation. Matrix components were visualized with fluorescein immunohistochemistry, while "synaptic" patches (AChR accumulations) were identified by rhodamine-bungarotoxin. Under these conditions, AChR clustering can be detected within 2-3 hours of agrin treatment, reaching maximal levels by ~ 8 hours.

The distributions of laminin and HSPG were markedly affected by agrin treatment. Untreated myotubes generally had a meshwork across the cell surface. After a few hours of agrin treatment, laminin and HSPG appeared in patches. Half to three-quarters of the AChR patches showed coincidence with laminin, and a similar amount showed coincidence with HSPG. However, laminin and HSPG patching (which require ~ 18 and ~ 22 hrs to reach maxima) occurs more slowly that of AChR so it is unlikely either matrix component serves to initiate synaptic organization.

Myotubes displayed limited amounts of fibronectin and type IV collagen; some cells had wisps while others had a more uniform distribution. In contrast to that of other matrix components above, fibronectin and collagen distributions were not affected by agrin treatment.

In summary, we find that agrin causes laminin and HSPG (but not fibronectin nor collagen) to accumulate at synapse-like sites on cultured myotubes in parallel (but not preceding) AChR accumulations.
[Supported by grants from NSF 87-07530 and Rutgers University]

210.7

ACCUMULATION OF A_{12} ASYMMETRIC AChE IN AGRIN-INDUCED SPECIALIZATIONS. B. G. Wallace. Dept. Neurobiology, Stanford University School of Medicine, Stanford CA 94305.

Agrin, a protein extracted from the electric organ of Torpedo californica, induces the formation of specializations on the surface of cultured chick myotubes at which several components of the postsynaptic apparatus, including acetylcholinesterase (AChE), accumulate. Our previous results suggest that motoneurons release molecules similar to agrin that induce the formation of the postsynaptic apparatus on developing myotubes. Although several molecular forms of AChE are found in muscle, it is the A_{12} asymmetric form that accumulates at synaptic sites, where it is localized in the synaptic basal lamina. It is of interest, therefore, to determine if agrin induces the accumulation of A_{12} asymmetric AChE.

We found that chick muscle cultures grown in medium without horse serum and embryo extract contained A_{12} AChE, as well as globular forms. Incubating cultures overnight with agrin did not change the level of AChE, the rate of AChE synthesis, accumulation, and release, or the relative proportions of the different molecular forms. By selectively inactivating intracellular AChE and extracting globular forms with detergent, we found that agrin-induced specializations contained both globular and asymmetric forms of AChE and that agrin caused A_{12} AChE to accumulate on the myotube surface in association with extracellular matrix material, as occurs at developing neuromuscular junctions. (Supported by MDA and NIH Grant NS14506).

210.8

LOCALIZATION OF ACETYLCHOLINE RECEPTOR mRNA BY IN SITU HYBRIDIZATION IN CULTURED MYOTUBES TREATED WITH A RECEPTOR-INDUCING FACTOR. D.A. Harris, D.L. Falls, and G.D. Fischbach. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

42-kD ARIA (AChR-inducing activity) from chick brain stimulates insertion of acetylcholine receptors (AChRs) into the surface of chick myotubes, and selectively increases the amount of mRNA encoding the α-subunit of the receptor. To assess the effect of ARIA on individual myotubes in culture, we have performed in situ hybridization of fixed embryonic myotubes with a 600-nucleotide ^{35}S-labeled cRNA probe for α-subunit mRNA. To reduce nonspecific hybridization, we found it essential to include 0.3M dithiothreitol or β-mercaptoethanol in the hybridization solution and washes, and to wash at high stringency (50% formamide, 0.3M NaCl, 65° C).

A grain density above background could be detected in control myotubes only during the first 2-3 days after plating, when mononucleated cells were fusing and Northern blot analysis indicated that α-mRNA levels were highest. In contrast, 4-day myotubes that had been treated for the previous 24 hrs with a partially purified preparation of ARIA displayed discrete accumulations of grains, which were consistently located near clusters of nuclei. Significantly, not all nuclear clusters were associated with high grain density. Quantitative fluorescence measurements of cells labeled with an anti-receptor monoclonal antibody indicated a correlation between surface receptor density (averaged over 100 um^2) and the local α-mRNA concentration.

Our results raise the possibility that ARIA differentially regulates expression of α-mRNA by individual nuclei, and that surface accumulations of AChRs may result from localized synthesis of receptor messenger.

210.9

QUANTAL ANALYSIS OF SYNAPTIC TRANSMISSION AT A DEVELOPING NEUROMUSCULAR JUNCTION. M. Laser* and M-m. Poo (SPON: J. Rothlein). Section of Molecular Neurobiology, Yale University School of Medicine. 333 Cedar St., New Haven, CT.06510

During the first few hours of synaptogenesis between Xenopus spinal neuron and myotomal muscle cells, the amplitudes of the evoked synaptic currents in physiologic saline show substantial stimulus to stimulus fluctuation suggesting a quantal mechanism with varying numbers of quata released per stimulus. Intrinsic limitations such as the poor viability of the synapse in low Ca^{2+}, the low numbers of trials due to synaptic fatigue, and the absence of a defined amplitude of spontaneous events prevented conventional approaches of quantal analysis. A new method of quantal analysis was developed. The procedure involves the following steps: A series of quantal sizes are chosen. For each quantal size the evoked histogram is quantized and its' mean quantal content is calculated. Theoretical Poisson, binomial or Gaussian distributions with the same mean and normalization are constructed. The fit of each theoretical distribution with the quantized evoked amplitude histogram is assessed by calculating the mean relative variance (MRV) between the two. The quantal size and choice of statistics which yielded the smallest MRV are the likely to be correct if the MRV shows a sharp minimum. The analysis showed that the evoked responses at this developing synapse are best described by Poisson statistics with a single defined quantal size. However, this quantal size is significantly larger than the amplitude of the majority of spontaneous synaptic currents. There was a minor peak in the spontaneous event histogram which corresponded with the predicted quantal size suggesting that only a small subpopulation of the spontaneous events were related to the quantal unit underlying the evoked response during this early phase of synaptogenesis.

210.10

THE CAPACITY OF NON-GROWTH-CONE PORTIONS OF NEURITES TO TRIGGER THE DEVELOPMENT OF A POSTSYNAPTIC SPECIALIZATION. E. Rodriguez-Marin* and M.W. Cohen. Dept. of Physiol., McGill Univ., Montreal, Quebec.

Does the capacity of neurons to induce postsynaptic specializations reside exclusively in their growth cones? To test this possibility spinal cord and myotomal muscle cells from Xenopus embryos were co-cultured at low density (day 0). One day later (d 1), newly-added muscle cells were manipulated to contact neurites which either (A) had not or (B) already had contacted other muscle cells more distally. The neurites belonged to single neurons which were free of contact from all other neurons. The next day (d 2) all muscle cells contacted by the test neurons were examined for neurite associated receptor patches (NARPs) after fluorescent staining of the AChRs.

Even though Type A contacts were less than 1-day-old and were never traversed by a nerve growth cone they had NARPs along ~15% of their length. A similar value was obtained for contacts formed by chance due to new neuritic growth between d 1 and d 2. By contrast, Type B contacts rarely exhibited any NARPs at all. These observations indicate that pre-existing portions of neurite can trigger clustering of AChRs as effectively as nerve growth cones, as long as the neurite has not established contact with other muscle cells more distally. Once neurite-muscle contact is established, the portion of neurite proximal to the contact loses its triggering capacity.

210.11

LOCALIZATION OF GAD TO PRESYNAPTIC SITES CORRESPONDS TO THE ONSET OF INHIBITORY SYNAPTIC TRANSMISSION IN VITRO. J.M. Dubinsky, S.M. Rothman and D.I. Gottlieb. Departments of Pediatric Neurology and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Mo. 63110.

The development of central inhibitory interneurons has been studied in tissue culture preparations of neonatal rat striatum. Glutamic acid decarboxylase (GAD; Chang and Gottlieb 1988), GABA (Wenthold et al., 1986) and synapsin I (DeCamilli et al., 1983) were identified at different times after plating by immunocytochemistry. At all days in vitro (div) 76% ±6.1% (n=24) of all neurons stained for GAD. This is consistent with the 50-90% seen in vivo. Initially GAD immunoreativity was confined to a weak labeling of neuronal somas, each containing an excentric patch of brighter stain. By 4 div, GAD label appeared uniformly in some processes. By 8-11 div, GAD staining became punctate within processes and almost disappeared from the somas. Similarly, synapsin I immunoreactivity was uniform in neuronal somas and processes initially but became punctate within the neurites after a week. Double labeling with GAD and synapsin I showed an exact coincidence of all GAD puncta with synapsin I puncta. In contrast, immunoreactivity to GABA was quite weak initially, but became uniform in neurites and somas by 11 div. Neuronal responses to pressure applied GABA were recorded using whole cell voltage clamp. GABA conductances normalized to cell capacitance did not change over 30 div. However, spontaneous and evoked inhibitory synaptic activity could only be detected after 8 div. Since postsynaptic responses remained stable, the late onset of inhibitory transmission may be attributable to the slower development of the mature pattern of presynaptic synthetic enzyme.

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210.12

A MODEL FOR SPATIAL CONSTRAINTS DURING DEVELOPMENTAL SYNAPTIC REDUNDANCY. J. Mariani and G. Waysand*. Inst. Neurosciences (UA CNRS 1199), Univ. P & M Curie, and Lab. Phys. Solides, Univ. P. VII, Paris 75005 France.

Transient redundancy of synaptic contacts seems to be a general feature of the development of the nervous system, the subsequent elimination of synapses leading to the adult connectivity (see review in Mariani and Delhaye-Bouchaud 1987). Until now, little attention has been paid to factors that introduce an upper limit to the synaptic redundancy, i.e. to the maximum number of axons converging on a given target cell. In this study we consider synaptogenesis as a problem of disordered media. We propose that the maximum of synaptic redundancy can be described, at least formally, as the whole set of contacts in an isostatic random stacking of non-penetrating hard spheres. Geometrical contraints in this model impose, on average, 3.5 contacts per sphere. For all the documented cases of synaptic redundancy, the ratio of the transient maximum to adulthood final connectivity is always simply related to this 3.5 value predicted by the model. When several axons innervate a target cell in the adult stage, a rigourous partition of synapses appears which could be interpreted as the existence of some hidden organisation below the macroscopic disorder.

Mariani J. and Delhaye-Bouchaud N., News in Phys. Sci., (1987) 2, 93-97.
Waysand G. and Mariani J., Comments in Dev. Neurobiology, (in press).

210.13

DEVELOPMENT OF CGRP IMMUNOREACTIVITY IN RELATIONSHIP TO THE FORMATION OF NMJ IN XENOPUS MYOTOMAL MUSCLE. H.B. Peng, Q. Chen, S. De Biasi, and D.-L. Zhu. Dept. of Cell Biology & Anatomy, Univ. of North Carolina, Chapel Hill, NC 27599.

Calcitonin gene-related peptide (CGRP) has recently been localized at the neuromuscular junction (NMJ). To understand the role of this peptide in postsynaptic differentiation, we examined its appearance at developing NMJs in the myotomal muscle of Xenopus larvae. Tadpole tails were double-labeled with fluorescent α-bungarotoxin and a monoclonal antibody against the 65 kD synaptic vesicle antigen (SV48) or a polyclonal antibody against human CGRP. AChR clusters as shown by the toxin staining began to appear at the rostral NMJs as early as stage 22 (24 hr) and were detectable at all myotomes at stage 40 (66 hr). On the other hand, CGRP first appeared at the most rostral NMJs at stage 32 (40 hr) and became detectable in all myotomes at stage 42 (80 hr). Preabsorption of antibody solution with 2.6 nM synthetic human CGRP abolished the staining. The onset of CGRP immunoreactivity closely paralleled that of SV48 and, at mature NMJs, both kinds of staining coincided precisely in all the myotomes. Thus, CGRP was localized within the nerve terminals concomitantly with the clustering of synaptic vesicles. Since AChR clusters develop prior to the presynaptic CGRP localization, this peptide is unlikely to participate in signaling the postsynaptic development.

210.14

THE INFLUENCE OF INNERVATION ON BEAD-INDUCED ACH RECEPTOR CLUSTERS. W. Rochlin* and H.B. Peng (SPON: R. Glasser) Dept. of Cell Biology and Anatomy, Univ. of North Carolina, Chapel Hill, NC 27599.

Previously, innervation of cultured Xenopus myocytes was shown to disperse pre-existing aneural receptor patches and prevent their formation. However, neurites from different neurons can induce clustering of ACh receptors on the same myocytes. Thus, the global influence of the nerve depends on the nature of the clustering stimulus. To characterize this global influence, we examined the ability of innervation to disperse pre-existing bead-associated receptor patches (BARPs) and to inhibit the formation of BARPs. When myocyte cultures were treated for 1 day with beads and then for 1-1.5 days with dissociated neural tube cells, the percentage of beads with BARPs was decreased on cells with nerve-associated receptor patches (NARPs) in all 5 trials, but the difference was significant in only 3 of these trials. Similarly, when the treatments were reversed, there was a decreased percentage of BARPs in all five experiments, and a significant difference in 3 of these experiments. BARPs appeared to be less frequent at beads close to a NARP. We conclude that innervation reduces, but does not eliminate, the survival and formation of BARPs. This effect is variable, and depends in part on bead-NARP distance.

TROPHIC INTERACTIONS II

211.1

INDUCTION OF TYROSINE HYDROXYLASE mRNA IN CULTURED RAT CORTEX BY A MUSCLE-DERIVED FACTOR(S). *L. Lyandvert*, M. Evinger, D.J. Reis and L. Iacovitti (SPON: I.R. Katz).* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

We have previously reported that a soluble muscle-derived factor(s) induces a dramatic 10-20 fold increase in the number of cultured cortical neurons which can express the catecholamine (CA) enzyme tyrosine hydroxylase (TH). Since this induction occurs without change in neuronal survival, it was concluded that muscle contains a "differentiation" factor(s) which promotes TH expression in a previously undetected class of cultured cortical neurons. In this study we sought to determine whether such enhanced TH expression occurred only at the protein level or instead required an increase in the steady state levels of TH mRNA. To do so, the cortices of embryonic day 13 rat were dissociated and grown in culture on control media or media supplemented with 10-14 mg/ml of soluble muscle fraction (prepared by homogenization and high speed centrifugation of cultured L6 muscle cells). Cultures were harvested at 2 days *in vitro* and TH mRNA levels were quantitated by Northern blot analyses using a TH cRNA probe (380 ntd). On Northern blots, levels of a single mRNA band of 1900 bases was increased in those cultures maintained on the muscle factor(s) as compared to controls. In addition, supplementation of the culture with this factor(s) resulted in an increase in the amount of 60 kD protein which could be precipitated with TH antibodies following *in vitro* translation. We conclude that the muscle-induced increase in TH containing cortical neurons found in culture is accompanied by increases in the cortical levels of TH mRNA, suggesting that the effects of this factor(s) may be mediated at the level of the TH gene.

211.2

A SOLUBLE MUSCLE FACTOR(S) INDUCES TYROSINE HYDROXYLASE ACTIVITY IN CULTURED SUBSTANTIA NIGRA NEURONS. *L. Iacovitti, M. Evinger and D.J. Reis* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

In our previous studies, we found that a soluble muscle-derived factor(s) will greatly induce the differentiation of catecholamine (CA) traits in cultured rat cortex, increasing both the number of tyrosine hydroxylase (TH)-containing cortical neurons and their levels of TH enzyme activity. In this study, we sought to establish whether this muscle factor(s) also increases TH expression in other brain CA neurons. Cells from the substantia nigra of embryonic day 14 rats were dissociated and plated in culture on control media or media supplemented with the cytosolic fraction of cultured L6 muscle cells (final concentration: 10-14 mg protein/ml). After 7 days *in vitro*, cultured cells were harvested for radioenzymatic assay of TH activity and Northern analysis of TH mRNA. Sister cultures were processed for immunocytochemistry and the number of TH- immunoreactive neurons determined. In contrast with cortex, supplementation of substantia nigra neurons with muscle factor(s), resulted in a 35-50% decline in the number of TH-immunoreactive cells. Although surviving TH neurons appeared healthy, their morphology was distinctly different from that of nigral neurons grown on control media; TH stained neuritic processes were more numerous, shorter and less varicose than in controls. Moreover, the levels of TH enzyme activity (calculated per TH neuron) were dramatically increased 33-fold in nigra cultures grown with muscle factor(s) (76.5 fmol/TH neuron/hr) as compared with control (2.3 fmol/TH neuron/hr). Northern analysis with a TH cRNA probe revealed an increase in the levels of 1900 ntd mRNA in these muscle-supplemented cultures. In addition, the amount of 60 kD protein immunoprecipitated with TH antibodies following *in vitro* translation was also significantly elevated following the addition of muscle factor(s). Although the survival of TH neurons is decreased in supplemented cultures, the results of these studies indicate that, in surviving dopamine neurons, the expression of TH mRNA and TH enzyme activity is greatly induced by the addition of soluble muscle factor(s). The similarity of cortical and nigral responses to muscle suggests a common factor may be mediating these effects.

211.3

NORADRENERGIC SYNAPSES REMAIN FOLLOWING TARGET NEURON DEGENERATION IN A HYPERINNERVATED MOTOR NUCLEUS. Y.P. Shao* and J. Sutin. Dept. of Anatomy & Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Axotomy of motor neurons results in synapse retraction of about 50% of the afferent terminals ending on the soma and proximal dendrites. Neonatal 6-OHDA treatment leads to a marked, permanent noradrenergic hyperinnervation of the trigeminal motor nucleus (MoV) in rodents. The super-nummary NE terminals displace other synaptic endings on the motor neuron soma (Hemmendinger & Moore, J. Comp. Neurol., 1986, 250: 462-68). The injection of the toxic lectin Ricin communis into the masseter nerve leads to the degeneration of virtually all masseter sub-nucleus motor neurons and results in an extensive proliferation of glial cells which express β-adrenergic receptors. Since MoV contains only motor neurons, we determined if the increased NE synapses produced by neonatal 6-OHDA treatment survive after complete removal of target neurons. NE levels were measured by HPLC in tissue punches from control and Ricin-treated animals and by catecholamine histochemistry. No major differences in NE levels were found after elimination of target neurons. Supported by NIH-NINCDS grant #NS 14778.

211.4

SEPTAL EXTRACTS REGULATE G_4 ACETYLCHOLINESTERASE ISOFORM SYNTHESIS IN MOUSE HIPPOCAMPAL CULTURES. K. Schegg,* J. Peacock, P. Hering,* and K. Futamachi.* Reno VA Medical Center, University of Nevada School of Medicine, Reno, NV 89520.

Mouse hippocampal (HIPP) neurons in cell culture produce most of their acetylcholinesterase (AChE) in the globular monomeric (G_1) isoform whereas cultured mouse septal (SEP) neurons predominantly synthesize globular tetrameric (G_4) isoform (Schegg et al, 1986). Addition of SEP to HIPP cells in coculture appears to induce a modification of isoform synthesis in HIPP neurons such that G_4 is the predominant isoform. In order to determine if SEP indeed regulates AChE isoform composition in HIPP neurons, we have searched for a soluble factor released by SEP neurons. Exposure of HIPP cultures to media conditioned by growth on SEP or SEP-HIPP cocultures is ineffective. Extracts were prepared from mouse SEP & HIPP (ages embryonic day 18 to postnatal day 59); each have at least a small positive effect in increasing the relative percentage of G_4. However, extracts from postnatal day 10 of SEP cause a major increase in G_4 such that the isoforms shift from the HIPP to the SEP pattern. Total AChE activity is not increased in treated cultures. The isoform-shifting effect can not be mimicked by adding nerve growth factor, epidermal growth factor, or carbachol to cultures. Thus these data support a positive regulatory effect of SEP on AChE isoform compositions in HIPP.

211.5

ACTIVATION OF EXCITATORY AMINO ACID RECEPTORS ENHANCES PROTEIN SYNTHESIS IN BRAIN STEM AUDITORY NEURONS OF THE CHICK. R. L. Hyson* and E.W Rubel. Department of Otolaryngology, University of Washington, Seattle, WA 98195.

Elimination of excitatory afferent input to second-order auditory neurons in nucleus magnocellularis (NM) of the chick results in a rapid and dramatic decrease in protein synthesis in NM neurons, followed by cell atrophy and cell death (Born, D.E. & Rubel E.W, J. Neurosci., 8:901, 1988). Similarly, using an in vitro slice preparation of the brain stem auditory system of the chick, we have shown that orthodromically activated NM neurons display greater protein synthesis than unstimulated neurons. Here we show that the enhanced protein synthesis appears to be the result of an excitatory amino acid released from auditory nerve terminals: 1) this enhancement is not observed when synaptic transmission is blocked by maintaining the slice in a low calcium, high magnesium medium, and 2) it is not observed when orthodromic activation of NM neurons is blocked by the general excitatory amino acid receptor antagonist, kynurenic acid (5mM). This receptor-mediated increase in protein synthesis also appears to be independent of those events involved in the generation of action potentials since antidromic activation of the NM neurons does not increase protein synthesis. These results suggest that in addition to their traditional role in synaptic transmission, excitatory amino acids may also serve to transneuronally regulate the metabolic activity of young neurons. (Supported by PHS grants NS 24518 and NS 07906.)

211.6

EFFECT OF BASIC FIBROBLAST GROWTH FACTOR (FGF) ON SECRETION OF PROLACTIN (PRL) AS ASSESSED BY THE REVERSE HEMOLYTIC PLAQUE ASSAY (RHPA). G.H. Larson*, M.A. Sortino, R.A. Koos*, P.M. Wise (SPON: I.R. Cohen-Becker). Dept. of Physiology, Univ. of Maryland, School of Medicine, Baltimore, MD, 21201.

Although particularly high concentrations of FGF are produced by folliculo-stellate cells of the anterior pituitary gland, its precise function is controversial. Co-incubation of folliculo-stellate cells with anterior pituitary cell aggregates suppressed secretogoque-induced secretion of PRL, whereas co-incubation of FGF and anterior pituitary cells in long term cell cultures stimulated secretion of PRL. Therefore, we utilized the RHPA to assess the acute effect of FGF on secretion of PRL from individual lactotrophs. Rats were ovariectomized and 1 week later implanted with estradiol (180 µg/ml). After 3 days of exposure to estradiol, rats were decapitated and medial portions of the anterior pituitaries were removed and prepared for use in the RHPA. Pituitary cells were incubated with various doses (0.055-3.000 µM) of FGF for different lengths of time (30-240 minutes). FGF inhibited secretion of PRL in a dose dependent manner with the greatest inhibition (42.2%) observed at the 3 µM dose of FGF. Maximal inhibition was observed by 30 minutes at all doses of FGF. Thus, the acute effect of FGF is to inhibit secretion of PRL, suggesting that the interaction between FGF and lactotrophs is complex.

211.7

EFFECTS OF LONG-TERM RETINAL BLOCKADE WITHOUT DENERVATION ON VISUAL SYSTEM GLUCOSE METABOLISM IN THE HOODED RAT. G.A. Thurlow and R.M. Cooper. Psychology Dept., University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

As assessed by the 2-deoxyglucose (2-DG) technique, both eye enucleation and retinal receptor elimination initiate a sequence of metabolic depression and "recovery" in cortical and subcortical visual centers in the hooded rat. Denervation and loss of retinal input occur in each procedure. To determine whether denervation (loss of trophic factors) or loss of retinal activity was sufficient to initiate the sequence of metabolic changes, tetrodotoxin (TTX) was monocularly injected every 48 h to silence retinal ganglion cells, and glucose uptake was determined after times ranging from 24 h to 2 mos. In all visual areas, TTX resulted in an initial metabolic depression, but unlike eye enucleation or receptor loss, only slight metabolic recovery occurred during the TTX administration; and within animal comparisons after bilateral TTX injections 24 h prior to 2-DG showed only a slight metabolic elevation on the long-term-TTX side. Enucleation reinstated the original depression levels. We conclude that, because denervation, direct or indirect, is critical to the metabolic increase, and because inactivity without denervation does not result in a similar increase, the "recovery" of metabolic levels must be an indication of physiological changes which occur as a direct result of loss of a trophic factor.

211.8

NGF AND NICOTINIC ACH RECEPTOR EXPRESSION ON RAT NODOSE NEURONS IN CULTURE. Allan Mandelzys and Ellis Cooper, *Dept. of Physiol. McGill University. Montreal, Que. H3G 1Y6.*

The nodose ganglion contains sensory neurons that innervate visceral tissues, such as, the heart, the lungs, the liver. Most nodose neurons from newborn rats, however, when grown in tissue culture without other cell types, express funtional nicotinic ACh receptors, and some form cholinergic synapses with one another. In this study, we have examined the role of NGF on the expression of ACh receptors by nodose neurons in culture. We find that NGF has little influence on the growth and survival of these neurons when grown in culture without other cell types for 3-4 weeks. At later times, however, small differences between +NGF and -NGF cultures were apparent: neurons with NGF grew to be about 10% larger in diameter than those grown without NGF and their neurite outgrowth was qualitatively greater. Although the effect of NGF on growth was small, most neurons, independent of whether grown with or without NGF, expressed high affinity NGF receptors as measured by I NGF binding. To test whether these NGF receptors are involved in the expression of functional nicotinic receptors, we measured the proportion of neurons expressing ACh receptors in sister cultures grown for 3 weeks either in the presence or absence of NGF. We found that over 60% (327/534 in 12 cultures) of neurons grown with NGF expressed ACh receptors, whereas, less than 12% (54/473 in 10 cultures) of neurons grown without NGF expressed ACh receptors. Futhermore, in preliminary experiments where we removed NGF from cultures at times when the neurons are acquiring ACh-sensitivity (the first 4 to 10 days in culture), we found that 2 days later these cultures had significantly fewer ACh-sensitive neurons compared to sister cultures that contained NGF throughout. In summary, this study suggests that activation of NGF receptors is a necessary step for nicotinic ACh receptor expression by nodose neurons, independent of NGF's action on neuronal growth.

211.9

THE EFFECT OF ELIMINATING AFFERENT IMPULSE ACTIVITY ON THE MORPHOGENESIS OF THE AMPHIBIAN MAUTHNER CELL. L.A. Goodman and P.G. Model, Dept. Neurosci., Albert Einstein Col. Med., Bronx, NY 10461.

Afferent innervation affects the properties of target neurons. For example, in the developing amphibian, the formation of extra vestibular (nVIII) contacts on the Mauthner cell (M-cell) enhances dendritic branching, while deprivation reduces it (Goodman and Model, *J. Neurosci., 8*:776, 1988). The mechanism underlying the interaction between afferent fibers and developing dendritic branches is not known; neural activity may be an essential component of the stimulating effect. In the developing axolotl *(Ambystoma mexicanum)*, we have examined the role of afferent impulse activity in the regulation of M-cell dendritic branching by interrupting neural activity while preserving nVIII contacts. In the medulla of the axolotl, M-cells occur as a pair of large, uniquely identifiable neurons at the level of entry of nVIII. Each cell receives synapses from the ipsilateral nerve; the terminals are restricted to a highly branched region of the M-cell lateral dendrite. We varied the amount of nVIII innervation as well as eliminated afferent impulse activity. First, a vestibular primordium was taken from a donor embryo and unilaterally implanted in a host embryo rostral to the *in situ* one: the experimental M-cell was deprived of nVIII input in the donor embryo while it was superinnervated in the host. Second, TTX-producing newt *(Taricha torosa)* embryos were surgically joined to axolotl embryos producing immobilization of the *Ambystoma* twin. Sodium channel blockade by TTX presumably eliminated action potential propagation allowing M-cell development in the absence of incoming impulse activity. Reconstruction of the M-cells in 18 mm donor larvae revealed that the dendritic branching pattern of "control" cells is normal and that of deprived cells is reduced. The preliminary data indicate that neural activity is not essential to transsynaptic stimulation of dendritic growth by afferent innervation. (Supported by the Martin Foundation & NIH grant NS-18823)

211.10

POSTNATAL MOTONEURONS SURVIVE LONG-TERM SEPARATION FROM THEIR TARGET. L.L. Crews* and D.J. Wigston (SPON: J. Manning) Emory Univ. Dept. of Physiology, Atlanta GA 30322

The survival of embryonic motoneurons is thought to depend on the acquisition of a peripheral trophic substance but it is somewhat uncertain whether this reliance persists after birth. To address this, we labeled motoneurons in postnatal mice then examined them up to six weeks after disrupting their peripheral connections. The biceps motoneurons were bilaterally labeled with Fluorogold before unilateral limb amputation, nerve transection or crush. The mice were fully anesthetized for all surgery.

Motoneurons retained their typical size and morphology after nerve crush, perhaps because the sheath surrounding the injured axons remained intact. However, following amputation or transection many of the prelabeled cells (10-80%, depending on age and survival time) were severely atrophied. The area of the atrophied somata was less than 10% that of control motoneurons. The remaining labeled cells on the injured side were, on average, 60% as large as their uninjured counterparts.

The number of labeled cell bodies on the injured side was equal to the number of labeled cells on the control side, indicating that few, if any, motoneurons died. It is unlikely that Fluorogold had been transferred from dying motoneurons to glia: the atrophied cells were not recognized by a polyclonal antiserum directed against GFAP from spinal cord astrocytes.

We conclude that when target access is compromised by amputation or axotomy, motoneurons do not die but rather persist in a protracted state of atrophy. The most intriguing implication of these results is that it may be possible to rescue these atrophied motoneurons.

211.11

EXPRESSION OF A SYNAPTIC VESICLE PROTEIN AFTER DEAFFERENTATION OF THE SUPERIOR CERVICAL GANGLION IN RATS DIFFERS WITH AGE. K. F. Greif and K. N. Flaherty*. Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

We have demonstrated previously that changes in the levels of a synaptic vesicle-specific protein (p65) in response to deafferentation of the superior cervical ganglion (SCG) differ in neonatal and adult rats. The time course of the shift in response has been investigated. Sprague-Dawley rats aged 1, 7, 14, and 28 d had bilateral deafferentation of the SCG. Similar surgery was conducted in 11 and 22-24 month old F344 rats, to study the response in aged animals. All surgical procedures were conducted using general anesthesia. At selected times after surgery, animals were killed and levels of p65 were determined using indirect radioimmunoassay. Antigen levels were measured in the SCG and in two SCG targets, the iris and pineal.

Results demonstrate that the shift from the neonatal to adult patterns in the SCG occurs during the first postnatal week. In neonatal animals, deafferentation produces a block in normal postnatal increases in p65 in the SCG. In animals deafferented at 7 d or older, a transient increase in p65 is observed. In the SCG of aged rats, responses resemble those in young adults, although the rate of response is slowed and magnitude increased. Patterns of response in target tissues suggest that development proceeds in a target-specific manner. These findings suggest that a critical developmental stage for SCG responsiveness to transsynaptic influences occurs during the first week of life. Studies in aged animals indicate that while the aged SCG retains the ability to respond to changes in neural activity, elements of patterns of response are altered.

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211.12

FACTORS GOVERNING THE ABILITY OF ASTROCYTES TO PROMOTE NEURONAL SURVIVAL AND PROCESS OUTGROWTH IN DISSOCIATED HYPOTHALAMIC CULTURES. D. S. F. Ling, D. Dickerman, R. E. Petroski, J. P. Grierson and H. M. Geller. Dept of Pharmacology, U.M.D.N.J. - Robert Wood Johnson Medical School and The Graduate School, Rutgers University, 675 Hoes Lane, Piscataway, NJ 08854.

The existence of glial-derived attachment and trophic factors is now well recognized and some of these are believed to play an important role in neuronal survival and process outgrowth. However, large variations in neuronal survival and growth on such monolayers has led us to investigate possible reasons for this heterogeneity. Astrocytes obtained from the newborn rat cerebral cortex were grown in flasks and are then plated (without prior passage) at various densities onto polylysine-coated glass coverslips and were allowed to form a confluent monolayer. Neurons obtained from fetal rat (E17) hypothalami were plated onto these monolayers and maintained in Dulbecco's-modified Eagle's medium with 10% fetal calf serum. Neuronal survival and process outgrowth were assessed and cultures were immunocytochemically examined for markers of glial and neuronal specific proteins as well as for extracellular matrix molecules. Our observations suggest that the competence of glial cells to promote neuronal growth is not reflected by the expression of GFAP or NCAM. Nor does the presence of laminin and fibronectin in the extracellular matrix seem to be of particular importance. Instead, neuronal attachment and process outgrowth appear to be related to glial cell density, such that low astrocyte density promotes neuronal survival. Supported by NIH NS25168 and a grant from the UMDNJ Foundation.

211.13

THE POSTNATAL ENRICHMENT OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) IS PRESERVED IN TRIGEMINAL GANGLION NEURONS THAT MAINTAIN A PROJECTION TO THE CEREBRAL ARTERIES. K. Horgan, T. P. O'Connor and D. van der Kooy (spon: J. A. Connolly), Dept. of Anatomy, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

The adult trigeminal ganglion's sensory projection to the cerebral arteries is enriched in the neurotransmitter CGRP. We asked if this CGRP enrichment is present in the arterial projection from the neonatal ganglion or if it is due to selective survival of a small number of CGRP containing neurons in the face of massive postnatal cell death and axon retraction in the overall arterial projection. Two populations of ganglion cells were retrogradely labelled from adult or neonatal flourescent tracer applications to the middle cerebral artery; the population that maintains an artery collateral postnatally and the composite population that either maintains or retracts an artery collateral postnatally. In the composite population, CGRP was present in 20.7±1.4% of retrogradely labelled cells at postnatal day (PND)5 and in 13.1±1.4% of labelled cells at PND90. In the population specifically maintaining an artery collateral, CGRP was present in 20.7±1.4% of labelled cells at PND5, and in 25.5±1.8% of labelled cells at PND90.Thus the CGRP enrichment is present in the neonatal arterial projection and there is a small increase in the proportion of CGRP positive cells by PND90 amongst the cells specifically maintaining artery collaterals. However, CGRP enrichment appears to be lost by the cells which retract artery collaterals. The presence of adult trigeminal ganglion projections to the forehead skin with low CGRP levels permits a test of this idea. In neonatal rats a significant number of single trigeminal cells have axon collaterals to both the cerebral arteries and the forehead. By PND90 almost all of these cells specifically retract their artery collaterals. A developmental decrease in CGRP positive cells amongst the population specifically retracting an artery collateral but retaining a forehead projection (identified by retrograde double labelling), will support the idea that arterial projections maintain enriched trigeminal CGRP levels.

211.14

SCHWANN CELL-NEURONAL INTERACTIONS INVOLVE NGF. B.A. Urschel and C.E. Hulsebosch. Dept. of Anat. & Neurosci. and Marine Biomed. Inst., Univ. of Tex. Med. Br., Galveston, TX 77550.

In an attempt to gain insight into the functions of Nerve Growth Factor (NGF), we suppressed the endogenous levels of NGF with antibodies. We were interested in the effects produced by the inactivation of NGF on both centrally and peripherally projecting fibers. Newborn rats were given subcutaneous injections (3 µl/gm body weight) of rabbit antibodies to purified mouse β-NGF (ANTI-NGF) daily for a period of one month. The relation of myelin thickness to fiber diameter of myelinated axons in T7-T11 dorsal and ventral roots and peripheral nerves was analyzed in the ANTI-NGF animals and in normal littermates (CONTROL). The results of the analysis are shown below as means ± standard error (n = 100).

Myelin thickness in:	ANTI-NGF	CONTROL
DORSAL ROOTS	.34µm ± .03µm	.59µm ± .02µm
VENTRAL ROOTS	.71µm ± .02µm	.81µm ± .02µm

Myelin thickness was significantly thinner in the dorsal and ventral roots ($p < 0.005$ and $p < 0.002$ respectively) of those animals treated with ANTI-NGF as compared to their untreated littermates. These results indicate that Schwann cell-neuronal interactions are altered by the inactivation of NGF. The peripheral nerve study will be presented. (Supported by SCRF, NIH grants NS11255 and NS25450, Hall Foundation and Bristol-Meyers).

211.15

SOMATOSTATIN EXPRESSION IN CULTURED CILIARY GANGLION NEURONS

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The avian ciliary ganglion contains two distinct neuronal cell types: ciliary neurons which innervate striated muscle of the eye's iris and ciliary body, and choroid neurons which innervate the vascular smooth muscle of the choroidal coat. Although much is known about the differences between ciliary and choroid neurons, how these differences arise has not previously been addressed. Recently, the neuropeptide somatostatin has been found within the choroid neurons *(Epstein et al 1988, Neuroscience in press)*. We are interested in how expression of this neuropeptide is controlled during development. We have found that a substantial proportion of ciliary ganglion neurons express somatostatin immunoreactivity when cocultured with dissociated cells from the choroid layer. In contrast, no somatostatin is detectable in ciliary ganglion cells cultured alone or when cocultured with striated muscle. This observation is consistent with the hypothesis that somatostatin expression depends upon an interaction with vascular smooth muscle target cells. Such interactions may be important for regulating the neuronal traits which differ between choroid and ciliary neurons.

211.16

GROWTH OF EMBRYONIC HEART CULTURED IN OCULO IS ALTERED BY SYMPATHETIC INNERVATION AND EPINEPHRINE STIMULATION. D.C. Tucker and C.H. Gautier . Department of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294

Control of embryonic rat heart growth and beating rate by sympathetic innervation and by circulating catecholamines was studied by grafting hearts into the anterior eye chamber of adult host rats. Embryonic hearts grafted into sympathetically denervated eye chambers have a faster intrinsic beating rate and are smaller than embryonic hearts grafted into unmanipulated eye chambers (Tucker & Gist, 1986).

To study the interaction of sympathetic innervation and circulating epinephrine, host rats were prepared by unilateral superior cervical ganglionectomy (SCGx) and bilateral adrenal medullectomy (Medx). Grafts into unmanipulated eye chambers grew equally well in Medx and sham-operated hosts (2.23 + .41 vs 2.40 + .51 mm^2). Grafts into sympathetically denervated eye chambers attained a larger size in Medx than sham-operated hosts (1.48 + .27 vs. 0.86 + .09 mm^2). Grafts cultured in hosts sympathectomized as infants also grew less well if the adrenal medulla was intact than if hosts were Medx before grafting (1.43 + .25 vs 2.58 + .43 mm^2 for atria grafts and 0.9 + .02 vs 2.83 + .55 mm^2 for ventricle grafts). These data suggest that embryonic heart growth may be inhibited by circulating epinephrine stimulation and promoted by sympathetic innervation.

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211.17

GROWTH OF RETINAL EXPLANTS ON WHEAT GERM LECTIN AND LAMININ IN THE PRESENCE OF TAURINE. L. Lima*, P. Matus* and B. Drujan*. (SPON: H. Vanegas). Lab. of Neurochemistry, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

The extracellular matrix plays important roles in controlling cell growth and organization of tissues. Minimal requirements for regeneration in vitro are substrate-related. Retinal explants from goldfish (Carassius auratus) were plated 10-14 days after crush of the optic nerve in Leibovitz medium. The plates were previously coated with poly-L-lysine (PLL, 0.1 mg/ml), as control for growth, or with wheat germ lectin (WG, 0.1-0.2 mg/ml), and laminin (LAM, 5-10 µg/ml). Attachment of explants was 80, 66 and 71% on PLL, WG, and LAM respectively. Taurine stimulates growth from explants on PLL in a concentration-dependant manner, but was much less effective in the rest of substrates used. After 5 days in culture taurine increases nerve growth index (NGI) in 225% on PLL. Explants on LAM started to grow at 7-8 days, by 10 days the NGI increases in 33% in the presence of taurine. Similar results from explants plated on concanavalin A, lentil and peanut lectins were previously reported. Fibers were of 6 µm on PLL and LAM, and 4 µm on WG. Clockwise directionality was only observed on PLL. These results demonstrate the dependance of extracellular matrix for the stimulation produced by taurine, and suggest a better interaction with polycation surface than with glycosides residues.

211.18

IDENTIFICATION OF GROWTH-RELATED OPIOID PEPTIDES USING NEUROTUMOR CELLS IN CULTURE. P.J. McLaughlin and I.S. Zagon. Dept. Anatomy, Penn. State Univ. College of Medicine, Hershey, PA 17033.

Tumorigenic events are regulated by endogenous opioid systems (i.e., opioids and receptors). To determine which opioid peptide(s) is (are) involved with growth, S20Y neuroblastoma (NB) cells were used in an assay system that studied growth in the presence of peptides representative of the three opioid precursor molecules: prodynorphin, proenkephalin A, and POMC, as well as a variety of exogenous opioids and non-opioid compounds. The pentapeptide, methionine enkephalin (ME), was the most active growth inhibitory agent, followed by slightly modified peptides (e.g., leucine enkephalin). Further examination indicated that ME had a dose-response effect that was naloxone reversible and stereospecific. Studies of thymidine incorporation and mitosis revealed a significant reduction in labeling and mitotic indexes in ME treated cultures relative to controls. RIA demonstrated that tumor cells produced a variety of peptides, including ME. Opioid receptors, including those for ME were also detected. These studies indicate that ME is a potent and natural trophic factor in growth, support the thesis that a target of opioid action is cell proliferation, and reinforce evidence that opioids and receptors function in an autocrine fashion to regulate growth. Support by NIH grants NS-20500 and NS-20623.

211.19

EXOGENOUS POLYAMINES. EFFECTS ON DEVELOPMENT AND PROTECTIVE EFFECTS AGAINST NEURONAL DEGENERATION. G.M. Gilad and V.H. Gilad. Dept. of Neurobiology, Coriell Inst. for Med. Res., Camden, NJ, USA.

Treatment of rats with exogenous polyamines (PA) for a limited time period after peripheral nerve injury can accelerate regeneration and functional recovery. Similar PA treatment can reduce programmed neuronal death as well as axotomy-induced death of sympathetic neurons in development. While the cellular site and mechanism of PA action is still unknown the basic working hypothesis is that when cells in general have an increased demand for PA, as evidenced by increased PA biosynthesis, they also develop a high affinity uptake system for extracellular PA; and, that this is a relatively short lasting event. Results of preliminary experiments are in support of this notion: 1) PA treatment during the first 12 days after birth can lead to earlier eye opening and earlier appearance of righting reflex, and, 2) similar treatment can prevent the monosodium glutamate-induced neuronal degeneration in the developing retina. PA may exert their effects directly on the reactive cells and/or indirectly through enhancing the effects of growth factors which are produced by other cells.

ALCOHOL, BARBITURATES AND BENZODIAZEPINES

212.1

PENTOBARBITAL PRODUCES TEMPERATURE-INDEPENDENT AND DEPENDENT CHANGES IN VISUAL EVOKED POTENTIALS OF RATS. B.E. Hetzler and L.K. Norris*. Psych. Dept. Lawrence Univ., Appleton, WI 54912

Flash evoked potentials (FEPs) have often been used to assess the effects of drugs on the nervous system, but few studies have examined pattern reversal evoked potentials (PREPs). Even in the case of FEPs, secondary influences of altered body temperature are often unknown. The present study examined FEPs, PREPs and body temperature in hooded rats following i.p. injections of saline, and of 15 and 30 mg/kg pentobarbital. Rats were tested in standard (23°C) and warm (31°C) environments. The 30 mg/kg dose produced hypothermia of 2.6°C at 23°C, but not at 31°C. Early components of FEPs were generally increased in amplitude by the 15 mg/kg dose, and decreased by the 30 mg/kg dose at 23°C. At 31°C, the 30 mg/kg dose no longer decreased early component amplitude. Late FEP components were decreased in amplitude at both ambient temperatures. The main PREP components N1P1 and P1N3 showed an amplitude increase at 15 mg/kg, but not at 30 mg/kg, at both temperatures. However, PREP component N2P2 was reduced by the 30 mg/kg dose only at 23°C. The 30 mg/kg dose increased FEP and PREP latencies at both temperatures, but the increases were roughly twice as large at 23°C. These data show that pentobarbital produces amplitude and latency changes in both FEPs and PREPs independent of hypothermia, and that some of the drug-induced effects are enhanced by hypothermia. The results also help explain differences in PREPs recorded previously in awake versus anesthesized rats.

212.2

EFFECTS OF PENTOBARBITAL ON FACILITATED AND SPONTANEOUS TRANSMITTER RELEASE IN CRAYFISH. B.D. Winegar and G.D. Bittner. Department of Zoology and Institute for Neurological Sciences, University of Texas, Austin, TX 78712.

The effects of pentobarbital (PB) on evoked excitatory post-synaptic potentials (EPSPs) and spontaneous miniature EPSPs (MEPSPs) were studied in central opener muscle fibers in the chelipeds of crayfish (Procambarus clarkii). Facilitated EPSPs evoked by 10 Hz stimuli were significantly decreased by 2 min exposure to 10^{-3} M PB $F(5,35)=3.89$, $p<0.01$. Non-facilitated transmitter release evoked by 1 Hz stimuli appeared to be unaffected by 10^{-7} to 10^{-3} M PB. PB at 10^{-4} M and 10^{-3} M significantly reduced the ratio of EPSP amplitudes at 10 Hz to those at 1 Hz (a measure of facilitation). EPSP time constants of decay (a post-synaptic measure) were unaffected by PB. MEPSP frequency was reduced by about 50% immediately upon addition of 10^{-4} M PB to the saline bath, while MEPSP amplitudes and their constants were unchanged. These results suggest that PB has a presynaptic action to depress facilitated and spontaneous transmitter release at excitatory synapses in crayfish opener muscle fibers.

Supported by NIAAA Grant # AA07746

212.3

FUNCTIONAL TOLERANCE TO THE ANXIOLYTIC EFFECT OF PHENOBARBITAL IN RATS. J.A. Richter, P.V. Hanford* and R.H.B. Sample*. Depts. of Pharmacology Psychiatry and Pathology, Indiana Univ. Sch. of Med. and Psychology, I.U.P.U.I., Indianapolis, IN 46223.

Functional tolerance develops to some but not necessarily all effects of the barbiturates. We wished to determine if functional tolerance develops to the anxiolytic effect of barbiturates which can be demonstrated in the Geller-Seifter conflict procedure.

Rats were reduced to 85% of their free-feeding weight and trained to lever press in a Skinner box for a reward of sweetened, condensed milk. Final performance was stabilized on a VI 1' schedule. Superimposed on the VI 1' schedule were periodic 2 min segments during which the subjects received 0.5 mA shocks for every fifth response (FR 5) and a non-contingent shock at the end of those segments. The rats received 73 days of twice daily i.p. injections of saline or sodium phenobarbital (NaPheB). NaPheB doses increased from 50 mg/kg at 8 am and 75 mg/kg at 5 pm to 80 mg/kg and 90 mg/kg respectively by day 22. For the final test for tolerance, we reduced the prior afternoon dose by half and injected all rats with 35 mg/kg in the am before testing. The two groups then exhibited equal anxiolytic effects but brain PheB levels determined by HPLC were 36% higher in the group given chronic PheB (37.0 ± 2.02 vs 27.2 ± 0.36 ug/g; mean ± SEM, N=6, 6). By this measure, functional tolerance was demonstrated to the anxiolytic effect. Grant RO1 DA00796.

212.4

BEHAVIORAL CONVULSIONS AND LONG-DURATION BURSTING ELICITED BY BRAIN REGIONAL INFUSIONS OF PENTOBARBITAL (PB). J.L. Lewis and F.S. LaBella. Dept. of Pharmacology, University of Manitoba, Winnipeg, Manitoba

Although PB is considered to be a CNS depressant, site-specific excitatory responses have been reported both in vitro and in vivo after peripheral administration, and in single neurons after iontophoresis. We have reported that general anesthesia induced by administration of β-endorphin is mediated by sites in or adjacent to the inferior third (I3V) and fourth (4V) ventricles. To assess the effect of PB at these sites, we administered either .004mM or .012mM PB into either the I3V or 4V of rats. The lower dose produced hyperresponsiveness and hyperactivity at either site. Animals infused in the I3V showed early ataxia. At the higher dose, infusions into the 4V consistently produced convulsions comprised of barrel rotations, flailing, forelimb clonus, and tonic extension accompanied by EEG sharp waves. Tactile stimulation precipitated convulsions for up to 40 min. Postictal depression and ataxia persisted up to 3 hr. I3 infusions resulted in hyperactivity followed by ataxia and flaccidity. In contrast to 4V animals, these animals showed EEG bursting which was still evident after seven days. At these doses, PB can induce excitation and convulsions in regions mediating anesthesia by other agents. (Work supported by the MRC of Canada.)

212.5

THE FACE TO FACE TEST FOR ANXIOLYTICS: A SOCIAL INTERACTION TEST IN MICE. P.J.K.D.Schreur. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The face to face test in mice is a quicker and less expensive adaptation of Sandra File's social interaction test in rats (File and Hyde, 1978; File, 1980). Male CF-1 mice (Charles River, 19-29g, 8 pairs per dose) were injected s.c. 30 min before testing. Pairs of mice from different home cages were placed together into a tiny plastic cage (7" x 5-1/2") with an opaque lid. Duration of face to face interaction was measured visually for 3 min. Groups were compared by Wilcoxon's rank sum (two-tailed).

Face to face interaction was increased by diazepam, chlordiazepoxide HCl, alprazolam, flurazepam HCl, buspirone HCl, gepirone HCl, ipsapirone HCl, 8-OH-DPAT (8-hydroxy-dipropylaminotetralin HBr), and a high dose of sodium pentobarbital. For some of these, high doses were less effective than lower doses. RO 15-1788, RO 16-6028, and RO 17-1812 had no significant anxiolytic effect in this test; in fact, RO 15-1788 and RO 16-6028 actually decreased the amount of face to face interaction without causing noticeable sedation (an anxiogenic effect?). In addition, RO 15-1788 also antagonized the anxiolytic effect of diazepam. Chlorpromazine HCl did not significantly increase face to face interaction; in fact, high (sedative) doses eliminated it. d-Amphetamine sulfate had no effect at all in this test. In conclusion, the face to face test in mice is a sensitive predictor for anxiolytic activity of classical benzodiazepine anxiolytics and the 5-HT1a agonists buspirone and 8-OH-DPAT.

212.6

EFFECTS OF SCOPOLAMINE ON PASSIVE AVOIDANCE IN OFFSPRING OF DIAZEPAM-TREATED MALE MICE. A. Márquez-Orozco*, M.C. Márquez-Orozco, M.* Ramos-Avila* and R.A. Prado-Alcalá. Embryol. Dept. and Physiol. Dept., Med. Sch, Natl. Univ. of México, P.O.B. 70250, México, D.F., México 04510.

To assess the effects of scopolamine (SCOP) on retention of passive avoidance in offspring of diazepam-treated male mice (DZP-T), male CD-1 mice were given diazepam (2.7 mg/kg, i.p.) daily during six weeks. Nine weeks later they were mated with females. Two months after birth, the litters were trained to avoid a footshock that was given in a passive-avoidance conditioning box. Retention of the task was tested 5 and 8 days later. A group of litters of untreated parents (UT) was also studied. Each of these two groups had four subgroups: SCOP-treated males, SCOP-treated females, Saline (SAL)-treated males and SAL-treated females (10/group). The SCOP and SAL i.p. injections were given within 1 min after training. There were significant differences in retention among groups only during the second retention test: the DZP-T-SCOP groups had lower retention than the DZP-T-SAL and the UT-SAL animals; the male UT-SCOP animals performed worse than its UT-SAL countepart, but the UT-SCOP females did not differ from the UT-SAL females. These results suggest that SCOP interacts with the effects of diazepam to produce deficits in long-term memory, and that the male mice are more affected by SCOP than females.

212.7

COMPETITION FOR SUCROSE-PELLETS IN TRIADS OF RATS: EFFECTS OF ACUTE AND SUBCHRONIC ADMINISTRATION OF CHLORDIAZEPOXIDE. C.Gentsch*, M.Lichtsteiner* and H.Feer* (SPON: A. Enz). Psychiatric University Clinic, CH-4025 Basel (Switzerland).

Within triads of male Wistar rats, clear and stable rank-orders have been discerned, based on each individual's competition-score for a limited number of sucrose-pellets (Gentsch et al. Behav.Brain Res.27:37,1988). Being interested in pharmacological manipulations which enable poor-performing rats to overcome their typical abstention from competition, we compared, in parallel, the effects of chlordiazepoxide (CDZ) in poor-performing (P) and high-performing (H) rats. After a single dose (0.1-20mg/kg,ip.,-1h), CDZ did not improve the P-rats' competition-rates but temporarily attenuated the competition-scores in H-rats (to 70 and 5% of the mean pre-drug levels at 6.7 and 20mg/kg, respectively). Subchronic treatment (5mg/kg for 5 consecutive days) produced, on the first day, no effect in P-rats and an attenuated mean competition-score in H-rats. On subsequent days, H-rats recuperated almost completely and, within that period, CDZ increased the P-rats' mean scores to 13,19 and 14 on days 3,4,5, respectively (as compared to a mean pre-drug level of 4). Following drug-withdrawal, both H- and P-rats re-attained their previous levels of competition. Thus, within the present "social experimental set-up" CDZ can, indeed, influence P-rats. However, these rats need to become tolerant to the drug's sedative effects (as apparent in H-rats), prior to being disinhibited (caused by CDZ's anxiolytic efficacy).

212.8

DIAZEPAM IMPROVES PERFORMANCE OF MAUDSLEY REACTIVE RATS IN A SHUTTLEBOX ESCAPE TASK. S. L. Cottingham and J. N. Crawley. Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892.

The Maudsley Reactive (MR) and Non-reactive (MNR) rat strains have been selected for their high (MR) and low (MNR) levels of "emotionality" when placed in a novel environment. In addition, NIH MR ratshave performance deficits in a shuttlebox escape task compared to MNR rats whose performance is comparable to Sprague-Dawley rats. In MR rats, latency to complete the escape task increases over the testing period. We therefore hypothesized that the shuttlebox deficit may be due to the stressful nature of the task in MR rats that have been bred for hyperemotionality.

The present study was undertaken to determine whether an anxiolytic drug could reverse the deficit that MR rats, but not MNR rats, show in the escape task. Rats were removed from the home cage and administered diazepam (DZP,1.25 mg/kg i.p.) or vehicle. 25 minutes later rats were placed in the shuttlebox and allowed to habituate for 5 minutes. The task consisted of 5 FR1 (one crossing) and 25 FR2 (two crossings) in which an 80 dB, 2.8 kHz tone was presented 5 seconds before shock onset. An animal was considered to have failed the task if the average latency over the 25 FR2 trials to perform the task was more than 15 seconds from the shock oriset.

The average latency for DZP-treated MR animals (n = 7) was 12.7±1.1 sec, compared with 17.6±2.2 sec for vehicle-treated animals (n = 13). 0/7 of the DZP-treated MR rats failed, as compared to 4/13 vehicle animals. The difference between the vehicle-treated group and the MR population response was not statistically signifcant but the average latency of DZP-treated MR rats was less than that of the MR population (t test, $p < .05$). There was no difference between vehicle and DZP-treated MNR rats, all of which learned the task.

These results suggest that anxiolytics partially reverse the performance deficit in MR rats, in this presumably stressful learning paradigm.

212.9

SWIMMING BEHAVIORAL ALTERATIONS INDUCED BY PRENATAL ADMINISTRATION OF DIAZEPAM. M.C. Márquez-Orozco*, A. Márquez-Orozco* and I. Zarco de Coronado. Embryol. Dept. and Physiol. Dept., Med. Sch. Natl. Univ. of México, P.O.B. 70250, México 04510. (SPON: J.A. Roig)

In a great number of studies it has been reported that benzodiazepines exposition during pregnancy causes morphological changes in almost all visceral and somatic structures, in newborn mice. Also, the central nervous system showed long lasting alterations specially in the cerebellum, mesencephalon, striatum and cortex. In order to detect how these alterations affect the postnatal somatic development swimming behavior was evaluated. Female mice CD-1, received a daily dose of diazepam (2.7 mg/kg b.wt., i.p.), from the 6th to 17th day of gestation. Gestation term remained unchanged. Control mice received 0.09% saline solution. After birth the pups were separated from their mothers and each litter was reduced to n=8 and transfered to a foster mother for nursing. From 5th to 12th day pups were placed for 15 to 30 sec in a swimming pool (40x30x25 cm) filled up to 15 cm deep (35°C). All the experimental animals exhibited hypotonia, fatigue, dyspnea and it was neccesary to apply reanimation techniques. They showed slow evolution in maduration of swimming behavior characteristics such as lifting the nose, maintaining straight direction and coordination of movements. These alterations were attributed to a deficient neuronal differentiation.

212.10

INTERACTIONS OF ETHANOL AND RO 15-4513 ON LOCOMOTOR ACTIVITY AND INTRACRANIAL SELF-STIMULATION IN RATS. **G.J. Schaefer and R.P. Michael, Dept. Psychiatry, Emory Univ., School of Medicine, Ga Mental Hlth. Inst. 1256 Briarcliff Rd, Atlanta, GA 30306.**

The behavioral effects of Ro 15-4513 alone and in combination with ethanol were evaluated. In the first study, rats (n=10) were habituated to an activity apparatus (Digiscan RXY) for 30 minutes. They were then administered either vehicle or Ro 15-4513 (0.1,0.3,1.0,3.0 mg/kg), and locomotor activity changes were recorded for the next hour. Increases in activity were observed at the 1.0 and 3.0 mg/kg doses. In a second study, rats (n=10) were tested similarly in an activity apparatus, but were administered either vehicle, ethanol (0.1,0.3,1.0,1.7 g/kg), or combinations of ethanol and 1.0 mg/kg Ro 15-4513. The combinations of Ro 15-4513 and ethanol produced increases in activity compared to those of ethanol alone at all ethanol doses. In a third study, rats (n=6) were implanted with stimulating electrodes aimed at the medial forebrain bundle and trained to lever-press for brain self-stimulation on a fixed ratio 15 schedule of reward. Animals were then tested with vehicle, ethanol (0.1,0.3.0.56,1.0,1.7 g/kg) or combinations of ethanol and 1.0 mg/kg Ro 15-4513. Ethanol alone produced a modest increase in lever-pressing at 0.3 g/kg, followed by a graded decrease at higher doses. Ro 15-4513 alone produced a large decrease to about 30% of vehicle scores. When combined with ethanol, Ro 15-4513 produced marked decreases in response rates at all ethanol dose-levels (< 20% of vehicle scores). The data showed that Ro 15-4513 has behavioral actions of its own, which are both antagonistic and synergistic to those of ethanol. (Supported by Georgia Dept. Human Resources and Emory Univ. Research Council).

212.11

THE EFFECTS OF RO15-4513 ON SMALL AND MODERATE DOSES OF ETHANOL IN A LOCOMOTOR ACTIVITY PARADIGM. T.K. McKean* and W.M. Bourn. Dept. of Pharmacology and Toxicology, Northeast Louisiana University, Monroe, LA 71209-0400.

The imidazobenzodiazepine Ro15-4513 has been reported to antagonize some of the acute effects of ethanol administration in a number of paradigms. The present experiments were designed to test the ability of Ro15-4513 to reverse the locomotor effects of ethanol in rats.

The effects of Ro15-4513 (1 or 10mg/kg ip) were investigated in Sprague-Dawley rats pretreated with small or moderate doses of ethanol (0.25 or 1.5g/kg ip). Ethanol, as a 25% w/v solution, was administered 10 minutes prior to Ro15-4513 (suspended in deionized water and 1% tween 80).

Ro15-4513, in a dose-dependent manner, blocked an increase in locomotor activity produced by the low dose of ethanol. However, Ro15-4513 alone produced a reduction in locomotor activity, suggesting that the ethanol "antagonism" could simply result from an additive effect of the two drugs. Ethanol alone at the higher dose reduced locomotor activity. The latter was attenuated by the low dose of Ro15-4513.

The results of this study are somewhat supportive of the hypothesis that Ro15-4513 is capable of antagonizing some of the effects of ethanol. However, the intrinsic mild behavioral effects may be partly responsible for reported ethanol antagonist actions.

212.12

EFFECT OF RO15-4513 ON ACUTE ETHANOL-INDUCED CHANGES IN INTOXICATION SCORE, AMBULATORY ACTIVITY AND MONOAMINE METABOLITE LEVELS. M. Gongwer*, J.M. Murphy and W.J. McBride. Inst. Psych. Res., Indiana Univ. Sch. Med., Indianapolis, IN 46223.

This study investigated whether the GABA-benzodiazepine (GABA-BZD) receptor complex is involved in the ethanol-induced (2.5 g/kg body wt, i.p.) increases in the levels of 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the anterior striatum (ASTR) and nucleus accumbens (ACC) of adult male Wistar rats (N=7 per group). This dose of ethanol (E) produced distinct signs of intoxication and significantly reduced spontaneous motor activity by 35%; administration of 10 mg/kg RO15-4513 restored ambulatory activity to control values and significantly reduced the degree of intoxication. In the ACC, the levels of DOPAC, HVA and 5-HIAA were increased by 69, 115 and 19%, respectively, one hour after E. Similar increases were observed in the ASTR. RO15-4513 did not alter the E-induced increases in these metabolite levels in either region. The behavioral data are consistent with the GABA-BZD-Cl^- receptor complex being a site of action of E, but the biochemical data indicate that activation by E of the DA and 5-HT neurons projecting to the ACC and ASTR is not mediated by this complex. (AA-03243, AA-07462).

212.13

ETHANOL INTOXICATION AND BENZODIAZEPINE ANTAGONISTS: DOSE RESPONSE EFFECTS OF RO15-4513 AND FG7142. **P. J. Syapin, B. L. Jones*, A. Sartorius*, K. W. Gee*, and R. L. Alkana. USC, Schs. of Med. and Pharm., L.A., CA 90033.**

The antagonism of ethanol hypnosis by the benzodiazepine (BZ) antagonist/partial inverse agonist Ro15-4513 is a BZ receptor mediated action (Brain Res. Bull. 19 603, 1988). To further characterize this effect, the dose-response curve for Ro15-4513 antagonism of ethanol hypnosis was determined. For comparison, the BZ antagonist/inverse agonist FG7142 was also studied. Male C57BL/6J mice were first given ethanol (3.6 g/kg i.p.) and ten minutes later were injected with either Ro15-4513 (1, 5, 10, or 20 mg/kg), FG7142 (20, 40, or 60 mg/kg), or their respective vehicles. Ro15-4513 dose-dependently antagonized hypnotic effects of ethanol. Each dose caused a significant increased in the wake-up blood ethanol concentration (BEC) and reduction in the duration of hypnosis. In contrast, the effects of FG7142 on ethanol-induced hypnosis were not systematic. The lowest FG7142 dose significantly increased the wake-up BEC without altering the duration of hypnosis, while the highest dose significantly increased the duration of hypnosis, with no change in wake-up BEC. The dose-response characteristics of Ro15-4513 and FG7142 as antagonist of ethanol hypnosis appear to be different, suggesting differences in their underlying mechanisms of antagonism. **(Supported by NIAAA Grant AA03972 and BRSG SO7 RR05792)**

212.14

RO15-4513 INDUCED DECREASE IN VOLUNTARY ETHANOL INTAKE IN RATS: EVIDENCE FOR NON-SPECIFICITY. B.R. Smith*, R. Segal* and Z. Amit., Ctr. Stud. Behav. Neurobiology, Dept. Psychol., Concordia Univ., Montreal, Que., CANADA.

The present paper examined the effect of the partial benzodiazepine inverse agonist, RO15-4513 and the GABA antagonist picrotoxin on voluntary ethanol intake in rats. It has been reported that RO15-4513 blocked ethanol induced physical intoxication (Suzdak et al., Science, 234:1243, 1986) and decreased operant responding for oral access to ethanol (Samson et al., Pharm. Biochem. Behav., 27:517, 1987). Rats were exposed to an ascending series of ethanol solutions presented in a free choice along with water and were later maintained in this paradigm at 9% v/v ethanol and water. Both, RO15-4513 (3 mg/kg, IP) and picrotoxin (2 mg/kg, IP) decreased voluntary ethanol consumption as measured by g/kg intake of ethanol. However, unlike picrotoxin treatment, this decrease produced by RO15-4513 was not accompanied by a reduction in preference for ethanol over water. The absence of a corresponding shift in preference is often indicative of a general reduction in fluid intake and RO15-4513 was seen to decrease general fluid intake. Picrotoxin did not alter fluid consumption. The present findings indicate that GABA ergic mechanisms may play some role in the regulation of voluntary ethanol intake. However, RO15-4513's effect on ethanol consumption may not represent a specific interaction with ethanol, but may rather reflect some general nonspecific action which decreases intake of fluids.

212.15

ROLE OF BENZODIAZEPINE (BZ) RECEPTORS IN TREATMENT BUT NOT ETIOLOGY OF ETHANOL-WITHDRAWAL ANXIETY. C.M. Harris, D. Benjamin,* I.S. Elkhayat,* S. Bhadra* and H. Lal. Dept. Pharmacol., Texas Col. Osteopath. Med., Ft.Worth, TX 76107.

Ethanol withdrawal produces a stimulus (Lal et al., Fed. Proc. 46:1301, 1987) similar to the anxiogenic pentylenetetrazol (PTZ). To assess the role of BZ receptors in modulating the withdrawal stimulus, rats were trained with food reward in a two-lever choice task to discriminate between PTZ (20 mg/kg, ip) and saline (1 ml/kg, ip), then given chronic ethanol (11-13 g/kg/day) in the diet for 4-6 days, and tested for lever selection after injection of test drugs at 12-20 hr after a last dose (3 g/kg, po) of ethanol. After saline (1 ml/kg, ip), rats selected the PTZ lever. PTZ lever selection was reduced by CGS 9896 (20 mg/kg, po; $p < 0.005$). Zopiclone (2.5 - 10 mg/kg, ip) and midazolam (0.64 - 2.5 mg/kg, ip) each reduced the PTZ lever selection dose-dependently. In contrast, flumazenil (10 - 40 mg/kg, ip) had no effect during this period of peak withdrawal or a day later when half of the rats select the PTZ lever after saline. Thus the PTZ-like withdrawal stimulus was blocked by anxiolytics acting at the BZ receptor but was not affected by a receptor antagonist. These data suggest that if ethanol-withdrawal anxiety is produced by endocoids acting at the GABA-BZ-TBPS receptor-chloride ionophore complex, they act at a site different from the BZ receptor.

Supported by Grant AA06890 from NIAAA.

212.16

ANTAGONISM OF THE ATAXIC EFFECTS OF ETHANOL BY DRUGS ACTING AT BENZODIAZEPINE RECEPTORS, THE PICROTOXIN SITE, AND BY ALPHA-2 RECEPTOR ANTAGONISTS. R.G. Lister, M.J. Durcan and M. Linnoila. Laboratory of Clinical Studies, NIAAA, DICBR, Bethesda, MD 20892.

The ability of benzodiazepine (BDZ) receptor partial inverse agonists (Ro 15-4513, FG 7142 and Ro 15-3505), of drugs acting at the picrotoxin site of the BDZ/GABA receptor complex (pentylenetetrazole (PTZ) and Ro 5-3663) and of alpha-2 receptor antagonists (atipamezole and idazoxan) to antagonize the ataxic effects of ethanol was investigated. NIH Swiss mice were injected with ethanol (2.4 g/kg) and 5 min later their ataxia was rated. The mice then received the various drug treatments, and ataxia was again rated for a further 20 min by an observer ignorant of the treatment each mouse had received. Ro 15-4513 (3 mg/kg), PTZ (20 and 25 mg/kg), Ro 5-3663 (4 mg/kg), atipamezole (1 and 3 mg/kg) and idazoxan (1 mg/kg) all significantly attenuated the ataxic effects of ethanol. In contrast Ro 15-3505 (1.5 mg/kg) and FG 7142 (20-40 mg/kg) failed to alter ethanol-induced ataxia. Further FG 7142 (20-40 mg/kg) reversed the antagonism of ethanol's effect by Ro 15-4513.

A comparison of these results with those obtained from other behavioral paradigms suggests that ethanol's ataxic effect is mediated by a mechanism different from that underlying its other behavioral actions (e.g. its anxiolytic and anticonvulsant effects).

212.17

THE BENZODIAZEPINE INVERSE AGONIST RO15-4513 EXACERBATES, BUT DOES NOT PRECIPITATE, ETHANOL WITHDRAWAL IN C3H MICE. H.C. Becker and R.F. Anton. VA Medical Center and Department of Psychiatry, Medical University of South Carolina, Charleston, SC 29403.

RO15-4513, an imidazobenzodiazepine that has been reported to antagonize several behavioral and biochemical actions of ethanol, was given to C3H mice at various times during withdrawal from chronic (72 hours) continuous exposure to ethanol vapor. The ethanol withdrawal response was scored for ambulatory behavior and convulsions (spontaneous as well as handling- and audiogenic-induced seizures). When administered immediately following chronic ethanol exposure, RO15-4513 (6 or 12 mg/kg) did not influence the withdrawal response. However, when given at subsequent times (3, 5, and 8 hours post-ethanol withdrawal), RO15-4513 significantly increased the severity of the withdrawal response in ethanol-exposed mice. Moreover, this exacerbation was completely reversed by pretreatment with the benzodiazepine receptor antagonist RO15-1788. RO15-4513 did not significantly influence the behavior of controls. Thus, these data indicate that the benzodiazepine inverse agonist, RO15-4513, is capable of exacerbating, but not precipitating ethanol withdrawal. Supported by the Veterans Administration and NIAAA.

212.18

FG-7142, A BENZODIAZEPINE PARTIAL INVERSE AGONIST, ANTAGONIZES ETHANOL-INDUCED DEPRESSIONS OF ELECTROPHYSIOLOGICAL ACTIVITY IN CEREBELLAR PURKINJE NEURONS. C.G. van Horne*, J.T. Harlan*, and M.R. Palmer. Univ. Colo. Hlth. Sci. Ctr., Denver Colorado.

It has been hypothesized that effects of ethanol may be mediated through a GABA receptor mechanism. It has been reported that the behavioral, biochemical, and electrophysiological effects of ethanol are antagonized by the benzodiazepine (BDZ) derivative, RO15-4513, a partial inverse agonist at the BDZ/GABA receptor complex. Our goal was to determine whether this antagonism was a unique property of Ro15-4513 or whether it could be attributed to partial inverse agonists in general. Both systemic and local applications of ethanol are known to cause a depression in the electrophysiological activity of cerebellar Purkinje neurons (CPNs). We therefore recorded the extracellular electrophysiological activity of CPNs through a multibarreled microelectrode and then compared pressure ejected local applications of ethanol (750 mM.) that caused repeatable and consistent depressions in baseline activity (between 30 and 70%) before and after the local application of FG-7142 (200 uM, pH 5.5-6.0), a BDZ partial inverse agonist. We found that FG-7142 application significantly antagonized ($p < 0.001$, paired t-test) the electrophysiological depressant effects of locally applied ethanol in 15 out of 17 neurons studied. Complete antagonism was observed in 47% of the FG-7142 sensitive neurons and a greater than 90% antagonism was seen in all but 3 sensitive cells. In comparison to our previous studies with Ro15-4513, FG-7142 was less potent but had a greater efficacy than Ro15-4513 in antagonizing ethanol. We therefore conclude that FG-7142 can antagonize the electrophysiological depressant effects of locally applied ethanol, and that this antagonism is not unique to Ro15-4513 but may be a property of partial inverse agonists in general. Grant support: AA05915 and AA03527.

212.19

ETHANOL-INDUCED DEPRESSIONS OF HUMAN CORTICAL NEURONS ARE ANTAGONIZED BY Ro15-4513: EVIDENCE FROM INTRACELLULAR TRANSPLANTS IN ATHYMIC RATS. A-C. Granholm[1], M. Eriksdotter-Nilsson[2], J. Stromberg[2], Philip Stieg[2], L. Olson, A. Seiger[3], M. Bygdeman[4], B. Hoffer[4] and M. Palmer[4]. [1]Department of Pharmacology, University of Colorado HSC, Denver, CO 80262, USA, [2]Dept. of Histology, Karolinska Institute, Stockholm, Sweden, [3]Dept. of Neurological Surgery, Univ. of Miami School of Medicine.

Human fetal tissue from the cerebral cortex was collected following elective abortions in the eigth to eleventh week of gestation using procedures approved by the Ethical Committee of the Karolinska Hospital conforming to USPHS guidelines. The human cortical tissue was transplanted to the anterior eye chamber of athymic nude rats. The cortical transplants increased significantly in size in oculo and became vascularized from the host iris. Different classes of neurons were identified with immunohistochemistry. Extracellular recording of single unit activity was performed in 9 transplants after 3-7 months in oculo. Single action potentials showed a more immature waveform in younger transplants, with long durations and very slow spontaneous discharges. Firing rates ranged from 0.5 to 6.0 Hz. Superfusion with known concentrations of ethanol elicited predominantly inhibitions of baseline firing rates, with occasional excitations at low concentrations (1-10mM). The transplants could be divided into two groups with respect to neuronal ethanol sensitivity, with EC50s of 28.9 mM (n=13) and 2.70 mM (n=4) respectively. Superfusion with Ro15-4513 caused a significant antagonism of ethanol-induced inhibitions. This antagonism lasted for 1.5-2 hours, whereafter the response to ethanol returned to control levels. In conclusion, ethanol elicits dose-dependent inhibitions of discharge in transplanted human cortical cells. This inhibition is antagonized by Ro15-4513.

212.20

RO15-4513 PREFERENTIALLY REVERSES ANTICONVULSANT EFFECTS OF ETHANOL IN RATS. S.K. Kulkarni* and M.K. Ticku (spon. R. Huffman), Dept. of Pharmacology, Univ. of Tx. Hlth. Sci. Ctr., San Antonio, TX 78284-7764.

RO15-4513, a novel imidazobenzodiazepine, is reported to antagonize the acute intoxicating effects of ethanol in rodents. In the present study the reversal by RO15-4513 (1-4 mg/kg, ip) of ethanol (2 g/kg, ip) protective effect against bicuculline (8 mg/kg) and picrotoxin (10 mg/kg) induced convulsions were studied in rats. Modification of onset of myoclonic jerks, duration of tonic extensors and mortality rate and time were recorded. Pretreatment with RO15-4513 (4 mg/kg), reversed the anticonvulsant effect of ethanol against bicuculline-induced tonic extensor phase and mortality (87%), a response sensitive to reversal by RO15-1788 (10 mg/kg). It reversed picrotoxin-induced mortality only upto 50%. As compared to ethanol it only partially reversed the protective effect of pentobarbital. The tonic extensor phase was not reversed. On the other hand FG-7142, another inverse agonist had least effect on the tonic extensor phase while it reversed ethanol protection against onset and mortality due to bicuculline-induced convulsions. FG-7142 failed to reverse the anticonvulsant effect of ethanol against picrotoxin. Both RO15-4513 and FG-7142 possessed proconvulsant effects against bicuculline but not against picrotoxin. These observations suggest that RO15-4513 preferentially reverses ethanol effects as compared to other inverse agonist.

212.21

[^{3}H]Ro 15-4513 BINDING TO CULTURED CEREBELLAR GRANULE CELLS. K. Nieminen, E.R. Korpi and O. Malminen*. Research Laboratories of the Finnish State Alcohol Company, Alko Ltd., POB 350, SF-00101 Helsinki, Finland.

The imidazobenzodiazepine Ro 15-4513 has been reported to block the anticonflict and intoxicating effects of ethanol in vivo as well as to antagonize ethanol-stimulated GABA-receptor mediated uptake of $^{36}Cl^-$ (Suzdak et al., Science 234: 1243-1247, 1986). Ro 15-4513 has not, however, proven to be an efficient alcohol antagonist in all studies (Hellevuo and Korpi, Pharmacol. Biochem. Behav. 30: in press). Pharmacologically, this controversial agent is classified as a partial inverse agonist of the benzodiazepine receptor. In addition to binding displaceable by traditional benzodiazepine agonists, such as diazepam, also diazepam-insensitive (d-is) binding of Ro 15-4513 has recently been reported (Sieghart et al., J. Neurochem. 48: 46-52, 1987).

We studied the properties of [^{3}H]Ro 15-4513 binding to intact granule cells from neonatal rat cerebellum. In cultured cells 7 d.i.v. about 60 % of the specific binding was diazepam-insensitive. Ro 15-1788 and βCCE displaced all specific binding with IC_{50} values near 100 nM and 1 μM, resp. Ethanol (30-300 mM) decreased the binding in the absence of diazepam, but not in the presence of it (10 μM).

The results suggest that Ro 15-4513 binds to two populations of sites in granule cells from the rat cerebellum; of these the binding displaceable by diazepam is sensitive to ethanol.

DRUGS OF ABUSE I

213.1

A DOSE-RESPONSE STUDY OF AMPHETAMINE-INDUCED LOCOMOTOR STEREOTYPY IN RATS. K. Mueller, P. Kunko*, D. Whiteside* and C. Haskett*. Dept. of Psychology, Texas Christian Univ., Fort Worth, TX 76129

We have recently proposed a new statistic ($\hat{\gamma}$) that quantifies the tendency of amphetamine-treated rats to exhibit repetitive patterns of locomotion in an open field. In our initial studies we found that higher doses of amphetamine (3.5 to 6.5 mg/kg) increased locomotor stereotypy (as measured by $\hat{\gamma}$) by producing repetitive trips around the perimeter of the open field.

In this experiment we injected rats (n=12 per group) with saline, 1.0, 2.0, 3.0, and 4.0 mg/kg amphetamine (subcutaneously) after the rats had been in the open field for 20 min. Each rat was immediately returned to the open field; its path through the open field was recorded.

Saline-treated rats tended to sleep during most of the observation period. The greatest locomotor stereotypy was produced by 2 mg/kg amphetamine; it was not unusual for some rats to repeat the same pattern of locomotion for 20 min. or more. Rats given higher doses of amphetamine exhibited locomotor stereotypy prior to exhibiting focused stereotypy (characterized by absence of locomotions and intense sniffing/biting of a restricted area of the open field).

We conclude that locomotor stereotypy is an important component of the behavioral effects of amphetamine in rats.

213.2

ENDOCRINE, IMMUNE AND NEUROCHEMICAL CHANGES IN RATS DURING WITHDRAWAL FROM CHRONIC AMPHETAMINE (AMPH) INTOXICATION. N.R. Swerdlow (1), R. Hauger (1), M. Irwin (1) K.T. Britton (1), G.F. Koob (2) and L. Pulvirenti (2), (1) Dept. Psychiatry, UCSD Sch. of Med., San Diego, CA 92093; (2) Preclin. Neurosci., RISC, La Jolla, CA 92037

Following cessation of chronic AMPH use or administration, both humans and infrahumans exhibit behavioral changes. Withdrawal from AMPH in humans is accompanied by symptoms similar to major depression, with disturbances in sleep, concentration and mood, including suicidality. Little is known regarding the neural substrates underlying the affective consequences of AMPH withdrawal.

In the current study, male Wistar rats (n=68) were treated with d-amphetamine sulfate (AMPH; 7.5 mg/kg i.p.) or saline (SAL) twice daily for 10 d. Withdrawal from this regimen of AMPH has been shown to disrupt responding for intracranial electrical brain stimulation in rats. One, 5, and 10 days following the final dose of AMPH, animals were challenged with either SAL or physostigmine (PHY; 0.1 mg/kg sc). PHY challenge has previously been shown to stimulate a "supersensitive" increase in plasma ACTH in depressed humans and dopamine-depleted rats. 20 min following PHY injection, animals were sacrificed. Trunk blood was collected and analyzed for levels of ACTH, prolactin and corticosterone. Pituitaries were collected and CRF receptors were assayed for K_D and Bmax. Brains were dissected and assayed for catecholamines in striatal and mesolimbic regions using HPLC. Spleens were collected and assayed for N_K cell activity.

Our findings indicate significant effects of AMPH withdrawal on neuroendocrine, neurochemical and immunological indices. AMPH withdrawal in rats provides information critical for understanding neural substrates of affective changes that accompany cessation of chronic AMPH use in humans.

213.3

IN VITRO REACTIVATION OF CENTRAL TRYPTOPHAN HYDROXYLASE AFTER IN VIVO INACTIVATION BY AMPHETAMINES. D.M. Stone*, G.R. Hanson and J.W. Gibb (SPON: W. Stevens) Dept. of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Within 1 h of systemic administration to rats, methamphetamine (METH) and several of its structural analogues, including 3,4-methylenedioxymethamphetamine (MDMA) and p-chloroamphetamine (PCA), induce a pronounced decrease in the activity of central tryptophan hydroxylase (TPH), the rate-limiting enzyme for serotonin biosynthesis. The mechanism of this effect currently remains unknown; however, it appears not to be a direct effect of the parent compound, since amphetamines have no inhibitory action on TPH activity in vitro (Sanders-Bush et al., *Biochem. Pharmacol.* 21:1501, 1972; Schmidt and Taylor, *Biochem. Pharmacol.* 36:4095, 1987). We have recently discovered that cortical TPH activity from MDMA-treated rats, which was reduced to < 60% of control 3 h after a 10 mg/kg dose, could be completely reactivated to control enzyme activity by a 20-h preincubation in vitro (25°C) under strong reducing conditions (5 mM dithiothreitol, 50 μM Fe^{+2}, N_2 atmosphere). Cortical TPH previously inactivated by METH or PCA could be similarly reactivated. However, enzyme activity which was decreased 30 days after multiple MDMA doses could not be reactivated, suggesting irreversible inactivation had occurred by this time. These results suggest that the in vivo activity of TPH may be regulated by the oxidation/reduction status of key sulfhydryl groups within the enzyme molecule, and that amphetamines inactivate TPH by inducing oxidation of these groups (formation of protein disulfides), most likely indirectly via an oxidized metabolite or liberation of a reactive endogenous species. The kinetics of enzyme reactivation will be presented. (Supported by USPHS grants DA 00869 and DA 04222).

213.4

REGIONAL EFFECTS OF ALPHA-BENZYL-N-METHYLPHENETHYLAMINE (ABNMP) ON DOPAMINE AND SEROTONIN METABOLISM IN RAT BRAIN. V. Jackson-Lewis, M. Katz*, C.R. Clark*, S. Fahn and J.L. Cadet. Columbia University, New York, New York 10032

Illicit synthesis of drugs of abuse often results in compounds that are neurotoxic. Alpha-Benzyl-N-Methylphenethylamine (ABNMP), a methamphetamine analog, is such a compound. We investigated the acute effects of this drug on Dopamine (DA) and Serotonin (5-HT) metabolism in rat brain.

Male rats were treated with ABNMP (5-20mg/kg i.p.) and decapitated 30 mins. later. Another group of rats were treated with ABNMP (20mg/kg i.p.) and sacrificed at varying times up to 8 hrs after drug. Brain regions were dissected out on ice and tissues were frozen at -80°C until assayed. ABNMP (10-20mg/kg) caused decreases in DA in the striatum (25-35%) and in the nucleus accumbens (NAc) (25-30%). DOPAC and HVA were increased in these brain areas.

ABNMP (5-20mg/kg) decreased 5-HT (17-26%) and 5-HIAA (16-23%) in striatum, but had no effect on NAc 5-HT. 5-HIAA, in NAc, was slightly elevated.

ABNMP (20mg/kg) caused a peak decrease in striatal DA at 60 mins. which was still present 8 hrs. later. NAc DA was less affected; there was a small decrease in 5-HT in the striatum but not in the NAc.

Results suggest that ABNMP may affect the DA and 5-HT systems in rat brain.

213.5

NEUROBIOLOGICAL COMPONENTS OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF d-AMPHETAMINE. C. Bimle*, C. Co* and S.I. Dworkin (SPON: L.A. Bittenger). Dept. of Psychiatry, LSU School of Med., Shreveport, LA 71130

Rats were trained to discriminate d-amphetamine (1.0 mg/kg IP) from saline using a standard two lever procedure. The subjects were then tested using a modified drug-titration procedure. A descending range of doses from 1.0 to .17 mg/kg and saline were tested for discriminative stimulus properties. The dose tested with individual rats was decreased during successive sessions until the rat choose the saline lever after a drug injection and was increased until the subject again choose the drug lever. Following the determination of this threshold dose, the rats were lesioned with 6-OHDA or its vehicle. The animals were then tested again using the dose-titration procedure.

The procedure resulted in generalization gradients similar to those previously reported. The 6-OHDA lesion resulted in a moderate depletion of dopamine in the nucleus accumbens and a small but significant attenuation of the discriminative stimulus properties of d-amphetamine. These changes were not as large as those previously reported for cocaine discrimination. Thus, it appears that destruction of the dopaminergic input into the accumbens only moderately alters the discriminative stimulus properties of d-amphetamine. Research Supported By USPHS Grant DA-03631.

213.6

EFFECTS OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) ON BRAIN AND HEART BIOGENIC AMINES IN MICE. T.D. Steele* D.E. Nichols, and G.K.W. Yim. (SPON: C.O. Rutledge) Dept. of Pharmacol. and Toxicol., and Med. Chem. and Pharmacognosy, Purdue University, W. Lafayette, IN 47907.

The effects of acute and chronic administration of (±)-MDMA on brain and cardiac neurotransmitter and metabolite levels were assessed in male CF-1 mice. Acute treatment with the highest dose of MDMA (40 mg/kg) transiently decreased norepinephrine (NE) levels in the brain and heart. Only the cardiac depletion was blocked by desipramine pretreatment. In contrast to reported effects in rats, brain levels of serotonin and dopamine were elevated 3 hrs post-MDMA but were normal 6 and 24 hrs post-treatment. Levels of 5-HIAA, DOPAC and HVA were significantly lowered 3 hrs post-MDMA and gradually returned to control values. A regimen consisting of four daily doses of MDMA caused a significant decrease only in amine metabolite levels one week following the last treatment, which suggests an alteration in amine turnover possibly due to drug effects on monoamine oxidase. This effect may represent one biochemical difference contributing to the species variation in the response of biogenic amine systems to MDMA. (Supported in part by USPHS BRSG RR05586)

213.7

EFFECTS OF METHYLENEDIOXYMETAMPHETAMINE (MDMA, "ECSTASY") ON THE FIRING RATES OF SEROTONERGIC, DOPAMINERGIC, AND NORADRENERGIC NEURONS IN THE RAT. J.T.Lum, J.R.Palmer, and M.F.Piercey. The Upjohn Company, Kalamazoo, MI 49001.

MDMA is a non-hallucinogenic drug of abuse with an ability to reduce anxiety, increase sociability, and aid psychotherapy in human subjects. It releases dopamine (DA) and 5-HT, although higher concentrations destroy 5-HT terminals (Science 239:864, 1987). To further understand MDMA's psychotropic effects, we measured i.v. MDMA effects on firing rates of 5-HT neurons in dorsal raphe, noradrenaline (NE) neurons in locus coeruleus, and DA neurons in substantia nigra pars compacta. MDMA depressed firing rates of all 5-HT neurons, but potencies varied widely. There was a high-sensitivity group (MDMA ED50 = approximately 100 ug/kg) and a low-sensitivity group (MDMA ED50 = approximately 2000 ug/kg). Neither spiperone, a 5-HT1A autoreceptor antagonist (Lum and Piercey, Neurosci. Abs. 13:1649, 1987), nor the 5-HT2 antagonist LY53837 reliably reversed MDMA effects in either group. NE neurons were also depressed (ED50 = 670 ug/kg); piperoxan, an alpha-2 antagonist, only partially reversed these effects. MDMA in doses up to 3000 ug/kg did not affect firing rates of DA neurons. MDMA's psychotropic effects may be due to a unique interaction with NE cells and a highly sensitive subpopulation of 5-HT cells. Effects may reflect feedback control following enhanced transmitter release from terminals in post-synaptic targets.

213.8

MDMA AND MDE MEDIATE SUPRASPINAL BUT NOT SPINALLY MEDIATED ANALGESIA: EVIDENCE FOR POSSIBLE 5-HT INVOLVEMENT. J.W. Boja, T. Crisp and M.D. Schechter, Dept. of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

Serotonin (5-HT) has been shown to facilitate the reflexive response to pain and inhibit ascending nociceptive transmission (Zemlan et al., 1983). The "designer" drugs MDMA and MDE have both been reported to release 5-HT. Additionally, the analgesic properties of MDMA have been demonstrated in the mouse. In order to test the analgesic properties of MDMA, and the related drug MDE, male rats were tested utilizing the hot-plate test that measures supraspinal mediated analgesia, whereas the tail-flick procedure was used to measure spinally-mediated analgesia. MDMA (3.0 and 6.0 mg/kg, i.p.) significantly elevated hot plate latencies in a dose-dependent manner. MDE (8.0 mg/kg, i.p.) produced analgesia using the hot plate test. However, neither MDMA nor MDE elevated tail-flick latencies in rats. In fact, a hypersensitive response of this measure was seen following administration of 6.0 mg/kg MDMA. These results suggest that the analgesic effects of MDMA are mediated via the release of 5-HT. Funded by grant DA 04181.

213.9

SIGMA RECEPTORS: A PUTATIVE SITE OF ACTION OF MDMA (3,4-METHYLENEDIOXYMETHAMPHETAMINE) AND RELATED ANALOGS M. Yousif*, G. Battaglia, E.B. De Souza and R.A. Glennon* NIDA Addiction Res. Cntr., Baltimore MD 21224 and Dept. Medicinal Chemistry, MCV/VCU, Richmond, VA 23298

Sigma receptors are believed to mediate some of the effects of PCP and benzomorphan opiates. We report here that some of the psychotomimetc effects of the "designer" drug MDMA, and related analogs (MDE and MDP), may likewise be mediated by brain sigma receptors. MDMA, MDE and MDP exhibited high affinity (Ki= 0.2-3uM) for sigma receptors; these drugs were 7, 28, and 84-fold more potent, respectively, than MDA at this site. In contrast, the affinities of MDA were similar to those of MDMA (< 2-fold difference) at each of 20 other receptors and recognition sites in brain. All of the analogs exhibited low affinity for PCP receptors (Ki > 100uM) with MDA, MDMA, MDE and MDP being 11, 115, 454 and 484-fold more potent, respectively, at sigma receptors compared to PCP sites. The selectivity exhibited by these drugs contrasts with drugs such as PCP and benzomorphan opiates which interact with both sigma and PCP receptors. The data from the present study suggest that some of the psychotomimetic effects of MDMA, MDE and MDP may be due to selective high affinity interactions with brain sigma receptors. The finding that these compounds are more potent than MDA at sigma receptors may explain some of the differences in behavioral and/or subjective effects that have been reported between MDA and drugs such as MDMA and MDE.

213.10

SIGMA OPIATES: IDENTIFICATION OF A PRIMARY PHARMACOPHORE AND THE DESIGN OF HIGH-AFFINITY SIGMA-SELECTIVE LIGANDS. R. A. Glennon,* G. Battaglia,(+) J.D.Smith,* A. Ismaiel* and J. Herndon*. Dept Medicinal Chemistry, MCV/VCU, Richmond VA 23298 and (+)Addiction Research Center, Baltimore MD 21224 (SPON: L.S.Harris)

Few classes of agents bind at σ receptors with high affinity; of those that do, selectivity is generally low and structure-activity relationships are poorly understood. Perhaps the best studied agents are the "sigma opiates" (e.g. NANM, cyclazocine, pentazocine). We have begun an investigation of the structure-affinity relationships (SAFIR) of the σ opiates and have identified what we believe to be a primary pharmacophore: PAP. Although PAP itself binds with relatively low affinity, amine-substituted derivatives bind with a significantly higher affinity. For example, for R(-)PPAP, a derivative of PAP, Ki = 6 nM for [3H]haloperidol-labeled σ sites (rat cerebellum). SAFIR studies reveal that aromatic substitution also plays a significant role. These studies led to the design and synthesis of, e.g., (±)C2-PAP which binds with moderate affinity (Ki = 290 nM) and (±)C2-PPAP, one of the highest affinity agents yet reported for these sites (Ki = 0.5 nM). In addition, unlike many of the σ opiates, these agents display a fairly low affinity (Ki > 10,000 nM) for [3H]TCP-labeled PCP sites. Additional SAFIR studies with PAP analogs promise to provide new high-affinity, site-selective sigma ligands.

213.11

THE DETERMINATION OF PHENCYCLIDINE (PCP) AND RELATED COMPOUNDS BY HPLC-EC. C. Stephen*, C.J. Drebing*, W. Phillips*, R. Freedman and G.A. Gerhardt. Depts. Psychiatry and Pharmacology, Univ. of Colo. Hlth. Sci. Ctr., Denver, CO 80262.

We have developed a new rapid and sensitive method for the measurement of PCP, its metabolites, and cocaine using high performnace liquid chromatography coupled with electrochemical detection (HPLC-EC). PCP and related species are separated on a cyano bonded phase column (Beckman, 4.6 mm X 7.5 cm, 3 micron); the mobile phase is a pH 6.5 citrate-phosphate buffer containing 2.0 g/l NaCl with 20% acetonitrile. All eluting species are detected with a dual-carbon electrochemical detector (ESA 5011 Cell; electrode #1 set at 0.65 volts and #2 set at 0.80 volts). Species are detected at the second electrode and methyl-PCP is used as an internal standard. The detection limit of the assay is currently less than 1 nanogram per injection with a total separation time of less than 10 minutes. We are currently applying this method to studies of PCP metabolism and excretion in CSF, plasma and postmortem brain tissue samples from rodents.

213.12

DOSE DEPENDENT BEHAVIORAL EFFECTS OF N-ALLYLNORMETAZOCINE (SKF10,047) ISOMERS IN A PRIMATE SOCIAL COLONY. D.J. McGinness*, R.F. Schlemmer, Jr., N.L. Katz & J.M. Davis, Univ. of Illinois at Chicago & Ill. State Psychiatric Institute, Chicago, IL 60612

(±)N-Allylnormetazocine (NAN) is a potent psychotomimetic in humans. Although this activity is usually attributed to its sigma agonist property, binding studies suggest that (+)NAN is a preferential sigma agonist, while (-)NAN is a mu antagonist and kappa opiate receptor agonist. The present study compared the behavioral effects of the isomers & racemate of NAN in a primate social colony. Following determination of normal colony behavior, 4 members of a stable Stumptail macaque social colony received 5 acute doses of (±)NAN, (+)NAN, & (-)NAN, 0.1-3.0 mg/kg, in a cross-over design. Each dose was given i.m. 15 min. prior to a 60 min. observation session conducted by a "blind" observer. All forms of NAN decreased initiated social interactions, grooming, locomotion & checking (visual scanning); and increased total resting time. Interestingly, (-)NAN was consistently more potent than (+)NAN. NAN failed to induce most of the behavioral changes induced by dopamine agonist psychotomimetics in this species, but instead induced changes more consistent with phencyclidine (PCP). The results of this study suggest that the mu antagonist and kappa agonist activity should not be disregarded when considering the clinical effects of the "sigma agonist" (±)NAN.

213.13

DO DEXOXADROL AND PHENCYCLIDINE PRODUCE DIFFERENT DISCRIMINATIVE STIMULI? D.A. Bennett, P.S. Bernard, C.L. Amrick and J. Lehmann. Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901.

Dexoxadrol (DEX) is a dissociative anesthetic for which discriminative stimuli have not been reported. In the following investigation, the discriminative stimuli of DEX were compared with those of phencyclidine (PCP). In generalization experiments, the representative dissociative anesthetics PCP, DEX and ketamine generalized to both cues. Two other compounds, dextrorphan (DT) and dextromethorphan (DM), were assessed for both generalization to and antagonism of the cues. Both compounds generalized to PCP discriminative stimuli but only DT generalized to DEX discriminative stimuli. In antagonism studies, DT did not block either the PCP or DEX cue, while DM produced dose-dependent antagonism of the DEX cue, with a maximum of only 22% antagonism of the PCP cue. These data suggest a qualitative difference between DEX and PCP discriminative stimuli. Dissociative anesthetics are know to produce some effects characteristic of psychosis. As such, an antagonist of these effects may have potential antipsychotic properties. It has been difficult to block PCP-induced behaviors. If the DEX cue is sensitive to compounds that antagonize the effects produced by dissociative anesthetics, it suggests that this assay might identify compounds with potential antipsychotic activity.

213.14

ANTIPUNISHMENT EFFECTS OF PHENCYCLIDINE AND PCP-LIKE COMPOUNDS. J.H. Porter, J.L. Wiley and R.L. Balster*. Depts. of Psychology and Pharmacology, Virginia Commonwealth Univ., Richmond, VA 23284.

Antianxiety drugs such as chlordiazepoxide (CDP) have been shown to increase punished responding in animals. The present study examined the antipunishment effects of phencyclidine (PCP) and related compounds in a Geller-Seifter (1960) Conflict test. Twenty male rats (80% BW) were trained to respond under a multiple fixed interval 60-sec (food only) fixed ratio 1 (food + shock) schedule of reinforcement. Significant increases in punished responding were produced by CDP (5 and 10 mg/kg), PCP (2 mg/kg), N-allylnormetazocine (10 mg/kg), etoxadrol (8 mg/kg), and (-)-B-cyclazocine (1 mg/kg). Morphine and (-)-a-cyclazocine did not produce any significant changes in punished responding, but both produced significant decreases in unpunished responding. Also, it was found that naltexone (1 mg/kg) did not block the antipunishment effect of (-)-B-cyclazocine. These results demonstrate that PCP and related compounds produce increases in punished responding, and that this effect does not appear to be related to the opiate properties of these compounds. The abuse of PCP and related compounds may be in part related to the antianxiety properties of some of these drugs. (Research supported by NIDA grant DA-01442)

213.15

IN VIVO BRAIN MICRODIALYSIS STUDY OF PHENCYCLIDINE ON PRESYNAPTIC DOPAMINE EFFLUX IN RAT CAUDATE NUCLEUS. J. Chen* and E.L. Gardner. Depts of Neuroscience and Psychiatry, Albert Einstein College of Medicine, New York, NY 10461.

Phencyclidine (PCP) is a potent CNS-acting drug with major abuse potential and the ability to induce psychosis claimed to mimic naturally occurring schizophrenia. Due to the postulated role of brain dopamine (DA) systems in mediating the rewarding properties of abused drugs (Spyraki, C., et al., *Psychopharmacology*, 79:278, 1983) and in the neuropathology of schizophrenia (Creese, I., et al., *Science*, 192:481, 1976), PCP has been suggested to affect brain DA systems. Gerhardt et al. (*J.Pharmacol.Exp.Ther.*, 241:714, 1987) showed PCP to mimic the action of the DA reuptake blocker nomifensine on *in vivo* electrochemical potentials presumed to represent DA in rat caudate. We studied PCP's effect on presynaptic DA efflux using *in vivo* brain microdialysis coupled with high performance liquid chromatography (HPLC), thus permitting precise biochemical identification of neurotransmitter efflux. Rats were implanted with microdialysis probes in the caudate, and dialysis experiments carried out as described by Zetterström et al. (*J.Neurochem.*,41:1769, 1983). 5 mg/kg PCP significantly raised basal DA efflux and robustly elevated K^+-stimulated DA efflux; these effects lasted up to 2 hrs and correlated with PCP-induced behavioral stereotypy. 10 mg/kg PCP produced more pronounced effects. We conclude that PCP has strong DA agonist effects and suggest these relate to its abuse potential and induction of psychosis.

213.16

DISRUPTION OF CENTRAL SEROTONERGIC FUNCTION BLOCKS THE ACTIVATION OF A_{10} BUT NOT A_9 DOPAMINE NEURONS BY PHENCYCLIDINE (PCP). A.Ceci* and E.D.French (SPON: F. Porreca Dept.Pharmacol., Univ.Arizona, Coll.Med., Tucson, AZ 85724

PCP's psychotomimetic effects have been suggested to involve the activation of the A_{10} mesocorticolimbic dopamine neurons. Since these cells receive a prominent serotonergic (5-HT) input from the dorsal raphe nucleus (DRN), the role played by this transmitter in PCP's A_{10} effects was investigated.

Single-unit extracellular recordings from A_{10} and A_9 neurons, identified according to well-established electrophysiological criteria, were made in chloral hydrate anesthetized rats. From these recordings cumulative dose-response curves for PCP were made: (1) 7-days following radiofrequency lesion of the DRN; (2) 2 hr after the administration of the selective 5-HT_2 antagonist, ritanserin (5 mg/kg, i.p.); and (3) 24 hr after the 2nd injection of the 5-HT depletor, p-chloroamphetamine (PCA) (10 mg/kg/day, i.p.). In controls, PCP increased the firing rate of A_{10} neurons by$\sim$50%. However, after all 3 treatments, this effect of PCP was virtually eliminated (+11% after DRN lesion, -7% after ritanserin and +12% after PCA). In contrast, the maximum activation of A_9 neurons by PCP ($\sim$+44%) was not attenuated following DRN lesion (+44%) or ritanserin (+48%) pretreatment. Also, none of the treatments significantly affected spontaneous firing rates.

These findings suggest that DRN 5-HT neurons play a pivotal role in PCP's stimulation of the mesocorticolimbic dopaminergic system, presumably through activation of 5-HT_2 receptors, and as such may be fundamentally involved in PCP's psychotomimetic actions.

213.17

THE EFFECT OF PHENCYCLIDINE ON DOPAMINE RELEASE IN THE CAUDATE-PUTAMEN OF THE RAT. X.Y. Xuan*, D. Chapman*, R.A. Gazzara, and S. Howard. (Sponsor: P. Lomax) MRRC, BRI, Department of Pharmacology, UCLA, Los Angeles, CA 90024.

Phencyclidine (PCP) has multiple pharmacological effects including: a psychotomimetic; a dissociative anesthetic; and a sympathomimetic. In these studies we used in vivo microdialysis to examine the effect of PCP on the extracellular levels of dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in the caudate-putamen of the rat. The dose-related effects of PCP (1, 10 and 20 mg/kg i.p.) in the urethane-anesthetized animal will be compared with the effects found in the unanesthetized preparation. Microdialysis samples were collected every 10 minutes and levels of DA, DOPAC, and HVA were measured using HPLC-EC. In the anesthetized preparation, PCP produced a dose-dependent increase in DA that was followed in time by an increase in both DOPAC and HVA. These preliminary data suggest that in the anesthetized preparation, PCP facilitates release of DA but does not inhibit uptake. Supported by USPHS Grant DA 03020.

213.18

BEHAVIORAL COMPARISON OF THE EFFECTS OF PHENCYCLIDINE, KETAMINE AND N-ETHYL-PHENYLCYCLOHEXYLAMINE IN THE CAT. *L.M. Hiraide*, S. Howard, D. Chapman* and M.S. Levine (SPON: M.K. Shellenberger). MRRC, UCLA, Los Angeles, CA 90024.*

This study compared the behavioral effects of phencyclidine (PCP), ketamine (KET) and an N-substituted analog of PCP, N-ethyl-phenylcyclohexylamine (PCE). KET has low binding properties to the PCP receptor while PCE has been demonstrated behaviorally and in binding studies in the rat to be a potent agonist of PCP. Experiments were performed on 15 adult cats. Each animal received 4 dosages of only one compound (0.1, 0.5, 1.0 and 2.0 mg/kg). There were 5 animals in each group. Behavior was assessed from 10 min pre-injection to 5 hr post-injection. Saline was administered to each cat on alternate weeks as a control. All compounds produced dose-related behavioral changes in postural integrity, locomotor activity, and stereotypic head movements. Responses produced by PCP and PCE were similar at all dosages. Overall the effects of PCE began with a shorter latency and outlasted those of PCP. The most intense and long-lasting effects occurred at the highest dose for both PCP and PCE. These results support previous data obtained in rats which have shown KET to be less behaviorally potent than PCP. Although in binding studies PCE has been reported to be six times more potent than PCP, the behavioral effects of these two compounds were similar in the cat. Supported by USPHS Grant DA03017.

213.19

EFFECTS OF PHENCYCLIDINE ON DOPAMINE IN THE CAUDATE NUCLEUS OF THE AWAKE CAT. D. Chapman*, R.A. Gazzara, X.Y. Xuan* S. Howard. MRRC, BRI and Department of Pharmacology, UCLA Los Angeles, CA 90024.

Phencyclidine (PCP) has been shown to inhibit the uptake of dopamine and at higher doses to release dopamine in vitro. Microdialysis was performed on chronically prepared awake restrained cats to evaluate the neurochemical basis of the behavioral effects of PCP. Cats adapted to restraint were each given 0.1,0.5,1.0, and 2.0 mg/kg PCP. Samples were collected every ten minutes using a microdialysis probe located in the caudate nucleus. Samples were analysed for dopamine, dihydroxyphenylacetic acid (DOPAC), and homovanillia acid (HVA) using HPLC-EC. PCP produced an increase in the extracellular concentration of dopamine, while the metabolites DOPAC and HVA decreased. These findings are consistent with earlier observations that demonstrate an inhibition of striatal dopamine uptake and a facilitation of release. The results of this study suggest that the neurochemical effects of PCP on striatal dopamine may be correlated with its behavioral effects in the cat. Supported USPHS Grant DA03020.

MONOAMINES AND BEHAVIOR II

214.1

POST-STRESS CHANGES IN RAT BRAIN MONOAMINES AND THEIR FREE AND CONJUGATED METABOLITES USING HPLC WITH SERIAL OXIDATIVE-REDUCTIVE ELECTROCHEMICAL DETECTION

W.W. Woodmansee, P.H. Desan, L.H. Silbert*, T.K. Smock and S.F. Maier, Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309

Exposure to various forms of stressful stimuli have been shown to induce behavioral and neurochemical changes. We examined the duration of monoamine alterations in the rat brain following a tailshock stressor. HPLC coupled with serial oxidative-reductive electrochemical detection was employed to measure norepinephrine (NE), dopamine (DA), and serotonin (5HT) and their respective metabolites (free MHPG, free DHPG, DOPAC, and 5HIAA) in the anterior cortex, cerebellum, hippocampus (structures whose chief noradrenergic input arises from the dorsal NE system), and hypothalamus (a region with a major innervation from the ventral NE system). Samples were collected from nonshocked controls and .5, 2, 6, and 24 hours following the termination of the stressor. Total (free and sulfated) MHPG and DHPG content was determined in cortex samples by enzymatic deconjugation prior to analysis. Stress was not found to alter DA and 5HT levels in any of the regions examined, but NE was dramatically reduced in the hypothalamus and hippocampus. Hypothalamic NE remained depleted for at least 24 hours. There were transient increases in 5HIAA and DOPAC in all regions examined, and transient increases in MHPG in all areas except the hypothalamus, suggesting an activation of all three monoaminergic systems. Hypothalamic MHPG and DHPG were significantly reduced up to 24 hours following stress. Large accumulations of free and sulfated MHPG were observed in the cortex, whereas significant increases in DHPG were evident in the conjugated, but not in the free form. Thus, stress, as employed in this laboratory to produce behavioral deficits, alters monoaminergic functioning in the rat brain that may persist for 24 hours. Supported by NSF grants BNS 85-07451 and BNS 85-20622.

214.2

THE EFFECT OF STRESS ON THE RELEASE OF ELECTROACTIVE SPECIES IN THE THALAMUS OF FREELY MOVING RATS. K.J.Renner*, L.Pazos* and R.N.Adams. (SPON: A.Oke) Dept. of Chemistry, University of Kansas, Lawrence, KS 66045.

The effects of shake-induced stress on the release of electroactive species was chronoamperometrically monitored in the ventral thalamic nuclear region. Male rats were bilaterally implanted with electrochemically pretreated, Nafion-coated carbon fiber electrodes and allowed to recover overnight. Chronoamperometric measurements, +0.12V vs Ag/AgCl, were taken at 15 sec intervals. In vitro, the Nafion-coated electrodes were minimally sensitive to ascorbic acid, serotonin, 5-hydroxyindole acetic acid and had low sensitivity to 3,4-dihydroxyphenyl acetic acid.

In vivo evaluation of electrode response to systemic drug administration showed that the baseline current decreased after α-methyl-p-tyrosine (αmpt) and FLA-63. Increases in the baseline current were observed following treatments with pargyline, yohimbine and yohimbine-injected 2 hr post-pargyline. These results suggest that an electrochemical signal, primarily due to norepinephrine (NE) release, can be monitored in thalamic regions.

Chronoamperometric responses to 2 min periods of the shake-stress were long lasting (ca. 20 min), reproducible and small in magnitude (ca. 200 nM). The signals were blocked by the administration of αmpt and FLA-63. These studies provide in vivo evidence suggesting that stressful sensory input to the thalamus is accompanied by the release of NE. (Supported by NSF).

214.3

ANGIOTENSIN AND DRINKING INDUCE DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS. D. S. Blander*, G. P. Mark, L. Hernandez and B. G. Hoebel (SPON. M. Lampert). Dept. Psychol., Princeton Univ., Princeton, NJ 08544

Angiotensin II can induce drinking when administered intracerebroventricularly (ICV). This experiment measured dopamine (DA) release in the nucleus accumbens (NAC) using microdialysis following ICV injections of angiotensin II or saline. Male, Sprague-Dawley rats were implanted with stainless steel cannulas in the third ventricle and guide shafts aimed at the posterior medial NAC. At least 1 week after surgery a microdialysis probe (0.2 mm x 2 mm tip) was inserted into the NAC and brain dialysate was analyzed every 20 min, for 1 hr prior to and 2 hr after ICV injections of either angiotensin II (100 ng/5 ul) or saline vehicle. One group (N=15) was tested with angiotensin or saline, and a second group was given angiotensin plus water to drink for 20 min. Angiotensin alone caused a small (25%) but statistically reliable increase in extracellular DA, and this effect dissipated 40 min post-injection. In contrast, with ICV angiotensin plus water to drink, NAC dopamine increased 70% above baseline, reaching a peak 40 min after injection. Extracellular DA remained elevated in these subjects for an additional 40 min. These results implicate dopaminergic involvement in reward-related behaviors [1,2] and suggest that DA may be released differentially in the accumbens depending on whether an animal is motivated (e.g. angiotensin alone) or rewarded (angiotensin plus water consumption).

1. Hernandez, L. & Hoebel, B. G. Life Sciences., 1988, 42, 1713-1723.
2. Chang, V. C., Mark, G. P., Hernandez, L. & Hoebel, B. G. Society for Neuroscience Abstracts, 1988, this volume.

214.4

EXTRACELLULAR DOPAMINE INCREASES IN THE NUCLEUS ACCUMBENS FOLLOWING REHYDRATION OR SODIUM REPLETION IN RATS. V. C. Chang*, G. P. Mark, L. Hernandez and B. G. Hoebel, Dept. Psychol., Princeton Univ., Princeton, NJ 08544

Studies have suggested dopaminergic involvement in rewarded behaviors such as food or fluid consumption [1,2]. The role of dopamine (DA) in salt and water intake was examined in this study using *in vivo* microdialysis in the nucleus accumbens (NAC). Male, Sprague-Dawley rats were implanted with stainless steel guide shafts ending dorsal to the posterior-medial NAC. One group (N=4) of animals was sodium deprived by combining diuretic, furosemide injections with a sodium-deficient diet. Another group (N=5) was deprived of water for 20 hr prior to testing. On the day of the experiment, a microdialysis probe (0.2 mm OD by 2 mm tip) was inserted into the NAC, and brain dialysate was analyzed at 20 min intervals for 1 hr before and 2 hr after giving 2% saline to salt-deprived animals or distilled water to water-deprived animals. Extracellular levels of DA increased significantly in both cases: 200% of baseline during saline drinking and 140% during water drinking. In both cases the increase was maintained for at least 1 hr following intake. These results suggest that dopamine in the nucleus accumbens is involved in rehydration and sodium repletion.

1. Hernandez, L. & Hoebel, B. G. Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life Sciences, 1988, 42, 1713-1723.
2. Blander, D. S., Mark, G. P., Hernandez, L. & Hoebel, B. G. Angiotensin and drinking induce dopamine release in the nucleus accumbens. Society for Neuroscience Abstracts, 1988, this volume.

214.5

EFFECTS OF DRINKING ON DOPAMINE, DOPAC AND HVA LEVELS IN THE RAT AS MEASURED BY IN VIVO DIALYSIS. K.E. Sabol and C.R. Freed, Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

This experiment was designed to determine whether water drinking results in changes in the release of dopamine (DA) and its metabolites using the in vivo dialysis technique. DA, DOPAC and HVA were measured in water deprived rats for a 220 min period on 2 separate days. Dialysis cannulae were located in either n. accumbens (NA) (n=5) or lateral anterior striatum (LAS) (n=5). For each rat, one day was designated a baseline session and no intervention was imposed. In the second session, the rat was given access to water for 24 min in the middle of the session. Consumption of approximately 15 ml of water caused increases of 30% for DOPAC and 24% for HVA in NA, and 24% for DOPAC and 27% for HVA in LAS. There were no changes in DA. In another experiment, the volume of water delivered was restricted to 0.05 ml of water every 30 sec. This fixed time paradigm was associated with equivalent motor activity though rats consumed only 16% of the water they received while free drinking. Preliminary results from the fixed time experiment showed increases in DOPAC and HVA even with the reduced water consumption. Changes in DA metabolites therefore appear unrelated to the amount of water consumed. Measures of overall activity made in both experiments suggest that changes in DA metabolites are instead related to motor activity induced by the drinking paradigms.

214.6

'IN VIVO' VOLTAMMETRIC RELEASE OF STRIATAL DOPAMINE (DA) IN INBRED MOUSE STRAINS. M.K. Sanghera;F. Crespi*+;K.Martin*+& C.A.Marsden*+. Dept Psychiat, UT Southwestern Med Ctr, Dallas, TX 75235; Dept Physiol, Univ Nottm Med Sch,UK+.

BALB/c and CBA mice differ in the spontaneous and drug-induced behaviors that are thought to to be mediated by DA mechanisms. In this study to sought to determine if this could be due to differences in the release of DA from terminals in the striatum. Carbon fiber electrodes (CFE) were implanted in the striatum of both strains (N=12) under chloral hydrate. Animals were pretreated with pargyline HCl (150 mg/kg,ip) and 3 hrs later K^+-stimulated DA release was measured using differential pulse voltammetry. The infusion of 2 µl of KCl from a Hamilton syringe inserted close to the CFE resulted in the immediate appearance of the DA Peak at +80 mV in both strains. In Balb/c this peak was 3.5x larger than in the CBA. The height of the peak corresponded to an 'in vitro' DA value of 24.5 ±7.6 µM for BALB/c and 7±2 µM for CBA. This DA release could be evoked every 10-15 mins with similar magnitude only in the BALB/c. Striatal DA after pargyline, measured with HPLC-ECD, was not different in the strains suggesting that in the BALB/c more DA may be present and released from the readily releasable pool than in CBA.

214.7

AMPHETAMINE SENSITIZATION IN THE NUCLEUS ACCUMBENS STUDIED WITH *IN VIVO* MICRODIALYSIS. Terry E. Robinson. Dept. of Psychology, The University of Michigan, Ann Arbor MI 48109.

The repeated use of amphetamine (AMPH) produces a hypersensitivity to the motor stimulant and psychotogenic effects of AMPH that can persist for months to years after the cessation of drug use. This suggests that AMPH may produce very long-lasting changes in those neural systems that mediate the motor stimulant and psychotogenic effects of AMPH. The present study was designed to explore the neurobiological basis of this sensitization phenomenon by studying rats that were pretreated with an escalating dose regimen of AMPH (from 1 to 10 mg/kg over 5 weeks). This regimen mimics the pattern of drug use associated with the development of AMPH psychosis. It was found that this treatment was not neurotoxic, because 25-30 days after the last pretreatment injection the postmortem tissue concentrations of dopamine (DA), serotonin and their metabolites were normal, and the animals showed relatively normal levels of spontaneous motor activity across the day-night cycle. However, AMPH pretreatment produced robust behavioral sensitization. Animals showed a marked hypersensitivity to the motor stimulant effects of an AMPH challenge, even after 15-21 days of withdrawal. Most importantly, this behavioral hypersensitivity to AMPH was accompanied by significantly elevated extracellular concentrations of DA in the nucleus accumbens, as indicated by *in vivo* microdialysis in freely-moving animals. In contrast, AMPH pretreatment had no effect on the basal extracellular concentrations of DA. It is suggested that the sensitization produced by chronic AMPH use may be due to enduring changes in the releasability of DA, and may represent an example of neural plasticity common to other forms of behavioral adaptation.

214.8

ELECTROPHYSIOLOGICAL ACTIONS OF SELECTIVE SIGMA RECEPTOR AGONISTS AND ANTAGONISTS ON MIDBRAIN DOPAMINE NEURONS. G.F. Steinfels and S.W. Tam. DuPont Co., Medical Products Department, Wilmington, Delaware 19898 U.S.A.

Receptor binding and autoradiographic studies have demonstrated that sigma and phencyclidine receptors have distinct binding sites and different anatomical distribution in brain. In vivo pharmacological studies, however, have been hampered by the lack of highly selective agonist and antagonist ligands for each site. (+)-Pentazocine, (+)-3PPP, and DTG bind to the sigma receptor but not to the PCP receptor. I.V. administration of these sigma agonists produced a dose dependent decrease in firing rate of A9 dopamine (DA) neurons. In contrast, MK-801, a selective PCP receptor agonist generally increased firing rate. Rimcazole and BMY 14802 are antipsychotic compounds which bind to sigma but not to PCP or DA receptors. I.V. administration of Rimcazole produced either no effect or increased DA neuron firing rate. BMY 14802 more consistently produced an increased firing rate of DA neurons compared to Rimcazole. Further, the rate reducing effects on DA neurons produced by (+)-3PPP were found to be partially or completely reversed following administration of BMY 14802. These data demonstrate different pharmacological properties of selective sigma or PCP receptor agonists on DA neurons. This study also demonstrates that a selective sigma receptor antagonist can antagonize the pharmacological response of a sigma receptor agonist.

214.9

MODULATION OF THE MASSETERIC REFLEX BY ENVIRONMENTAL CONDITIONS. I.L. Stafford and B.L. Jacobs, Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

The masseteric or jaw closure reflex is a monosynaptic brainstem system that receives dense monoaminergic inputs. We have proposed it as a model system for examining functional changes in central neurotransmission. Here we describe the basic methodology and explore changes in the reflex produced by conditions known to physiologically activate NE neurons. The next abstract directly explores the role of NE in mediating these changes. The reflex is elicited in behaving cats by pulse stimulation of MesV delivered every 6 sec for 8 trials at each of 3 current levels. This stimulation monosynaptically activates MoV, causing a masseter muscle contraction, which can be measured on an oscilloscope. Following baseline measures of the reflex amplitude, the reflex was once again elicited while cats were exposed to 15 min of loud white noise (100 db), a dog, or clicks presented at various intervals prior to MesV stimulation. The first two conditions dramatically facilitated the reflex response. The clicks produced reflex facilitation at 100 & 150 msec and suppression at 20 msec. The next study examined whether these effects could be directly attributed to changes in brain NE.

214.10

MODULATION OF THE MASSETERIC REFLEX BY BRAIN NOREPINEPHRINE. B.L. Jacobs and I.L. Stafford, Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ

Two approaches were employed to determined whether NE mediated, at least in part, augmentation of the masseteric reflex produced by environmental conditions known to activate NE neurons (see preceding abstract). In the first, cats were given either the α-adrenergic antagonist prazosin (5 mg/kg i.p.) or the 5-HT antagonist methysergide (0.5 mg/kg i.p.) and then exposed to loud white noise (100 db), a dog, or clicks presented at various intervals prior to elicitation of the reflex. In all cases prazosin blocked the reflex augmentation whereas methysergide was without effect. In the second study, the catecholamine neurotoxin 6-OHDA (4 μg in 1 μl) was employed to produce unilateral NE denervation of the motor component (MoV) of the reflex pathway. One week later, the reflex was elicited bilaterally while the cat was exposed to the aforementioned conditions. The normal augmentation of the reflex produced by these conditions was either blocked or markedly diminished on the denervated side. In both experiments, the suppression produced by the 20 msec click-reflex interval was maintained, indicating that these effects were not non-specific. These data represent the first demonstration that the release of NE, at a specific site and under physiologic conditions, facilitates behavioral output in the intact organism.

214.11

SEROTONERGIC FACILITATION OF THE MASSETERIC REFLEX IN BEHAVING CATS. L.E. Ribeiro-do-Valle, C.W. Metzler*, I.L. Stafford and B.L. Jacobs. Prog. Neurosci., Dept. Psych., Princeton Univ., Princeton, NJ 08544.

Serotonin (5-HT) has been shown to facilitate the activation of spinal and facial motoneurons in anesthetized animals. Preliminary results in our laboratory with awake cats indicate that 5-HT also increases the response of trigeminal motoneurons to their mesencephalic trigeminal nerve (MesV) input. This action of 5-HT was systematically examined in the present work. Cats were prepared as described in the preceding two posters. The amplitude of the electrical response of the masseter muscle (Mas) to stimulation of MesV was recorded before and at various times after injection of 0.125 ug of 5-HT directly into the motor nucleus of the trigeminal nucleus (MoV). We observed a mean increase of 250% in the magnitude of the reflex response, one and five min after 5-HT injection into MoV. The response approached baseline levels in the next 30 to 60 min. Three to six hours after the drug injection, we observed a second increase of 300% in the reflex response. The following day the response was again near baseline levels. The present results indicate that 5-HT has short- and long-latency facilitatory actions upon the trigeminal motoneurons. The short-latency effect may be related to the well known slow depolarization of motoneurons caused by 5-HT, while an intracellular second messenger system may be responsible for the long-latency effect.

214.12

SENSORY-EVOKED PHASIC ACTIVATION OF LOCUS COERULEUS NORADRENERGIC NEURONS IN BEHAVING CATS. E.S. Levine, W.J. Litto, C.A. Fornal, D.A. Morilak, K. Rasmussen and B.L. Jacobs, Prog. Neurosci., Princeton U., Princeton,NJ 08544.

Phasic presentation of noxious and non-noxious sensory stimuli elicits a brief activation of locus coeruleus noradrenergic neurons (LC-NE) in awake animals. In the present study using behaving cats, the tail-flick response to radiant heating of the tail was used to examine LC-NE neuronal responses to a mild, short-lasting noxious stimulus. Heat intensity was adjusted to produce baseline tail-flicks within 3-5 sec. All trials began when the cat was in a quiet waking state based on polygraphic and behavioral criteria. LC-NE neurons consistently showed a phasic activation around the time of the tail-flick. Unit activity was significantly elevated ($\sim$ 300%) during the two sec period centered at the time of the tail-flick compared to a two sec period centered at the time of heat onset. Systemic administration of an analgesic dose of morphine (2.0 mg/kg, i.p.) increased baseline unit activity and tail-flick latency, but did not alter the phasic activation of LC-NE units during the tail-flick. In contrast, a low dose of diazepam (0.125 mg/kg, i.p.) significantly reduced the phasic activation of LC-NE neurons at the time of the tail-flick, but did not significantly alter baseline unit activity or tail-flick latency. We are presently examining LC-NE neuronal responses to noxious and non-noxious stimuli as a function of changes in the level of behavioral arousal.

214.13

EFFECTS OF HYPERGLYCEMIA ON SINGLE UNIT ACTIVITY OF LOCUS COERULEUS NORADRENERGIC (LC-NE) AND DORSAL RAPHE SEROTONERGIC (DRN-5HT) NEURONS IN BEHAVING CATS. W.J. Litto, C.A. Fornal and B.L. Jacobs, Prog. Neurosci., Dept. Psychology, Princeton Univ., Princeton, NJ 08544.

Central noradrenergic and serotonergic neuronal systems have been implicated in the regulation of blood glucose levels through an interaction with feeding, neuroendocrine, and autonomic mechanisms. In this study, the effects of glucose loading on the single unit activity of LC-NE and DRN-5HT neurons were determined in catheterized, behaving cats. Single unit activity was sampled when cats were in a quiet waking state based on polygraphic and behavioral criteria. Single unit activity and blood samples were obtained prior to, and at 5, 15, 30, 45, 60, and 90 min following a bolus injection of dextrose (500 mg/kg, i.v.). Blood glucose levels were increased four-fold over baseline levels (89.4 $\pm$ 6.8 mg/dl; n=8) 5 min after dextrose injection, were significantly elevated for 60 min, and returned to preinjection levels by 90 min. Single unit activity of LC-NE and DRN-5HT neurons was not significantly different from baseline levels at any time following dextrose injection. These data suggest that LC-NE and DRN-5HT neurons are not directly glucose-sensitive, and that changes in their activity are not a component of counterregulatory mechanisms invoked by hyperglycemia.

214.14

SINGLE-UNIT ACTIVITY OF SEROTONERGIC DORSAL RAPHE NEURONS IN RELATION TO THE HYPOTENSIVE ACTION OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)TETRALIN (8-OH-DPAT) IN BEHAVING CATS. C.A. Fornal, W.J. Litto, H. Nagasaki*, L.E. Ribeiro-do-Valle and B.L. Jacobs, Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

Central serotonergic neurons are thought to play an important role in blood pressure regulation. Recently, 5-HT_{1A} agonists like 8-OH-DPAT have been shown to decrease mean arterial pressure (MAP), presumably by inhibiting the neuronal activity of serotonergic neurons via a direct action on somatic 5-HT autoreceptors. In this study, the effects of intravenous administration of 8-OH-DPAT (1-100 ug/kg) on MAP and single-unit activity of serotonergic neurons recorded in the dorsal raphe nucleus (DRN) were examined. The lowest dose of 8-OH-DPAT had no effect on MAP, but produced a brief, moderate inhibition of serotonergic DRN unit activity. Larger doses (>5 ug/kg) produced dose-related decreases in MAP within 30 sec that reached a maximum between 2-4 min. The fall in MAP was preceded by a complete suppression of serotonergic DRN unit activity. The maximum decrease in MAP ($\sim$50 mm Hg) was observed following 100 ug/kg, whereas maximum suppression of unit activity was produced by lower doses ($\sim$ 10 ug/kg). The hypotensive action of 8-OH- DPAT was relatively long (>30 min) and usually outlasted the unit suppression. These data support the hypothesis that inhibition of serotonergic neurons may contribute to the hypotensive action of 8-OH-DPAT.

214.15

REPEATED MILD STRESS MAY CAUSE TERMINAL SPROUTING OF LOCUS COERULEUS NEURONS: PHYSIOLOGICAL EVIDENCE. S. Nakamura, T. Sakaguchi* and F. Aoki*. Dept. of Physiology, Fac. of Medicine, Kanazawa Univ., Kanazawa 920, Japan.

Stress has been known to cause a variety of neurochemical changes in the central noradrenergic system. We now report physiological evidence that in repeatedly stressed rats, the number of axon terminals arising in the locus coeruleus (LC), a main nucleus of central noradrenergic neurons, increased in the cerebral cortex. Male Sprague-Dawley rats (8-to-14 weeks of age) were used. For stress treatment, animals restrained in a small cage were bathed up to the neck in warm water (36-37°C) for 10 min daily. The stress groups received stress treatments for either 1 or 2 weeks. The amount of projections of LC neurons to a given brain site was assessed by the physiological measure "projection index" (P-index), which is defined as the percentage of LC neurons activated antidromically from the given brain site. The P-indices for the frontal and occipital cortex and thalamus were determined under urethane anesthesia. The P-indices for each stimulating site did not differ between the control and 1-week stress group, whereas those for the cerebral cortex significantly higher in the 2-week stress than control group. These results suggest that repeated mild stress causes terminal sprouting of LC neurons in the cerebral cortex.

214.16

EFFECTS OF PHENTOLAMINE ON BEHAVIORAL THERMOREGULATION. S. Kent, C. Ricks*, D. Freedman* and E. Satinoff. Dept. of Psychology, Univ. of Illinois, Urbana-Champaign, IL. 61820.

We have shown that phentolamine (PHEN), an α-adrenoceptor antagonist, suppresses REM sleep. This effect was secondary to the drop in body temperature (Tb) which was ambient temperature (Ta) dependent. The present study examines whether the Tb changes reflect an altered thermal setpoint or altered effector capabilities. Five male Long-Evans rats (b.w. 300-500 g) were housed on a 12:12 LD cycle at a Ta of 23±1°C with food and water available ad lib. They had previously been trained to press a bar for warm air in the cold (0-4°C: cold escape) and for cool air in the heat (38-42°C: heat escape). Tb and Ta were monitored by telemetry every 2 minutes and 5 seconds, respectively, for at least 1 hour pre- and 2 hours post-injection (PHEN 10 mg/kg i.p. or an equivalent volume of saline).

Within 15 minutes of a PHEN injection in the cold rats increased the number of cold escapes by over 750%. Ta rose from a range of 2-4°C to 16-18°C within an hour, controls did not increase the Ta. After PHEN Tb dropped 2.5°C within ½ hour and then began to rise. Without access to the bar Tb dropped a mean of 7°C and the animals had to be removed from the cold. There are no differences between treatments in the heat. We conclude that PHEN does not alter thermal setpoint.

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214.17

EFFECTS OF THE NEUROTOXIN DSP4 ON CATECHOLAMINE LEVELS, ALPHA-TWO ADRENERGIC RECEPTOR BINDING AND STEROID-DEPENDENT REPRODUCTIVE PROCESSES IN THE JAPANESE QUAIL. P. Sante*, G.F. Ball B.S. McEwen, and J. Balthazart. Univ. of Liège, Liège, Belgium and Rockefeller Univ., New York, N.Y. 10021 (SPON: B. Nock).

Several lines of evidence implicate catecholamines (CA) in the regulation of reproductive hormones and behavior. We tested this possibility by administering the noradrenergic neurotoxin DSP4 (50 mg/kg) to castrated and testosterone-treated quail. The efficacy of the neurotoxin was assessed by measuring monoamine levels with high performance liquid chromatography combined with electrochemical detection in selected microdissected brain regions. Significant decreases in norepinephrine (NE) and epinephrine (E) concentrations were detected while dopamine and serotonin were unaffected. Plasma luteinizing hormone (LH) levels were decreased and copulatory activity was increased in DSP4 treated birds confirming a significant role for the noradrenergic system in these processes. The behavioral effect of the drug was only apparent in the T-treated birds and did not affect other androgen dependent behaviors. α-2 Adrenergic receptors as assessed by quantitative autoradiography ([³H]para-aminoclonidine binding) were not affected by the administration of the neurotoxin in any of the areas examined. In that a significant decline of NE and E was observed in most brain regions, this strongly suggest that these α-2 receptors are in general post-synaptic. These receptors are primarily located in steroid sensitive areas which play a major role in the regulation of both copulatory behavior (e.g. preoptic medial nucleus) and LH secretion (e.g. infundibulum). These receptors may mediate many of the effects of NE and E on steroid dependent reproductive processes. Supported by grant # MH41256 to B.M.

214.18

NORADRENERGIC AGONISTS AND LHRH STIMULATE MALE REPRODUCTIVE BEHAVIOR IN JAPANESE QUAIL. M.A. Ottinger, L. Cortes-Burgos* and C.S. Rawlings*. Dept. of Poultry Science, University of Maryland, College Park, MD 20742.

Pharmacological agents were given to castrated male Japanese quail to determine if activation of mating behavior would occur with low plasma testosterone. Males were castrated under anesthetic at 2 weeks of age, maintained in controlled environment (photoperiod of 15L:9D), with feed and water available ad libitum. At 8 weeks of age, males had low circulating testosterone and showed no sexual behavior. Phenylephrine and isoproterenol were injected peripherally into 50 castrate males. Both phenylephrine (0.075 mg/kg) and isoproterenol (7.5 mg/kg) stimulated mating behavior in 40% of the males. Testosterone implants were given to stimulate mating behavior in castrates. Propranolol (12.5 mg/kg) administration resulted in a depression of the number of males showing behavior (100% to 10%).

Avian LHRH was administered into the third ventricle (111 ng/1 µl PBS) to castrate males given testosterone implants. The number of mating attempts and completed matings increased significantly at 30 and 60 min., post-injection and then declined by 90 min. These data further support the regulatory role of the noradrenergic system in the expression of reproductive behavior and the modulatory effects of the LHRH system in behavior.

214.19

ALPHA-ADRENERGIC FACILITATION OF ROUGH-AND-TUMBLE PLAY IN THE RAT. S.M. Siviy, J. Menendez and D.M. Atrens*. Psychology Dept., Univ. of Sydney, Australia.

Previous research from our laboratory has shown that acute blockade of alpha-1 adrenoceptors with the selective antagonist prazosin reduces rough-and-tumble play in juvenile rats (Soc. Neurosci. Abst., 13:409). The effects of prazosin on play are both pharmacologically and behaviorally specific. Reduced adrenergic activity thus appears to be incompatible with active play in the rat. It follows that increased adrenergic activity should facilitate play. Idazoxan is a specific alpha-2 adrenergic antagonist which results in increased release of forebrain norepinephrine (Brain Res, 426:103-111). Accordingly, the effects of idazoxan on play were assessed. Juvenile rats (29-38 days old) were housed individually and given a daily 5 minute opportunity to play. Rats were injected IP with either the vehicle or one of four doses of idazoxan (1 - 8 mg/kg) 30 minutes prior to a play session. Idazoxan increased pinning, an indicator variable of play in this species, at all doses tested. The largest increase, 56% over vehicle levels, was after 4 mg/kg. Dorsal contacts, an index of play solicitation, were not affected by any of the doses. These data suggest that increased noradrenergic activity at alpha receptors is facilitatory to rough-and-tumble play in the rat. Experiments designed to assess the behavioral specificity of this effect are currently in progress.

Supported by an ARGS Programme Grant to DMA.

214.20

EFFECTS OF FOREBRAIN NOREPINEPHRINE DEPLETION ON NEOPHOBIA IN THE RAT. J.D. Steketee and A.C. Swann. Department of Psychiatry and Behavioral Sciences, University of Texas Medical School, Houston, Texas 77030.

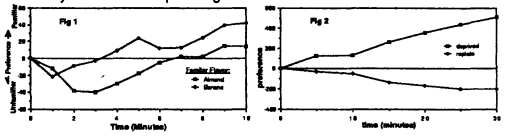
Forebrain NE depletion has been reported to increase (Britton et al. Physiol. Behav. 33:473, 1984) or decrease (Sahakian et al. Neurosci. 10:1405, 1983) neophobia. This may result from differential effects on environmental and food neophobia (Robbins et al. Physiol. Psychol. 13:127, 1986). In order to investigate this possibility we examined environmental and food neophobia in rats after NE depletion by bilateral infusions of 6-OHDA into the dorsal bundle. After a minimum of 2 weeks of post surgical recovery, environmental and/or food neophobia was measured in food deprived rats. Environmental neophobia was measured in a novel open field with familiar food mounted on a centrally placed food pedestal. Food neophobia was measured in a different novel environment with 2 food cups placed in the center, one for familiar rat chow and the other for novel cheddar cheese. Lesioned rats reared less and approached familiar food fewer times than controls showing increased environmental neophobia in lesioned animals. Lesioned animals preferred novel cheese (20/21 chose cheese first, $p<0.0001$) while controls showed no food preference (11/20 chose cheese first). Lesioned animals spent more time eating the novel food ($p<.0001$) while controls spent more time eating the familiar food ($p<.02$). Results of this study support an opposite role for forebrain NE in environmental and food neophobia.

215.1

A ROLE FOR BOTH NEOPHILIA AND POST-INGESTIVE FEEDBACK IN DIET SELECTION BY PROTEIN DEFICIENT RATS. S.C. Heinrichs*, B.O. Moore and J.A. Deutsch*(SPON:R. Boynton) Psychology Dept., UCSD, C-009,CA 92093.

A new method allows second by second monitoring of responses made by protein deficient rats to obtain either a protein diet or a non-protein, carbohydrate alternative. If fed an experimental diet lacking in protein for at least 4 days, animals eat more of at least seven protein alternatives in a 30 minute meal (Moore, See Abstract). Pregnant dams fed inadequate amounts of protein also prefer a new protein alternative. On the other hand, if control or pregnant dams are protein replete, they choose more of the carbohydrate.

These results indicate preference for a novel flavor (neophilia) since 1) each group prefers a novel alternative depending on experimental diet and protein need, 2) the preferred choice occurs within 1 minute's time, insufficient for post-ingestive consequences, and 3) the two choice responses are concurrent, again avoiding a post-ingestive contingency. However, if the novel flavor cue is inert rather than nutritive (e.g. almond extract), an initial neophilia decays rapidly in protein deprived animals and is reversed within minutes (Fig 1). In contrast, protein deprivation produces a consistent preference for protein continuing over 30 minutes (Fig 2). Thus, evidence will be presented for a compound protein selection strategy in which neophilia persists only if beneficial post-ingestive feedback is available.



215.2

DIET-INDUCED ELEVATIONS IN BODY WEIGHT AND FAT DEPOSITION ASSOCIATED WITH CHRONIC REDUCTIONS IN SYSTOLIC BLOOD PRESSURE BUT INCREASED BLOOD PRESSURE RESPONSIVENESS IN SHR RATS. R. J. Contreras. Dept. of Psychology, University of Alabama, Birmingham, AL 35294.

Interest is in the contribution of diet and patterns of consumption in influencing blood pressure and blood pressure responsiveness in Spontaneously Hypertensive Rats (SHR). Male 60-day old SHR rats were maintained on an unrestricted diet of either (1) Agway Prolab Chow pellets; (2) high fat mash and sweetened milk (HF/M); or (3) alternate 2-week periods of HF/M and Agway pellets. Body weight and caloric intake were measured weekly as were systolic blood pressure and heart rate using tail plethysmography under light ether anesthesia. After 16-weeks, mean arterial pressure (MAP) and heart rate (HR) responses to the pressor effects of bolus intravenous infusions of phenlyephrine hydrochloride (50, 100, 200, 400 ng/100 g BW) were obtained from the femoral artery of urethane-anesthetized rats.

The consumption of the HF/M diet resulted in significant elevations in body weight and in terminal brown fat pad, white fat pad, and heart organ weights. In addition, HF/M feeding resulted in significantly faster heart rates; for rats on the alternate feeding regimen, HR was elevated during HF/M feeding and reduced during pellet feeding. However, the indirect blood pressure levels of the two groups fed the HF/M diet tended to be lower than those of the pellet-fed group. Despite these chronic reductions in indirect blood pressure, the rats fed the HF/M diet were significantly more responsive to the pressor effects of intravenous phenylephrine.

Supported by NIH Grant HL-38630.

215.3

SATURATED FAT DIETS DECREASE CARBOHYDRATE AND INCREASE PROTEIN SELECTION IN RATS. C.D. McGee* and C.E. Greenwood (SPON: N. Scott) Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Rats consuming 20% (w/w) lard diets select less carbohydrate (CHO) and more protein (PRO) than rats fed 20% (w/w) soybean oil (SBO) diets (Pharm Biochem Beh 27:1). To ensure this effect was not specific to fat source, male Wistar rats were stabilized to 20% (w/w) SBO or beef tallow (TAL) diets containing 24% PRO and 48% CHO for 2 weeks. During self-selection from 2 diets with the fat previously fed but different PRO and CHO composition (5% PRO, 67% CHO and 55% PRO, 17% CHO), TAL fed rats selected more PRO (119±9 vs 62±3 g/14 d, mean±SEM, TAL and SBO respectively) and less CHO (98±12 vs 170±6 g/14 d) than SBO fed rats. A second study was conducted to rule out the possibility that differences were due to taste preference. When rats selected from diets with identical PRO and CHO composition but differing in sources of dietary fat, there was a slight preference for TAL at all PRO levels. When rats selected between diets described in experiment 1 from first exposure, TAL fed rats selected significantly more PRO and less CHO than SBO fed rats during the last 2 weeks but not the first 2 weeks, suggesting physiological adaptation must occur prior to expressing selection differences. These results confirm that rats fed high saturated fat diets select more PRO and less CHO than rats fed high polyunsaturated fatty acids. Further, these data suggest this difference is not due to taste preference. (MRC)

215.4

I.V. FAT- AND WATER-SOLUBLE NUTRIENTS AFFECT FOOD INTAKE DIFFERENTLY. E.K. Walls and H.S. Koopmans, (SPON: C. Riphagen) Dept. of Medical Physiology, Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1

Nutrients infused directly into the bloodstream bypass the usual absorptive mechanisms of the gut. To assess the effect of gut signals on daily food intake, two different types of i.v. nutrients were infused during 17.5 hours of feeding. In the first experiment (n = 5), food intake during 6 days of saline infusion was 80.0 ± 2.7 kcal and the rats gained 1.4 ± 0.8 gms/day. Thereafter, a solution of 25% dextrose and 4.25% amino acids (Travasol) was infused i.v. at levels of 27 and 54 kcal/day for two consecutive four day periods during night-time feeding. During the infusion periods, the rats reduced their food intake by 20.7 ± 1.7 and 40.0 ± 2.4 kcal per day, respectively, and gained weight at a rate of 3.5 ± 1.2 gm/day. Thus, oral intake was reduced by an amount that was equivalent to 75% of the total i.v. calories infused ($p<.05$), showing that gut signals play a small but significant role in limiting food intake. In the second study with fat infusion (n = 4), the rats ate 63.5 ± 2.0 kcal during 6 days of saline infusion and their body weight was stable. When infused i.v. with 40 kcal of a fat emulsion (Intralipid), the rats showed a significant ($p<.01$) but smaller reduction, 12.1 ± 1.1 kcal/day, in oral food intake and gained 3.0 ± 0.8 gm/day. Their reduction in food intake was only 30% of the fat calories infused. The results show that gut signals play a major role in the control of daily food intake, particularly for dietary fats.

215.5

EFFECT OF PROTEIN AND CARBOHYDRATE BREAKFASTS ON PLASMA TRYPTOPHAN RATIO IN MALES. K.L. Teff and S.N. Young, Dept. of Psychiatry, McGill Univ., Montreal, Canada H3A 1A1.

To enter the brain, TRP must compete with other large neutral amino acids for uptake across the blood-brain barrier. The plasma ratio of TRP to the sum of the competing amino acids (TRP/NAA) has been used as an indicator of tryptophan availability to the brain. Protein and carbohydrate have opposite effects on brain TRP. Protein tends to lower brain TRP while carbohydrate increases it. We have looked at the effect of breakfasts containing varying amounts of protein and carbohydrate on the plasma tryptophan ratio in normal human males. Isocaloric breakfasts were given in the form of a chocolate pudding that contained either 0, 4, 8 or 12% protein with carbohydrate as the remaining macronutrient. An additional treatment consisting of a Danish pastry and coffee was given to determine the effect of a typical breakfast on the TRP/NAA ratio.

We found that only the 0% protein treatment (pure carbohydrate) significantly increased the TRP/NAA. The addition of even 4% protein was sufficient to inhibit the rise in the ratio. As all normal meals contain 4% or more carbohydrate, there are no normal meals which are "carbohydrate meals" as far as the TRP/NAA ratio is concerned. We conclude that behavioral changes observed after intake of meals high in carbohydrate in humans are not mediated by increases in the TRP/NAA ratio.

215.6

Threonine (Thr) injection into the prepyriform cortex increases the intake of thr imbalanced diet. J.L.Beverly*, D.W.Gietzen, P.M.B.Leung and Q.R.Rogers. Dept. Physiol. Sci. and Food Intake Lab, Univ. California, Davis, CA 95616.

An intact prepyriform cortex (PPC) is essential for the normal reduction of food intake observed 2 hrs after feeding rats a thr imbalanced diet. More recently the concentration of thr in the PPC of rats fed a thr imbalanced diet (IMB) was found to be significantly reduced by 2.5 hr of feeding IMB. In two trials thr or buffered aCSF (vehicle) were injected bilaterally into the PPC of rats and the effect on intake of a thr basal diet (BASAL) or IMB measured. Bilateral chronic guide cannulae were implanted 3 mm above the PPC of male Sprague-Dawley rats which were then fed BASAL for 10-14 days. Rats were anesthetized with Metofane 30 min. prior to the onset of dark cycle and 2.0 nmoles (Trial 1) or 2.2 nmoles (Trial 2) thr or vehicle injected (1 ul/5 min) into the PPC. Although the reduction in 24 hr intake of IMB was not completely attenuated intakes of IMB by rats receiving thr were greater than by rats receiving vehicle. 24 hr intakes of IMB by rats receiving thr were 151% of vehicle rats in Trial 1 ($p<.01$) and 138% of vehicle rats in Trial 2 ($p<.05$). The differences in intake were not apparent in either trial until at least 2 hr of feeding. These results indicate that increasing thr content in the PPC partially alleviates the reduction in intake of a thr imbalanced diet. Supported by NIH Grants AM-07355, DK-13252 and USDA Grant CRCR-1-2418.

215.7

EFFECT OF DIETARY PROTEIN ON POST-MEAL GLUCOSE, INSULIN AND TRYPTOPHAN AVAILABILITY IN RATS. L. Thibault, G. Bergeron* and A.G. Roberge. Sch. of Dietetics and Human Nutrition, McGill Univ., Montreal; Lab. Neurochemistry & Nutrition, Laval Univ., Quebec, Canada.

An inverse relationship between the dietary protein/carbohydrate ratio and the fasting serum tryptophan level and a direct relationship between the former and the fasting brain tryptophan level and 5-HT turnover in limbic structures was previously observed in rats fed single casein diets (Thibault & Roberge,J.Amer.Coll.Nutr.In Press,1988).Selection of nutrients from a dietary choice is a fundamental characteristic of behavior in animals and in man.Adult male Sprague-Dawley rats were allowed to choose for a 8 h daily period and for 16 consecutive days between pairs of diets containing either 0 and 60% casein or 0 and 60% soya. On day-16, diets were removed after the 8 h feeding period and arterial and veinous blood samples were collected under anesthesia at intervals of 30, 60 and 90 min after the end of eating.The total food intakes were similar in casein and soya fed rats whereas a significantly higher food intake of the 60% soya diet was observed when data were compared to the 60% casein diet.The post-meal arterioveinous glucose,insulin and tryptophan differences were assessed and the results obtained suggested: 1)a higher glucose utilization in casein fed rats than in soya fed rats within 30 min after eating, and 2)▲-insulin values at 30 and 60 min post-meal and arterial and veinous tryptophan levels at all the post-meal intervals significantly higher in soya fed rats than in casein fed rats. These results suggested that the protein/carbohydrate selection is regulated differently according to the type of dietary protein and that the preference for selecting protein with soya is possibly mediated by an increased tryptophan availability related to a high insulin secretion but paralleled by a glucose utilization that is similar to the one observed with casein. Supported by NSERC of Canada.

215.8

NUTRIENT COMPOSITION: EFFECTS ON TEMPORAL PATTERNS OF FEEDING. G. Brennan*, G. Shor-Posner, C. Ian*, R. Jasaitis*, P. Eyih*, K. Madhu*, & S. F. Leibowitz. (SPON: J. Brudny) The Rockefeller University, New York, NY 10021

Evidence suggests that some foods may be more effective than others in their capacity to promote satiety. To examine this further, we have utilized computerized techniques to investigate the influence of pure nutrients (fat, protein, and carbohydrate) upon nocturnal patterns of feeding in 30 adult male Sprague-Dawley rats.

Microstructural analyses have revealed that the nutrient content of a meal produces different effects upon some, but not all, standard meal parameters. The latency to initiate eating, rate, and feeding duration were similar for all meal compositions. However, a first meal of the dark period composed primarily of protein resulted in a reliably ($p<.05$) longer intermeal interval (83 min) than was associated with a carbohydrate (40 min) or fat (41 min) meal. This effect was unrelated to the size of the meal, as the protein-rich meal (11 Kcal) was similar in size to the carbohydrate-rich meal (10 Kcal) and significantly smaller than a fat-predominant meal (18 Kcal, $p<.05$). Thus, protein appears to be more effective than carbohydrate and fat in producing satiety in rats.

215.9

EFFECT OF CONTROL (C), HIGH FAT (HF) AND HIGH SUCROSE (HS) DIETS ON WEIGHT GAIN IN LIGHT AND HEAVY RATS. J.H. Carlton and J.L. Howard. Wellcome Res Labs, Res Tri Park, NC 27709.

Study of food intake and body weight is complicated by the fact that rats of similar age, sex and strain display a wide range of body weight, a range that increases across the life span. Regarded as inconvenient variability, little has been done to characterize this phenomenon.

Three weight-matched sets of 10-wk old, female, Sprague Dawley rats were fed ad lib one of the following diets: C - Wayne Lab Ration (WLR); HF - ground WLR, vegetable oil, cream and cheese; or HS - ground WLR, sweetened non-fat milk and cookies. After 12 wks, rats had maintained their initial rank order of body weight within groups, HF and HS were equal in mean weight gain and both had gained 40% more than C. Based on final body weights, the lighter (L) and heavier (H) rats in each dietary group were selected for comparison. For all groups, L rats had weighed initially more than 90% of what H rats weighed. After 6 wks on C, L rats gained only 38% of what H rats did; on HF and HS, L rats gained 60% of what H rats did. These weight difference ratios were maintained at 12 wks. During wk 12, HS mean caloric intake was 68% above C, HF 107% above C. In C, H rats consumed significantly more calories than did L rats. In HF and HS, H rats maintained elevated weights without consuming more calories than did L rats. Data on metabolism will be presented. In summary, body weight varies widely in ad lib C-fed rats. HF or HS diets increase body weight and reduce divergence between L and H rats.

215.10

GLUCOSE AND FRUCTOSE SUPPRESS THE EXCITATORY EFFECT OF CONDITIONED CUES ON MEAL INITIATION. M. Bedard and H.P. Weingarten. Dept. of Psychology, McMaster University, Hamilton, Ontario, L8S 4K1 Canada.

Since feeding behavior is under multifactorial control, the appropriate analysis of meal initiation requires examination of the interaction of factors involved in this behavior. External stimuli (CS+'s) conditioned to food reliably elicit large meals (Weingarten, 1983). We examined the ability of various factors to block conditioned meal initiation. This strategy analyzes the interaction between excitatory and inhibitory influences on meal initiation and provides insight into mechanisms involved in suppressing eating during the intermeal interval. Caloric preloads, administered intragastrically 15 min before onset of the CS+, suppressed meal initiation in a dose-dependent manner. Further data helped specify the mechanism by which nutrient in the gut suppressed meal initiation. Specifically, both glucose and fructose injected ip suppressed meal initiation and reduced meal size by as much as 59%. Although the exact nature of the inhibitory signal gating the excitatory influence on meal initiation is unclear, our results are congenial with the speculation of others that this event is postabsorptive and that the liver plays a key part in this process.

215.11

EFFECT OF LOCUS CAERULEUS (LC) LESIONS ON FOOD INTAKE RESPONSES AND DIETARY CHOICE OF RATS FED DIETS CONTAINING PROTEIN IN EXCESS. P.M.B. Leung, D.W. Gietzen and Q.R. Rogers. Department of Physiological Sciences, University of California, School of Veterinary Medicine, Davis, CA 95616.

Bilateral electrolytic lesions were placed in LC of adult Sprague Dawley rats (7.4 to 7.8 mm posterior to bregma, ±0.7 mm from sagittal suture and 6.0 mm from dura). Rats with LC lesions were fed the 6% casein diet (LP) pre- and post operatively and showed mild transient hyperphagia during recovery. Following equilibration with LP, animal with lesions and intact controls (INT) were fed a 75% casein diet (HP) for 19 days before reequilibration with LP. Then rats with lesions and INT were offered, in sequence, choices between LP and HP (28 days), LP and 36% casein diet (MP) (21 days) or LP and 15% casein diet (NP) (13 days). Rats with LC lesions and INT showed similar marked initial food intake (FI) depression (39.1±2.8% and 39.6±5.7% of their respective baseline intake of 23.4±0.7g and 22.9±0.6g) and slow adaptation when fed HP. During LP and HP choice, PE% (protein calories as % of total caloric intake) selected were 11.6%, 16.2% and 16.6% for rats with lesions and 10.7%, 20.6% and 12.3% for INT on the 1st, 15th and 28th day respectively. For LP and MP choice, PE% selected on 1st, 14th and 21st day were respectively 24.0%, 18.6% and 22.0% for lesioned rats and 23.1%, 18.5% and 24.5% for INT. During LP and NP choice, PE% selected were 12.6%, 12.8% and 11.7% for lesioned rats and 12.1%, 13.3% and 11.0% for INT on the 1st, 7th and 13th day respectively. Results indicate that LC does not appear to be involved in the control of FI of rats fed diets containing protein in excess. (Supported by NIH grant DK13252).

215.12

GUSTATORY NEURAL RESPONSES TO POLYSACCHARIDE AND STARCH IN THE RAT B.K. Giza*, R.F. Antonucci*, A. Sclafani and T.R. Scott (SPON: K. Campbell). Dept. Psychol. and Inst. Neurosci., Univ. Delaware, Newark, DE 19716

Polycose and amylopectin are highly palatable to the rat. The tastes however, do not generalize well to that of sucrose or of the other prototypical stimuli--NaCl, HCl and quinine--in behavioral tests. We used these chemicals in addition to a standard array of taste stimuli during recordings from 63 single neurons in the NTS of anesthetized rats to determine their neural effectiveness and relative taste qualities. Amylopectin (2%) elicited only a few spikes, always at stimulus onset; Polycose, at 0.1 M, evoked a burst of activity followed by a moderate tonic response. The time courses of both responses were similar to the phasic-tonic sequence of non-sweet stimuli. The profile of activity evoked by Polycose across neurons was most similar to those elicited by Na-Li salts and by quinine; it showed moderate similarity to the patterns representing HCl and MSG, and modest correlations with the patterns for sugars. The results of behavioral studies have led to the suggestion that Polycose represents a unique taste quality. Our electrophysiological data align Polycose with all non-sweet chemicals and, to a lesser extent, with sugars as well, implying a complex quality. The basis for its highly appetitive nature is not apparent from the neural response Polycose evokes.

Supported by research grant DK30964 from the NIH.

215.13

CAPSAICIN-SENSITIVE NEURONS MEDIATE FEEDING INDUCED BY DECREASED FATTY ACID UTILIZATION BUT NOT BY GLUCOPRIVATION. S. Ritter, J. Taylor* and S. Stone*, Dept. of VCAPP, Washington State Univ., Pullman, WA 99164

Surgical lesions and capsaicin, a toxin that destroys small unmyelinated sensory neurons, were used to evaluate the participation of visceral sensory neurons in glucoprivic and lipoprivic feeding. Rats were maintained on a fat supplemented diet and feeding was measured after systemic blockade of fatty acid or glucose utilization with mercaptoacetate (MA) or 2-deoxy-D-glucose (2DG), respectively. Capsaicin abolished MA feeding, but not 2DG feeding. Area postrema/nucleus of the solitary tract (AP/NTS) lesions abolished both MA- and 2DG-induced feeding. Hepatic vagotomy did not impair responses to either challenge. Low suprathreshold doses of 2DG and MA given simultaneously were additive in their effects on feeding in controls and hepatic vagotomized rats. Capsaicin-treated rats ate the same amount after 2DG plus MA as after 2DG alone. AP/NTS lesioned rats did not eat in the combined drug test. Thus, glucoprivic and lipoprivic feeding are mediated by chemically and anatomically distinct receptors. Glucoprivic feeding requires neurons in the AP/NTS region, but is not vagally-mediated. Lipoprivic feeding requires sensory neurons that project to the AP/NTS, but not those in the hepatic vagal branch. Additivity of MA and 2DG in stimulating feeding requires participation of both receptor populations.

215.14

INCREASE IN BODY WEIGHT AND SWIMMING DEFICIT FOLLOWING INTRAPERITONEAL CAPSAICIN IN NEONATAL RATS. I.A. Coronado-Zarco* and I. Zarco de Coronado. Physiol. Dept. Med. Sch. Natl. Univ. of Mexico. P.O. Box 70250. 04510 México, D.F.

Capsaicin was administered in order to elucidate the participation of unmyelinated sensory somatic or visceral neurons on the regulation of food intake as revelead by the control of body weight or the motor swimming hability during the postnatal development.

On the 5th. postnatal day rats received capsaicin or vehicle intraperitoneal injection. From day 7 to 22 experimental animals showed increase in gnawing activity and body weight. The swimming behavior observed maintained inmature characteristics for longer time than control animals. Hypophysis was found bigger in the trated rats. These findings suggest that capsaicin damages afferent pathways, alter food intake and swimming activity by acting at a neuroendocrine level.

215.15

THE EFFECT OF CHRONIC DEXAMETHASONE-21-ACETATE TREATMENT ON MEAL SIZE, MEAL FREQUENCY AND MACRONUTRIENT SELF-SELECTION IN RATS. P.S. Grigson*, D.F. Johnson*, K. Ackroff*, G.H. Collier* and C.F. Flaherty. Psychology Department, Rutgers University, New Brunswick, NJ 08903.

Chronic treatment with dexamethasone (a synthetic glucocorticoid) leads to suppressed levels of circulating corticosteroids, elevated blood glucose and a dramatic loss in bodyweight (Bryson, unpublished dissertation, U. of Cal. 1981). We examined the effects of chronic dexamethasone-21-acetate (dex) treatment on bodyweight, food intake and macronutrient selection in two experiments. Experiment 1, which examined the effects of dex (.5 mg/kg, ip) on 24 hour intake in two animals, replicated the decrease in bodyweight and suggested it was at least in part due to a decrease in food intake --owing to both a decrease in meal frequency and meal size. Experiment 2, which examined macronutrient self-selection, indicated that chronic dex treatment (.5 and 1.0 mg/kg, ip) led to a decrease in total kcals which was due entirely to a decrease in the consumption of fat, with no change in carbohydrate consumption and an elevation in protein intake.

215.16

SHIFTS IN RATS' PREFERENCES FOR SUGAR AND POLYSACCHARIDE SOLUTIONS. K. Ackroff* and A. Sclafani (SPON: I. Abramov). Dept. of Psychology, Brooklyn College and Graduate School, CUNY, Brooklyn, NY 11210.

In 2-bottle preference tests (24 hr/day), rats initially prefer a 32% sucrose solution to an isocaloric 32% hydrolyzed starch (Polycose) solution. However, over days, the animals reverse their preference and drink more Polycose than sucrose (Sclafani et al., 1987). We conducted several experiments to determine the role of taste and postingestive factors in this preference shift. Adult female rats were given ad lib access to chow and two carbohydrate solutions. At 4% and 8% concentrations sucrose was persistently preferred to Polycose, but at 32% a shift to Polycose was observed. Rats also persistently preferred saccharin-sweetened Polycose to plain Polycose. These data suggest that taste factors alone do not account for the preference shift effect. When given iso-osmotic solutions of 32% sucrose and 16% Polycose + 16% glucose rats rapidly switched their preference from sucrose to the mixed solution. Rats also show a preference shift in 32% glucose vs. 32% Polycose tests. These data indicate that neither the osmotic effects nor the unique metabolic effects of sucrose are responsible for the preference shift. In the above studies, the Polycose preference was lost after an 8-day break but reappeared with additional testing. This negates a simple learning explanation of the preference reversal effect. The shift phenomenon appears to represent a complex interaction of the taste and postingestive actions of carbohydrates.

215.17

POLYSACCHARIDE AND SUGAR TASTE PREFERENCES IN MONKEYS. A. Sclafani and G. Sunderland*. Dept. of Psychology, Brooklyn College and Dept. of Psychiatry, SUNY-Health Science Center, Brooklyn, NY 11210.

Rats are very attracted to the taste of starch-derived polysaccharides (e.g., Polycose), whereas humans are not attracted to Polycose. The present experiment compared the taste preferences of nonhuman primates to Polycose, sucrose, and maltose. Maltose was of interest because to rats maltose appears to have a Polycose-like rather than sucrose-like taste.

Adult male squirrel monkeys (n=9) and bonnet macaques (n=6) were given 24 hr/day saccharide vs. water preference tests. The saccharides included Polycose, sucrose and maltose (.01, .05, .10 and .20 or .50 M). Chow was ad lib.

Squirrel monkeys displayed stronger preferences for sucrose (61.6% to 78.2% of total intake) than for Polycose (49.8% to 69.3%) or maltose (53.9% to 61.4%). The bonnet macaques, on the other hand, displayed equivalent preferences for Polycose (63.9% to 91.8%), maltose (75.1% to 95.6%) and sucrose (64.5% to 100%). In addition, the bonnet macaques showed greater preferences for Polycose and maltose than did the squirrel monkeys. Sucrose preferences did not reliably differ between species. These different taste preference profiles are consistent with the natural diets of squirrel monkeys (frugivores) and bonnet macaques (omnivores). The results suggest, but do not establish, that bonnet macaques, like rats, have taste receptors for polysaccharides whereas squirrel monkeys, like humans, lack such receptors.

215.18

GLUCOCORTICOIDS POTENTIATE THE DIPSOGENIC ACTION OF ANGIOTENSIN II. R. Ganesan* and C. Sumners [Spon: W.W. Dawson] Dept. of Physiology, Univ. of Fla., Coll. of Medicine, Gainesville, FL 32610.

Previous studies from this laboratory have indicated that long-term peripheral infusions of the mineralocorticoid, deoxycorticosterone acetate, enhanced the drinking response to peripherally- and centrally-administered angiotensin II (AII). We have now found that the synthetic glucocorticoid, dexamethasone, can similarly potentiate A II-induced drinking in rats. In addition, we obtained this effect using a single injection of the steroid. Initial studies indicated that a 750 μg/kg intraperitoneal dose of dexamethasone caused a reduction in body weight and inhibited feeding for up to 48h after the injection. An enhancement of AII-induced drinking was observed when the glucocorticoid was given 3h or 6h prior to the dipsogen (200 μg/kg, sc). However, when subjects were tested 24h after the administration of the steroid, the amount of water consumed was no different from controls. Subsequently we confirmed that a subcutaneous injection of the steroid was equally effective, and that the magnitude of its action on AII drinking was dose-dependent (100 μg-1600 μg/kg). Dexamethasone also potentiated drinking to intracerebroventricular (icv) AII (2.5 ng), but it had no effect on icv carbachol-induced drinking. This result renders less likely the possibility that dexamethasone has a general stimulatory effect on all drinking. A notable point is that dexamethasone not only increased drinking to AII but also lengthened its duration up to 2h after the icv injection. Recent work has uncovered interactions between several different aspects of the angiotensin system and glucocorticoids, but further examination is required to pinpoint the exact locus of interaction in this instance. (Supported by NIH grant HL-36645).

215.19

RELATIVE CONTRIBUTIONS OF HIGH AND LOW AFFINITY CORTICOSTEROID RECEPTORS TO LONG TERM REGULATION OF FEEDING, METABOLIC EFFICIENCY, AND ADIPOSITY. L. Devenport, T. Thomas, A. Sundstrom*, and A. Knehans*. Department of Psychology, University of Oklahoma, Norman, OK 73019.

Relatively pure or mixed Type 1 or 2 corticosteroid receptor agonists exhibited variations in their effectiveness across time and regional tissues. Young adrenalectomized male rats were infused for 4 weeks with doses of dexamethasone (DEX, Type 2 agonist), aldosterone (ALDO, Type 1), DEX+ALDO, or corticosterone (B, dose-dependent mixed agonist). ALDO's anabolic and appetite-stimulating effects were undiminished across the 28 d. In contrast, DEX's strong catabolic action subsided during the first 14 d. Declines either ceased (high dose) or weight began increasing (other doses) during the last two weeks. Carcass analysis indicated that the abatement of Type 2 effects were unevenly distributed. Infusion of B at lower doses mirrored the ALDO results. Higher doses at first produced a net catabolic (Type 2) effect but as it waned, Type 1 effects were unmasked. Body composition analysis revealed that high dose B rats were locally obesified, and in other regions, wasted. The particular distribution was a joint product of the uneven remission of Type 2 effects, and the unremitting Type 1 activity. This Cushing's pattern was mimicked by combined DEX+ALDO infusion. The results closely match the reported patterns of receptor autoregulation and pituitary modulation of ligand capacity, and are probably best understood in those terms.

215.20

ROLE OF THE SYMPATHETIC NERVES IN THE ACTION OF ESTRADIOL ON WHITE ADIPOSE TISSUE. S. Lazzarini* and G. Wade. (SPON: A. Barto). Neuroscience and Behavior Program and Psychology Department, Univ. of Massachusetts, Amherst, MA 01003.

Estrogens have both central and peripheral effects on body weight in rats. Ovariectomy (OVX) induces weight gain, primarily by increasing fat stores, an effect which can be reversed by estradiol replacement. Sympathetic activity also regulates the mobilization of free fatty acids in vivo. Furthermore, estrogens potentiate catecholamine-induced lipolysis in vitro. The present experiments were designed to determine whether sympathetic nerves mediate estrogen-induced lipolysis by influencing adipose tissue cytosol estrogen receptor (CER) concentration. Rats were OVX and 3 weeks later, the retroperitoneal fat pads (RTRO) were unilaterally denervated. After 14 days of EB treatment (2 ug/day), the denervated pad lost 23% less weight than the innervated pad. There was no effect of denervation in the oil treated animals. Measurement of fat pad CER concentration in the oil treated groups revealed no differences between intact and denervated fat pads. These data suggest that the sympathetic nerves play a role in estrogen-induced reductions in fat pad weight but not via changes in adipose tissue CER.
(Supported by NS 10873, AM 32976 and MH 00321).

TRANSMITTERS IN INVERTEBRATES III

216.1

KNEFIRFamide, A NOVEL FMRFamide-LIKE NEUROPEPTIDE FROM THE NEMATODE ASCARIS SUUM. C. Cowden* and A.O.W. Stretton. Dept. of Zoology, Univ. of Wisconsin, Madison, WI 53706.

Several FMRFamide-like peptides have been extracted from Ascaris heads where they are found in neurons but not in muscle or hypodermis (Cowden et al., 1987 Amer. Zool. 27:127A). The first of these to be purified and sequenced is the heptapeptide KNEFIRFamide. An acid methanol extract from 10,000 Ascaris heads was fractionated on C18 cartridges with acetonitrile (ACN)-TFA. The first and second steps of HPLC used a C18 column eluted with gradients of butanol-TFA and ACN-TFA. The next step was HPLC gel filtration in ACN-TFA and the final step was an isocratic elution from a C18 column with ACN-HFBA. Fractions were monitored with an RIA. A liquid phase sequenator was used to determine the amino acid sequence (U.W. Biotechnology Center). This Ascaris peptide is different from all known FMRFamide-like peptides. Synthetic peptide co-elutes with Ascaris peptide. Its physiological role in the Ascaris motornervous system is now being studied (Davis et al., this meeting).
Supported by NIH postdoctoral award #5 F32 NS07954-02 and NIH grant #AI 20355.

216.2

ELECTROPHYSIOLOGICAL EFFECTS OF KNEFIRFamide, A PEPTIDE FROM THE NEMATODE ASCARIS SUUM. R.E. Davis*, C. Cowden*, and A.O.W. Stretton. (SPON: H.M. Sobkowicz). Dept. of Zoology, University of Wisconsin - Madison, Madison, WI 53706.

The heptapeptide, KNEFIRFamide, has been isolated from Ascaris (Cowden and Stretton, this meeting). The physiological effects of synthetic KNEFIRFamide on the Ascaris motornervous sytem were investigated using intracellular recording techniques. The peptide had a dramatic effect on the electrical properties of ventral inhibitory motorneurons: slow potentials, whether spontaneous or induced by depolarization, were rapidly and reversibly blocked by nanomolar concentrations. Neuromuscular transmission was not effected. No effects on the electrical properties of dorsal excitatory motorneurons (which do not exhibit slow potentials) or their synapses were observed.
Supported by NIH grant #AI 15429.

216.3

EFFECTS OF FMRFamide ON INTACT AND ISOLATED LONGITUDINAL MUSCLE CELLS OF THE MEDICINAL LEECH. B.J. Norris and R.L. Calabrese. Dept. of Biology. Emory Univ. Atlanta, GA 30322

The neuropeptide Phe-Met-Arg-Phe-NH_2 (FMRFamide), when bath-applied onto longitudinal muscle of the medicinal leech, causes a tonic contraction and occasionally triggers a weak myogenic rhythm (Norris and Calabrese, J Comp Neurol, 266:95, 1985). In intact muscle cells, under current clamp, FMRFamide causes a depolarization which is initially accompanied by a conductance increase. This increase is followed within seconds by a conductance decrease, that has little effect on membrane potential. Depolarization persists as long as FMRFamide is present, even after the conductance decrease has appeared, but terminates quickly when FMRFamide is removed. The conductance decrease, however, can last for many minutes after FMRFamide is removed. Using isolated cells, we will attempt to identify the ions involved in both conductance changes. This work is supported by NIH grant no. NS24072-04 to RLC.

216.4

STRUCTURE-ACTIVITY RELATIONS OF FMRFamide-RELATED PEPTIDES (FaRPs) ON CRUSTACEAN HEART. K.G. Krajniak* and M.J. Greenberg. The Whitney Laboratory, Univ. of Florida, St. Augustine, FL 32086.

The FaRPs were first discovered in molluscs, but members of this peptide family have now been identified and sequenced in crustaceans and insects. The crustacean FaRPs are cardioexcitatory, and we have therefore compared their activities with those of the molluscan peptides, and with related analogs, on isolated, perfused hearts of the blue crab, Callinectes sapidus. All peptides studied caused dose-dependent increases in rate, amplitude, or both. Two known crustacean peptides, TNRNFLRFa and SDRNFLRFa [a = amide], had the lowest thresholds: 10^{-9}-10^{-8} M. Three of the heptapeptide FaRPs (SDPFLRFa, NDPFLRFa, pQDPFLRFa) characteristic of the pulmonate molluscs are also N-terminally extended analogs of FLRFa, but they are (like FLRFa and its tetrapeptide analogs, FMRFa and FIRFa) 1000-fold less potent than the crustacean peptides: threshold was 10^{-6} to 10^{-5} M. RNFLRFa and pQNFIRFa are shorter than the crustacean FaRPs, but they have the Asn residue in the same position and are only about ten-fold less active; threshold was 10^{-8}-10^{-7} M. The insect FaRP, leucomyosuppressin (pQDVDHVFLRFa) had a very high threshold (10^{-5} M) on the crab heart. Neither YGGFLRF nor FLRF were active (up to 10^{-4} M), although their amidated analogs were. Therefore, FaRP receptors of crustacean hearts seem to require an amidated peptide with the minimum sequence: NFXRF-NH_2 (X is L, M, or I).
[Supported by NIH grant HL-28440 and NSF grant DCB-8616356.]

216.5

TOWARD THE GENETIC BASIS OF THE FMRFamide-RELATED PEPTIDES (FaRPs) IN MOLLUSCS. T.R. Nuttall*, D.A. Price and M.J. Greenberg. The Whitney Laboratory, Univ. of Florida, St. Augustine, FL 32086.

A survey of molluscan FaRPs has revealed that the phyletic distribution of the peptides is not uniform; in particular, a set of heptapeptide analogs of FLRFamide is restricted to the Pulmonata. The gene encoding FMRFamide in the opisthobranch Aplysia californica includes no heptapeptide sequences, but the 5' region of the gene does code for 1 copy of FLRFamide and 1 of a long peptide with the C-terminal sequence -GYLRFamide. Duplication and subsequent mutation of this region could have given rise to a heptapeptide gene. The distribution of FMRFamide-like genes in molluscs was examined by Southern blotting; a fragment of a FMRFamide cDNA from Aplysia was the probe. Under low stringency conditions (i.e., 50-56°C, 1xSSC, 0.1%-0.5%SDS), hybridization signals were detected by autoradiography in all gastropod subclasses and in bivalves. A well-defined hybridization signal was detected in both the Eco RI and Pst I digests of DNA from Veronicella floridana, a systellommatophoran pulmonate; DNA fragments of approximately 4 Kb (Eco RI digest) and 2.5 Kb (Pst I digest) occurred at low stringency (56°C, 1xSSC, 0.5% SDS). A restricted DNA library is being made. Since the Systellommatophora diverged from the main pulmonate line close to the time of the opisthobranch/pulmonate split and heptapeptide FaRPs have been detected in Veronicella, examination of this species should provide clues to the evolution of the FaRPs.

[NIH grant HL-28440 and NSF grant DCB-8616356.]

216.6

PROCTOLIN IN THE CNS OF LYMANTRIA DISPAR AND OTHER LEPIDOPTERA. N.T. Davis* and H. Keshishian (Spon: W. Chapple). Dept. Physiol. Neurobiol., Univ. Ct, Storrs, CT 06268.

The neuropeptide proctolin has important functions in many arthropods, but it is reported to be absent in Lepidoptera. Its occurrence in these insects was reinvestigated and a proctolin-like substance was recovered from ganglia of Lymantria dispar. The substance has the same chromatographic retention time as proctolin, enzymatic degradation indicates that it is a peptide, it is bound by proctolin antisera, and, thus it is indistinguishable from authentic proctolin.

A small number of proctolin-like immunoreactive (PLI) neurons was stained in the larval CNS of L. dispar, Manduca sexta, Trichoplusia ni, Galleria mellonella, and Vanessa cardu, and included a median cluster of A-neurons of the brain, paired neurons of the frontal ganglion, an anteriomedial and ventromedial cluster of neurons in the subesophageal ganglion, paired lateral neurons in the thoracic ganglia, and dorsomedial neurons in the abdominal ganglia. Also varicose PLI axons were found in the corpora cardiaca, perivisceral organs, and transverse nerves. In L. dispar, PLI neurons were found in the corpora cardiaca and on the transverse nerves. The A-neurons are a subset of median neurosecretory cells of the pars intercerebralis, and these and PLI neurons of the subesophageal ganglion project into the retrocerebral complex. PLI neurons of the transverse nerves and the thoracic neurons project into the perivisceral organs.

216.7

STATE-DEPENDENT MODULATION OF THE CRAB PYLORIC RHYTHM BY MODULATORY PROCTOLIN-CONTAINING NEURONS. M.P. Nusbaum and E. Marder. Dept. Biol. Brandeis Univ., Waltham, MA 02254.

Stimulation of the two modulatory proctolin-containing neurons (MPNs) initiates or enhances the pyloric rhythm of the crab stomatogastric ganglion. Simultaneous recordings of both MPNs indicate that the two MPNs have similar effects and appear electrically coupled. The MPNs have state-dependent excitatory effects on the pyloric cycle frequency. In rhythmic preparations, MPN stimulation causes larger increases in the cycle frequency of slow rhythms than of fast ones because MPN stimulation causes maximal pyloric cycle frequencies of approximately 1 Hz. This ceiling effect occurs at the same cycle frequency as the maximal effect attained by bath-applied proctolin on the lobster pyloric rhythm (Hooper & Marder, J. Neurosci. 7:2097-2112). MPN continues to enhance the impulse activity of its pyloric network targets, however, even when it causes little or no increase in cycle frequency. MPN causes both small amplitude (<1 mV) postsynaptic potentials and long lasting depolarizations in the LP and IC neurons. We are studying these two MPN effects to determine: 1) whether they are both caused by proctolin or whether the MPN's also release a second neuroactive substance, and 2) how each contributes to MPNs state-dependent effects on the activity of the intact pyloric network. Supported by NRSA NS-07446 (MPN) and NS-17813.

216.8

PROCTOLINERGIC MODULATION OF NEUROMUSCULAR TRANSMISSION AT AN OVIPOSITOR MUSCLE OF *LOCUSTA MIGRATORIA*. J.H. Belanger and I. Orchard. Dept. of Zoology, Univ. of Toronto, Toronto, ON, Canada M5S 1A1.

The only behaviour for which the muscles of the locust ovipositor are used appears to be egg-laying. These muscles have associated with them 0.68 to 1.97 pmol proctolin/muscle (Belanger and Orchard, *Neurosci. Abs.* 13:235, 1987). The opener muscles of the ovipositor of laying females appear to be modulated by proctolin.

At low proctolin concentrations (10^{-10} M), neurally-evoked contractions of the muscle are enhanced three- to fourfold above controls. There is no significant change in the resting potential of the fibres or the input resistance of the fibre membrane, nor is there a significant change in tonus.

At higher doses (10^{-8} M), a slow (0.3 - 0.5 Hz) myogenic rhythm appears, superimposed upon a large increase in tonus. These are accompanied by a large (30 mV) depolarization, and an enhancement of neurally-evoked contractions by up to 300X.

We are currently investigating the physiological relevance of this modulation, as well as the possibility that the ovipositor muscles of non-laying females are not (as) sensitive to proctolin.

216.9

ACTIVATION OF CRAYFISH SWIMMERET RHYTHM IS ASSOCIATED WITH RELEASE OF A PROCTOLIN-LIKE SUBSTANCE. L.D. Acevedo, M.E. Adams,+ W.M. Hall+ and B. Mulloney. Zoology Dept., University of California, Davis, CA 95616 and +Entomology Dept., University of California, Riverside, CA 92521-0137.

Excitatory command neurons activate the swimmeret rhythm in the crayfish, *Pacifastacus leniusculus* (Wiersma and Ikeda, 1964). The neuropeptide proctolin mimics stimulation of these neurons (Mulloney et al., 1987). To determine whether proctolin is the transmitter used by these command neurons, we measured proctolin-like bioactivity (PLB) in the abdominal ganglia, looked for proctolin-like immunoreactivity (PLI) in appropriate areas of each ganglion, and quantified release of PLB following command neuron stimulation.

We used a locust leg bioassay (O'Shea and Adams, 1981) to determine if PLB exists in the abdominal ganglia. All ganglia contained PLB, with an average of 10 pmol per ganglion. Depolarization of the abdominal ganglia with high-potassium saline released about 1% of the total PLB.

Immunohistochemical staining of the abdominal nervous system revealed PLI in the Lateral Neuropil, which contains most of the swimmeret circuitry. Several pairs of axons in the interganglionic connectives showed PLI; some of these axons sent branches into each Lateral Neuropil.

Stimulation of axon bundles containing excitatory command neurons resulted in release of PLB. In some cases, release of PLB was associated with command activation of the swimmeret rhythm. The released PLB was 4% of that releasable by high-potassium depolarization.

Supported by NSF grant BNS 84-06931 and NIH grant NS 21194 to B. Mulloney.

216.10

ISOLATION AND CHARACTERIZATION OF A PIGMENT DISPERSING HORMONE FROM THE SHRIMP, PENAEUS AZTECUS. J. M. Phillips*, K. R. Rao, J. P. Riehm*, W. T. Morgan.*† Dept. of Biology, Univ. of West Florida, Pensacola, FL 32514. †Dept. of Biochemistry and Molecular Biology, Louisiana State Univ., Med. Center, New Orleans, LA 70112.

Utilizing the methods employed for isolation of pigment dispersing hormone (PDH) from Uca (Rao et al., 1985), we have purified and characterized an analog of β-PDH from lyophilized eyestalks of Penaeus. After initial extraction and liquid partition, the PDH was purified by gel filtration, cation-exchange chromatography, partition chromatography, and reversed-phase HPLC. Automated gas-phase sequencing and identification of C-terminal amide established the sequence as NSELINSLLGIPKVMNDAamide, and we confirmed this sequence by chemical synthesis.

Penaeus PDH differs from β-PDH (NSELINSILGLPKVMNDAamide; Uca/Cancer PDH) at positions 8 and 11. When tested for melanophore pigment dispersion, these peptides were nearly equipotent. In the crabs Uca and Cancer, β-PDH is the major form of pigment dispersing hormone. Similarly, the β-PDH analog characterized here is the major form present in the brown shrimp Penaeus; in contrast, α-PDH is the major form of PDH in the eyestalks of the prawn, Pandalus (Fernlund, 1971, 1976).

Supported by NSF Grant DCB-8711403.

216.11

SUBSTANCE P IS NOT AN EFFERENT NEUROTRANSMITTER IN THE *LIMULUS* EYE. H.K. Lehman, T.J. Lewandowski* J.K. Johnson and S.C. Chamberlain. Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

Immunoreactive Substance P (ir-Subst P) has been widely reported from *Limulus polyphemus*. Immunocytochemical investigations have detected the presence of ir-Subst P in the brain and retina (Chamberlain and Engbretson, J. Comp. Neurol. 208: 304-315). Ir-Subst P has also been detected by radioimmunoassay in *Limulus* retinal extracts and was proposed to be an efferent neurotransmitter (Mancillias and Brown, J. Neurosci. 4: 832-846). We report here that indeed ir-Subst P is found in the *Limulus* retina, but isolated ir-Subst P does not play a role in modulating visual function as an efferent neurotransmitter.

Brain and retinal extracts were extracted with boiling acetic acid and the residue was passed through a column of Sephadex G-25. Five ml fractions were collected and tested for 1) bioactivity that increases the ERG amplitude of *Limulus* and 2) ir-Subst P by radioimmunoassay. Three bioactive peaks elute from the Sephadex column, in .6, 1.0 and 1.2 column volumes; ir-Subst P elutes from the column in the void volume (0.1 column volumes). The binding curves of isolated ir-Subst P and synthetic Subst P are similar. Ir-Subst P elutes as single peak from reverse phase HPLC, but does not coelute with authentic Substance P.

Ir-Subst P has also been studied with immunocytochemical techniques. At the light microscopic level, ir-Subst P fibers penetrate the retina and run between the ommatidia to the level of the corneal epidermis. Ir-Subst P is not contained within the efferent fibers. Moreover, ir-Subst P is absent in fibers innervating photoreceptors not derived from epidermis. We are in the process of confirming these results at the ultrastructural level.

We conclude that an ir-Subst P molecule exists in the *Limulus* retina, but does not have the proper physiological actions or anatomical localization to be considered an efferent neurotransmitter, but may be part of a more general epidermal innervation.

This work has been supported by the NIH.

216.12

MONOCLONAL ANTIBODIES RAISED TO DROSOPHILA EMBRYOS RECOGNIZE NEURAL ANTIGENS IN THE MARINE SNAIL BULLA GOULDIANA. B. Ibrahim*, V. Bedian* and M.H. Roberts (SPON: R. Konopka), Department of Biology, Clarkson University, Potsdam, NY 13676

We have attempted to map the positions of immunohistochemically identifiable neurons in the CNS of the marine snail Bulla gouldiana, as a prelude to studies of the neurophysiological control of rhythmic behavior by central circadian pacemakers.

Using the avidin-biotin (ABC) technique, we find that monoclonal antibodies raised against Drosophila embryos recognize a number of neural antigens in the Bulla CNS. Out of thirty-five antisera screened, we find seven that stain cells in the Bulla nervous system. Five of the antisera recognize neuron nuclei, with the staining appearing uniform over the entire nucleus. Two of the antisera recognize the cytoplasm: one stains all cells in a non-uniform pattern; the staining is dense near the nucleus and decreases toward the periphery. The other cytoplasmic antigen, recognized by antibody E2F8.E5, is found exclusively in a small number of neurons in the pleural and pallial ganglia.

These results, particularly with antibody E2F8.E5, illustrate the utility of using immunohistochemistry with novel antisera as a method of identifying unique neurons in the molluscan nervous system. Current work is aimed at identifying the antigens recognized by these antisera.

216.13

FUNCTIONAL CONNECTIONS EXIST BETWEEN CONTRALATERAL BAG CELL CLUSTERS WITHIN THE HEAD GANGLIA OF APLYSIA. S. B. Shope and J. E. Blankenship, Marine Biomedical Institute, Univ. of Tex. Medical Branch, Galveston, TX 77550.

The bag cells of the marine mollusc Aplysia californica form two clusters of about 400 cells each on the rostral border of the abdominal ganglion. Bag cells discharge synchronously due to electrical coupling both within and between clusters. In recent experiments we removed the intact central nervous system from the animal and lesioned the abdominal ganglion longitudinally, separating the left and right bag cell clusters. In 6 of 6 experiments, electrical stimulation of one bag cell cluster resulted in a discharge in the other bag cell cluster. The effect depended only upon an intact pathway between each half of the abdominal ganglion through the pleural and cerebral ganglia. This suggests that there is a functional connection between the bag cells within the head ganglia. We are unable to determine if the connection is electrical or chemical. No obvious synchrony was noted between the clusters, however, any synchrony induced by electrical coupling in the head ganglia may be overridden by stronger electrical coupling within each cluster. Supported by NINCDS NS23169 and NSF BBS 8711368.

216.14

COMPARISON OF POTENCIES AND EFFICACIES OF ALPHA BAG CELL PEPTIDE ANALOGUES ON VOLTAGE CLAMPED NEURONS OF APLYSIA. M. K. Rock, S. B. Shope, and J. E. Blankenship. Marine Biomedical Institute, Univ of Tex Medical Branch, Galveston, TX 77550.

Alpha bag cell peptide (αBCP) is synthesized in the bag cells of the marine mollusc, Aplysia californica as a 9 amino acid peptide (αBCP_{1-9}) but active fragments αBCP_{1-8} and αBCP_{1-7} can be isolated from bag cell releasate as well. αBCP analogues were superfused onto voltage clamped rostral white cells (R3-R13) of the abdominal ganglion at 10^{-9} to 10^{-4} M. Dose-response curves demonstrated that αBCP_{1-8} and αBCP_{1-7} had similar potencies (dissociation constants) but that αBCP_{1-7} was more efficacious, ie, it produced a maximal current about twice that of αBCP_{1-8}. Scatchard and Hill analysis of the αBCP_{1-8} dose-response curve suggests two binding sites each requiring a single molecule for activation. Preliminary results suggest that αBCP_{1-9} is much less potent than the other two analogues. Supported by NINCDS NS 23169 and NSF BBS 8711368.

216.15

LOCALIZATION AND ACTIONS OF CATECHOLAMINES IN THE FEEDING SYSTEM OF APLYSIA CALIFORNICA. M.M. Rathouz*, K.P. Cohen* and M.D. Kirk. Dept. of Biology, Boston University, Boston, MA 02215.

The glyoxylic acid histofluorescence technique was used to localize catecholamines in Aplysia buccal ganglia and nerves and the esophagus. We observed high levels of blue-green fluorescence, indicating the presence of catecholamines, in three identifiable cell bodies (two symmetrically paired and one unpaired), and 10 smaller lateral cells (five in each ganglia). The esophageal nerve, the cerebro-buccal connective, and the neuropil of the ganglia contained processes which fluoresced blue-green as well. A plexus of blue-green axons is present in the area where the esophageal nerve joins the esophagus. This net of processes may act as a neurohemal organ releasing catecholamines diffusely in the outer wall of the gut.

Previous biochemical studies found that dopamine is the only catecholamine which accumulates to significant amounts in gastropod molluscs, therefore, we suggest that the blue-green fluorescence is due to dopamine. Our physiological studies show that bath-applied dopamine increases the strength and frequency of contractions in strips of Aplysia esophagus, crop, and gizzard. Actions of dopamine on the esophagus resemble actions of bath-applied acetylcholine. The localization of catecholamines in cells and processes of the buccal system and the actions of dopamine on the digestive apparatus suggest a modulatory role by these neurotransmitters in feeding and digestion. Supported by NIH grant NS24662 to MDK.

216.16

FURTHER LOCALIZATION OF SCPBERGIC NEURONS IN THE BUCCAL GANGLIA OF APLYSIA CALIFORNICA. P.J. Church and M.D. Kirk. Dept. of Biology, Boston University, Boston, MA 02215

The Small Cardioactive Peptide B (SCPB) is present in buccal neurons of Aplysia californica and has profound effects on buccal motor activity. Previous studies have localized SCPB to the identified neurons B1, B2, and B15 and shown its absence in B4/5 and B16. Immunocytochemical studies on juveniles have shown several small dorsal cells and an additional three or four medium to large ventral cells to contain SCPB, but the cells have yet to be characterized.

To determine the functional roles of SCPBergic buccal neurons, we are identifying and physiologically characterizing the remaining buccal neurons with SCPB-like immunoreactivity. Ganglia from 200-300 gram animals were treated either as whole mounts or in 20 um frozen sections. Cell identification was based on size, position, and physiology, and in some cases was combined with lucifer yellow injection for double-label experiments.

Based on cell body position and size, positive staining of the identified neurons B15, B1 and B2 was confirmed in adult animals. In addition, double label experiments show SCPB-like immunoreactivity in B11 and B9, as well as an absence of staining in B3. Consistant staining also appeared in cells on either side of B9 and B3 and a lone cell located on the lateral edge of the ganglia. The neuron medial to B3 appears to be B7. Several (approx. 25) small cells in the dorsal-rostral group stained positive as did at least one cell located peripherally at a branch point of the esophogeal nerve. Numerous processes in the neuropil, buccal nerves, commissural arch, and sheath above the arch also show SCPB-like immunoreactivity. Supported by NIH grant NS24662 to MDK.

216.17

CHARACTERIZATION OF SCP_b IMMUNOREACTIVE CELLS IN THE BUCCAL GANGLION OF *APLYSIA*.

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A cluster of 15 to 30 neurons which exhibits immunoreactivity to the small cardioactive peptide SCP_b was identified on the rostral surface of the buccal ganglion of *Aplysia* (Lloyd et al.,1985). Lucifer yellow injected cells from this group typically have a bipolar appearance, with one process ramifying extensively in the lateral region of the ganglion, and a second process extending toward the buccal commissure. In addition to sending a branch into the contralateral hemiganglion, the axon bifurcates into each branch of the radula nerve.

In preparations exhibiting spontaneous rhythmicity or in which repetitive bursting was produced by radula nerve stimulation (1-2 Hz), the rostral SCP_b immunoreactive ($iSCP_b$) cells were depolarized 10-15 mV in phase with bursting in the identified B4/B5 interneuron pair. This depolarization usually results in synchronous bursting of the $iSCP_b$ cells during the late phase of the B4/B5 burst. Synchrony of $iSCP_b$ cell bursting appears to result from common synaptic inputs and non-rectifying electrotonic connections among members of the cluster (somatic coupling ratio: 0.02-0.04). No dye coupling was noted with 6-carboxyfluorescein or Lucifer Yellow. Firing individual cells did not produce detectable muscle contractions. These cells may serve a sensory function, may be involved with peripheral (Lloyd, et al.,1984; Richmond et al., 1986) or central (Sossin et al., 1987) modulatory actions of SCP_b, or may play a role in a developmental stage of feeding behavior, as suggested by their relative prominence in juvenile specimens (Lloyd et al., 1985).

216.18

THE R20 NEURONS, WHICH MODULATE RESPIRATORY PUMPING IN *APLYSIA*, SYNTHESIZE SCP.

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Respiratory pumping in *Aplysia* consists of transient, synchronous pumping actions of the gill, siphon, mantle shelf and parapodia. This behavior previously has been shown to be driven by a network of about 30 electrically coupled interneurons in the abdominal ganglion, the R25 and the L25 cells. The R20 cells are a pair of electrically coupled interneurons which, when active, can initiate respiratory pumping or increase its spontaneous rate of occurrence. We describe here in more detail the properties of the R20 cells.

The R20 cells, which have somata that are approximately 120-160 µm in diameter, are located on the right caudal edge of the abdominal ganglion, just medial to the branchial nerve. They increase respiratory pumping activity by producing a slow, long-lasting excitation of the endogenous burst mechanism of cells in the R25/L25 network. They also make slow inhibitory connections to the RB cells in the abdominal ganglion and to the gill motoneurons in the branchial ganglion. They are weakly inhibited by cell L10.

The R20 cells stain positively with an anti-serum raised against the molluscan neuropeptide SCP_B. Biochemical purification with HPLC analysis of ^{35}S labeled cells demonstrates that each of R20 cells synthesizes not only SCP_B, but also SCP_A, a closely related molecule that is known to be encoded by the same gene as SCP_B. Both R20 cells also synthesize in abundance identical sets of several other low molecular weight, methionine-containing peptides.

The actions of the R20 cells on the R25/L25 network are mimicked by pharmacological application of SCP_A and SCP_B. However, the actions of the R20 cells on the RB cells are not mimicked by the SCPs: SCP produces excitation while firing the R20 cells produces inhibition. Likewise, firing the R20 cells inhibits the branchial ganglion cells, while bath application of SCP_B causes them to burst (Colebrook and Lukowiak, 1987). Thus the R20 cells probably release SCP_A and SCP_B and at least one other unidentified transmitter.(Supported by grants NS14385 and MH35564).

216.19

MONOAMINERGIC AND PEPTIDERGIC MODULATION OF MOLLUSCAN MOTOR FUNCTION.

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Serotonin (5-HT) and small cardioactive $peptide_B$ (SCP_B) are known to evoke patterned motor activity in identified neurons (e.g. B19) in the buccal ganglia of *Helisoma*, while FMRFamide has inhibitory effects (Granzow & Kater, 1977; Murphy *et al.*, 1985). This study shows that these neuroactive substances also have potent modulatory effects on the muscle targets of *Helisoma* buccal motoneurons.

In vivo, action potentials in neuron B19 evoke one for one excitatory junction potentials (EJPs) in the supralateral radular tensor (SLT) muscle that are reduced in amplitude by tubocurare. Application of 5-HT and SCP_B (10^{-6}M) has no effect on the amplitude of the EJP but significantly enhances neuron B19-evoked muscle contractions of supralateral radular tensor (SLT) muscle.

To determine whether these modulators act directly on muscle, a neuron-free culture system of single dissociated SLT fibers was established. Collagenase dissociated SLT muscle fibers maintain normal physiological properties including resting membrane potentials, excitatory potential changes in response to applied ACh, and contractile responses to both ACh and depolarizing current injection. Depolarizing potential responses to pressure applications of ACh are unaffected by either 5-HT and SCP_B (10^{-6}M) while FMRFamide (10^{-6}M) reduces the amplitude of the ACh depolarization. Although 5-HT and SCP_B have little effect on ACh-evoked depolarizations they potently enhance the magnitude of ACh-evoked SLT fiber contractions.

These results demonstrate that neuroactive substances in *Helisoma* not only effect the generation of patterned buccal motorneuron activity, but also directly modulate the peripheral targets influenced by this motor activity.

This work was supported by NIH grant NS24233.

216.20

ACETYLCHOLINE INDUCES OUTWARD CURRENT IN THE TAIL SENSORY NEURONS OF APLYSIA.

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The effects of acetylcholine (ACh) on tail sensory neurons in the pleural ganglion of Aplysia kurodai were characterized by whole-cell patch-clamp. Micro-application of ACh induced an increased conductance outward current. Repeated application at 1-2 min intervals produced little desensitization. The threshold was 10^{-7} M. The reversal potential in normal seawater is close to -80 mV. The reversal potential shifted to -63 mV in 20 mM $[K^+]$ and -33 mV in 50 mM $[K^+]$. Tetraethylammonium (1 mM) reduced the response to 22% of control. The reversal potential did not shift in Cl-deficient seawater. Atropine (1 mM) and d-tubocurarine (1 mM) partially blocked the response. Hexamethonium (1 mM) had no effect. Carbamylcholine induced a smaller outward current (71% of Ach response). Nicotine and muscarine had almost no effect. Arecoline induced a small outward current (12% of ACh response). We conclude that tail sensory neurons have an H type receptor to ACh which opens the K^+ channel.

REGULATION OF AUTONOMIC FUNCTION II

217.1

EFFECTS OF LUMBOSACRAL DEAFFERENTATION ON BRAINSTEM EVOKED MICTURITION IN THE DECEREBRATE CAT.

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The manner in which descending brainstem and segmental pelvic afferent systems interact during voiding has been subject of extensive speculation (de Groat et al., J. Aut. Ner. Sys. 3:135,1982; Floyd et al. J. Physiol. 322;45,1982). The purpose of this study was to determine if electrical stimulation of the pontine micturition facilitatory region could produce adequate detrusor contraction and external urethral sphincter (EUS) relaxation resulting in coordinated voiding in the absence of lumbosacral afferents.

Ten decerebrate male cats were used for the study. Bladder pressure was monitored using a suprapubic catheter inserted into the fundus of the bladder. Electromyographic records from the EUS muscle were also obtained. Lower lumbar and sacral deafferentation was done by cutting the dorsal roots bilaterally from L6-S3 (extending to upper Ca segments). In several cats the dorsal root ganglia of L7, S1 and S3 were also removed. Following deafferentation, no amount of distension of the bladder could produce a reflex void; only overflow leakage was observed. However, the ability for brainstem stimulation to evoke a bladder contraction of a magnitude and duration sufficient to empty the bladder remained. In addition, the reciprocal inhibition of the EUS activity was also observed during this voiding. These results suggest that the pontine micturition facilitatory region is capable of activating neural systems within lower brainstem regions and/or the spinal cord which can coordinate sacral parasympathetic activation and pudendal somatic inhibition independent of sacral segmental sensory pathways. This research is supported by the Medical Research Council of Canada.

217.2

SYMPATHETIC MODULATION OF CHOLINERGIC TRANSMISSION IN VESICAL PARASYMPATHETIC GANGLIA (VPG) OF THE CAT MEDIATED BY $ALPHA_1$ AND $ALPHA_2$ ADRENERGIC RECEPTORS.

W.C. de Groat, J.R. Keast and S. Smerin*. Dept. Pharmacol., Univ. Pittsburgh, Pittsburgh, PA, 15261.

Previous studies have shown that exogenous catecholamines can facilitate and inhibit transmission in cat VPG, in vitro, via α_1 and α_2 adrenergic receptors, respectively. The present experiments revealed that similar responses can be elicited by electrical stimulation (10-30 Hz, 20-40 V, 10-20 sec) of sympathetic pathways in the hypogastric nerves (HGN) in the anesthetized cats. HGN-stimulation or intraarterial (I.A.) injections of norepinephrine (2-10 µg) commonly depressed the postganglionic potentials elicited by preganglionic stimulation, although in some cats the responses consisted of initial inhibition (15-20 sec) followed by facilitation (0.5-2 min). Phenylephrine (5-50 µg I.A.), an α_1 agonist, facilitated transmission or produced an initial inhibition followed by facilitation. Yohimbine (20-50 µg, I.A.), an α_2 antagonist, blocked the inhibitory effects of drugs and HGN-STIM and unmasked or enhanced the facilitatory effects. Prazosin (20 µg), an α_1 antagonist, blocked the facilitatory responses but not the inhibitory responses. It is concluded that adrenergic neurons in VPG can modulate cholinergic transmission via α_1 facilitatory as well as α_2 inhibitory mechanisms.

217.3

OPIOID MODULATION OF BLADDER REFLEXES AT THE LEVEL OF THE PONTINE MICTURITION CENTER (PMC). J.R. Roppolo, H. Noto, B. Mallory and W. C. de Groat. Depts. of Pharmacol., Behav. Neurosci. and Ctr. Neurosci., Univ. of Pittsburgh, PA 15261.

Studies using intracerebroventricular injections of opioid agonists and antagonists have suggested a supraspinal site for opioid modulation of bladder reflexes. The present study was undertaken to determine if the PMC (in the dorsolateral pons) is a site where opioid modulation of micturition occurs.

Opioid agonists and antagonists were microinjected into the PMC of decerebrate cats while recording either isovolumetric rhythmic bladder contractions or cystometrograms (CMG). Both fentanyl (2-15 nM), a mu receptor agonist and DSLET (0.5-5 nM), a delta receptor agonist inhibited bladder contractions or increased the capacity of the bladder as demonstrated by the CMG. These effects could be reversed by naloxone either administered IV (30 to 100 µg/kg) or microinjected into the PMC (500-1000 nM). Microinjections of morphine (10 to 1000 nM) had only weak effects on bladder reflexes. Microinjections of naloxone (100-1000 nM) without prior administration of opioid agonists, increased the duration or frequency of rhythmic bladder contractions or reduced bladder capacity. These studies suggest that enkephalinergic inhibitory mechanisms are important in modulating bladder reflexes at the level of the PMC.

217.4

BRAINSTEM CONTROL OF URINARY BLADDER AND EXTERNAL URETHRAL SPHINCTER IN CAT. H. Noto, J. Roppolo, B. Mallory, M. Kruse and W. de Groat. Depts. of Pharmacology & Behav. Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

The present study examined the pontine control of urinary bladder (UB) and external urethral sphincter (EUS) coordination in decerebrated cats. Electrical stimulation (ES) (50-150 µA, 0.3 ms, 300 ms train at 50 Hz) of the pontine micturition center (PMC, P 1-3, L 1.5-3, H 0- -3) elicited a UB contraction at a latency of 0.4-1.5 sec, but evoked various responses in the EMG of the EUS. ES of the optimum site in the PMC induced an inhibition of the EUS (shortest latency 40-60 ms) which preceded the evoked UB contraction; this mimics the normal micturition pattern. The threshold intensity for EUS inhibition was lower than that for UB excitation, and the inhibition was induced with a full or empty bladder. ES of adjacent areas of the PMC occasionally evoked EUS excitation (0.1-2 sec latency). ES of the lateral reticular formation evoked EUS excitation. ES of the ventral reticular formation applied during a UB contraction inhibited the ongoing contraction and activated the EUS (latency 95-120 ms), whereas application of the same stimulus in the absence of a bladder contraction inhibited the EUS (latency 40-60 ms). These results indicate that the EUS receives inhibitory and excitatory descending inputs from the pons, and suggest that the PMC has an important role in the coordination of the UB and EUS.

217.5

PONTINE CONTROL OF MICTURITION IN THE RAT. M. Kruse, H. Noto, J. Roppolo and W. de Groat. Depts. Pharmacol. & Behav. Neurosci., Univ. Pittsburgh, Pittsburgh, PA 15261.

Micturition in rats differs from that in many other species by exhibiting a coactivation of the urinary bladder (UB) and the external urethral sphincter (EUS). The present study examined the role of the pons in coordinating the activity of these two structures. Short trains of electrical stimulation (50 Hz) in the medial pons (~ B -8.3, L 0.8, H 5-6.5) elicited contractions of the UB and an increase in EUS activity (latency < 1 sec). The EUS responses occurred with a full or empty UB, indicating that the EUS receives a direct descending input from the pons. Continuous stimulation (50 Hz) with intensities at or just below the threshold for inducing UB contractions markedly reduced the UB capacity as measured by cystometrogram (UB capacity decreased by 44±9%, n=5). Similar effects were noted with stimulation in a more lateral area (~ B -8.3, L 2.2, H 5-6.5). Electrical stimulation of regions ~2 mm ventral to these sites evoked short latency EUS activity (100-200 ms); however, continuous stimulation of these ventral areas increased the UB capacity by 62±30% (n=5). These data indicate that the rat's UB and EUS can be coactivated by descending inputs from the pons, and demonstrate that pontine stimulation can elicit responses that mimic physiologic behavior, including modulation of bladder capacity.

217.6

EFFECTS OF DIABETES MELLITUS ON URINARY BLADDER FUNCTION IN THE RAT. A.M. Mackway*, W.D. Steers, J. Ciambotti*, and W.C. de Groat, (SPON: O. Reinmuth). Depts. Pharmacol., Urol., and Behav. Neurosci., Univ. of Pittsburgh, Pittsburgh, PA 15261.

Streptozotocin-induced diabetic male rats (8 wks. duration) underwent cystometrographic (CMG) and electrophysiologic evaluations to determine etiology of bladder dysfunction. Diabetic rats (D, n=6) in comparison to controls (C, n=8) had significantly greater bladder (BL) capacity (D, 3.0+/-0.84 ml vs C, 0.7+/-.4 ml, P<.001), and weight (D, 112.6+/-10.7 mg vs C, 56.1+/-9.0 mg, P<.01). No differences between D and C were found for latencies or thresholds of hypogastric and pelvic nerve-evoked peripheral and central responses in BL nerves. However, alterations in central micturition reflexes were manifested as an absence of spinal reflexes in D rats (present in 38% C) and a lack of facilitation of the supraspinal reflexes by bladder distension in D (seen in 38% C).

These functional and electrophysiological changes in D differ from those observed in the enlarged BL due to outlet obstruction, and are consistent with an underlying autonomic dysfunction induced by diabetes.

217.7

INTRATHECAL CLONIDINE INDUCES INHIBITION OF MICTURITION REFLEXES AND SPASTICITY IN SPINAL CORD PATIENTS MADE TOLERANT TO SPINAL MORPHINE. R.M. Herman, D.W. Coombs*, R. Saunders*, M.C. Wainberg*. Catholic Med. Ctr., Manchester, NH 03102, Dartmouth/Hitchcock Med. Ctrs., Hanover, NH 03756.

Intrathecal (i.t.) clonidine (CLO) produces analgesia in patients tolerant to i.t. morphine (MOR). In patients with suprasacral spinal cord lesions (SSCL), an acute injection or chronic infusion of i.t. MOR attenuates spontaneous motor discharges ("spasticity") and hyperactive micturition reflexes, leading to enhanced bladder capacity. This study was undertaken to examine the effect of a bolus injection of i.t. CLO (150 µg) on micturition reflexes and spontaneous motor discharges when administered to 2 patients with SSCL, made tolerant to i.t. MOR by chronic infusion of the substance. Volume-induced micturition reflexes and EMG discharges from the striated ano-urethral sphincters and various lower limb muscles were assessed during medium fill cystometry. Within 30 minutes, i.t. CLO suppressed all vesical reflexes and spontaneous motor contractions prior to depression of blood pressure. The action of CLO potentiated the anti-spasticity action of i.t. MOR; in contrast to the action of i.t. MOR, i.t. CLO reduced peak detrusor pressure and vesico-sphincter dyssynergia. By manipulating the doses of MOR-CLO and the selection of opiate-α_2 adrenergic receptor agonists, functional synergism may be attained. (Partial Support: Spinal Cord Soc., Dartmouth/Hitchcock Med. Ctrs., Catholic Med. Ctr., Good Samaritan Med. Ctr., Phoenix, AZ 85006.

217.8

BED NUCLEUS OF STRIA TERMINALIS INFLUENCE OVER GASTRIC FUNCTION **G.E. Hermann, M.J. McCann and R.C. Rogers, Dept. Physiology, Ohio State Univ.**

Anatomic studies by Ricardo and Koh (1978) had indicated that the bed nucleus of the stria terminalis (BST), paraventricular nucleus of the hypothalamus (PVH), and the central nucleus of the amygdala (CNA) have reciprocal connections with the nucleus of the solitary tract (NST) and the dorsal motor nucleus of the vagus (DMN); nuclei that are involved autonomic control of gastric function. Our previous electrophysiological experiments have demonstrated that both PVH and CNA maintain direct connections with NST and DMN; thus, they are able to modulate the basic circuitry controlling gastric secretion and motility.

Our recent physiological experiments have revealed that brief electrical stimulation of the BST elicits an increase in the contractility and/or tone of the gastric musculature. This increase in gastric activity is mimicked by micropressure injection of glutamate into the same region of the BST and can be abolished following systemic administration of atropine methyl nitrate. Thus, the BST may be another forebrain structure that can modulate the function of the stomach via the dorsal medullary vagal complex.

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217.9

THYROTROPIN-RELEASING HORMONE (TRH) POTENTIATES THE GASTRIC RESPONSES EVOKED BY DORSAL MEDULLARY SEROTONIN (5HT). M.J. McCann*, G.E. Hermann, & R.C. Rogers, Dept. of Physiology, Ohio State University, Columbus, OH 43210. (Spon: D. Lim).

TRH and 5HT are colocalized in terminals in the dorsal motor nucleus (DMN), thus both may influence the output of this nucleus. We studied this by measuring gastric function after injections of TRH and 5HT into the DMN. Microinjection of 5HT (8pm/4nl) produced a small increase in motility (44%) and tone (367 mg) above baseline, whereas TRH (1nm/1ul) applied to the dorsal medulla evoked large increases in both indices (323% and 908 mg). After baseline levels returned following TRH application, subsequent 5HT injections now evoked large elevations in motility (291%) and tone (817 mg). This potentiation was blocked by a peripheral muscarinic antagonist. These results suggest that TRH augmentation of 5HT-mediated effects on autonomic nuclei may be a significant feature of the alterations in gastric function that accompany stress-related gastric pathology. (Supported by NINCDS-NS24535).

217.10

TRH EFFECTS ON PHYSIOLOGICALLY-IDENTIFIED NEURONS IN THE DORSAL VAGAL COMPLEX: IN VIVO AND IN VITRO STUDIES. R.C. Rogers, M.J. McCann and G.E. Hermann Dept. Physiology, Ohio State Univ.

Recently, our laboratory showed that TRH micropressure injected into the DMN in picomolar amounts significantly increased gastric secretion and motility. Though direct action of TRH on DMN neurons was implied, there has been no electrophysiological data available to support this contention. Evidence for direct action of TRH on dorsal vagal complex neurons in vivo was obtained by pressure injecting TRH (10 to 200 femtomole in 10-200 picoliter pulses) onto single electrophysiologically-identified DMN or NST cells. With in vitro studies, coronal slice preparations were superfused with TRH (10 micromolar). 46% of DMN cells were activated by TRH; none were inhibited. Conversely, 53% of NST neurons were inhibited by TRH; none were excited. These results indicate that central TRH pathways may "command" autonomic changes by suppressing vagal sensory inputs while activating vagal-motor outputs.

Supported by NINCDS and Ohio State grant funds.

217.11

INTRACEREBROVENTRICULAR (I.C.V.) ANGIOTENSIN II INCREASES FLUID ABSORPTION FROM RAT ILEUM. D.R. Brown and M.A. Gillespie*. Dept. of Veterinary Biology and Neuroscience Graduate Program, Univ. of Minnesota, St. Paul, MN 55108.

Angiotensin (A) II acts at peripheral and CNS sites to maintain extracellular fluid homeostasis. We examined the actions of A-II, A-III and other peptides on intestinal fluid absorption and mean arterial pressure (MAP) in rats after i.c.v. administration. In urethane-anesthetized rats (275 - 400 g), the carotid artery was cannulated for MAP determination and the jejunum (J) or ileum (I) was isolated and perfused in situ with a modified Ringer-HCO_3 solution containing 5 μCi/L of [^{14}C]polyethylene glycol 4000 as a fluid transport marker. Gut perfusates were collected 15' before and after peptide or saline i.cv. injection. A-II and A-III, but not A-I or angiotensinogen (1 μg/rat) increased net water absorption from I; A-II did not alter J transport. Somatostatin (1 μg) decreased I absorption; bombesin and TRH had no transport-related activity. All peptides ($\leq$ 1 μg) increased MAP. The pressor and proabsorptive actions of A-II were inhibited by the α-adrenoceptor blocker phentolamine (1 mg/kg, i.v.) or the A-II antagonist [Sar^1, Val^5, Ala^8]A-II (5 μg, i.cv.); atropine methylnitrate (0.1 mg/kg, i.v.) inhibited A-II actions on I transport but not MAP. Thus, A-II appears to increase I absorption through actions at CNS sites distinct from those mediating its hypertensive effects.

217.12

GABA RECEPTORS IN THE CENTRAL NERVOUS CONTROL OF GASTRIC FUNCTION: MICROINJECTIONS OF BICUCULLINE. H.-S. Feng,* J. Han,* and F.P. Brooks.* (SPON: J.R. Brobeck). Departments of Medicine and Physiology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Bicuculline methiodide 250 ng. injected into the dorsal motor nucleus (DMV) of the vagus was reported to increase acid output and gastric contractions in cats (Washabau et al, Gastroenterology 92: 1807, 1987) but 100 ng failed to increase gastric contractions in other experiments (Williford et al, Science 214: 198. 1981). We have made microinjections by micropressure injections of 200 nl. of saline containing 0-1000 ng. of bicuculline into the DMV, NTS and LHA of 29 anesthetized cats. Gastric content was collected from a gastric fistula and gastric contractions recorded with extraluminal force transducers on the antrum and corpus. Gastric content was titrated to pH7 with 0.1N NaOH with an autoburette. The force of antral contractions increased 6x (5.1$\pm$1.1 to 29$\pm$2gm) at 50 ng. bicuculline, but no increase in acid secretion occurred until 100 ng. was injected in either the DMV or NTS. The maximal gastric acid output was 2x greater (1.0$\pm$0.2 vs. 0.58$\pm$0.08mM/15 min)with injection of the NTS than the DMV. The LHA responded to 250-1000 ng. in a dose-related fashion with an increase in force of contractions (5.6$\pm$0.3 to 32$\pm$2gm). These results suggest that GABA receptors exert a more powerful inhibitory control of gastric motility than acid secretion in the DMV and NTS. However, acid secretion is inhibited to greater degree by GABA receptors in the NTS than the DMV. GABA receptors also participate in the tonic inhibition of a vagally mediated gastric motor excitatory pathway from the lateral hypothalamus. (NIH GRANT R01-36693-2)

217.13

AUTONOMIC NERVOUS SYSTEM INVOLVEMENT IN THE EFFECTS OF NPY IN THE PARAVENTRICULAR NUCLEUS ON GASTRIC ACID SECRETION. G.A. Humphreys*, J.S. Davison* and W.L. Veale. Dept. Med. Physiol., University of Calgary.

Interdigestive gastric acid output is suppressed significantly after injection of 200 pmole of NPY into the paraventricular hypothalamic nucleus (PVN) of anesthetized rats (Brain Research, in press). The purpose of these experiments was to characterize the magnitude and duration of this inhibitory response in gastrin stimulated rats and to determine the autonomic pathways which mediate this NPY effect. In gastrin stimulated rats, gastric acid output dropped by 10 $\pm$ 1.4 μeq/10 min 20 minutes after injection of 200 pmole NPY into the PVN and remained significantly lower than saline injected controls for the duration of the 180 min post-injection measurement period. The inhibitory effect of NPY in gastrin stimulated rats was abolished by intravenous injection of atropine and by coeliac ganglionectomy. The inhibitory response in unstimulated rats was abolished by subdiaphragmatic vagotomy, atropine, coeliac ganglionectomy and by phenoxybenzamine treatment. These results demonstrate that NPY inhibits both unstimulated and gastrin stimulated acid output in the anaesthetized rat. The effect is dependent on the integrity of, and may be mediated by, both the sympathetic and parasympathetic branches of the autonomic nervous system.

217.14

CENTRAL ADMINISTRATION OF BOMBESIN, DERMORPHIN, AND SALMON CALCITONIN REDUCES THE SEVERITY OF PAF-INDUCED GASTRIC ULCERS. A. Guglietta, B. J. Irons* and L. H. Lazarus, LMIN, NIEHS, Research Triangle Park, NC 27709.

Platelet Activating Factor (PAF) is a potent ulcerogenic agent that might be important for the formation of some gastric ulcers in humans. In this study we investigated whether the central administration of peptides (bombesin, 500 ng; dermorphin, 100 ng; salmon calcitonin, 250 ng) which suppress gastric acid secretion might influence PAF-induced gastric ulcers. Peptides were injected icv in rats and 1 h later PAF (3.2 μg/kg) was administered iv. Control animals received vehicles icv and iv. One h after PAF injection the animals were sacrificied, stomachs removed and graded macro- and microscopically for gastric lesions according to an arbitrary scale. PAF induced extensive gastric lesions which did not extend to the duodenum. Previous injections of bombesin, dermorphin or salmon calcitonin significantly reduced the severity of PAF-induced gastric ulcer macro- and microscopically. These data indicate that central injection of these peptides might influence the development of some type of gastric ulcer.

217.15

PARAVENTRICULAR NUCLEUS STIMULATION CAUSES GASTRO-DUODENAL ULCERS. A.V.Ferguson, P.Marcus*, J.Spencer*, and J.L.Wallace* (SPON: D.Bakker). Dept.Physiol. Queen's Univ. Kingston, Ont. CANADA. K7L 3N6.

The hypothalamus has long been known to play a role in the regulation of gastrointestinal function presumably through descending neural projections to medullary autonomic centres. In fact the elimination of such control over gastric acid secretion through bilateral vagotomy, has been a surgical procedure widely used for treatment of peptic ulcer for decades. More recent studies involving intracerebroventricular injections of various peptides, lesions in specific regions of the hypothalamus and stimulation in such regions have provided information on the specific hypothalamic nuclei involved in these regulatory control mechanisms.

The present studies have examined the effects of stimulation in the PVN on gastroduodenal ulceration in urethane anaesthetised male Sprague Dawley rats. Electrical stimulation (60 m, 200 uA, 60 Hz) in PVN resulted in damage in both the stomach (1.75±0.29) and duodenum (1.68±0.4) as assessed by histologically derived damage scores. Similar stimulation in immediately adjacent regions was without significant effect on the gastric mucosa. PVN stimulation following bilateral vagotomy resulted in damage scores (stom. 0.23±0.1, duod. 0.06±0.04) which were significantly reduced compared to the PVN stimulated vagi intact group ($p < 0.05$), but were not different to the group stimulated in regions outside the PVN ($p < 0.05$). These data suggest an important role for PVN efferents influencing medullary vagal preganglionic neurons in the development of gastric ulcers.

217.16

ELECTROPHYSIOLOGICAL RESPONSE OF NEURONS IN THE LATERAL HYPOTHALAMUS TO GASTRIC VAGAL INPUT. C.S. Yuan*, W.D. Barber and B.J. Cammarata* (SPON: S. Hsiao). Department of Anatomy, College of Medicine, University of Arizona, Tucson, AZ 85724.

Gastric vagal input from the proximal stomach was electrophysiologically evaluated in neurons in the lateral hypothalamus in anesthetized cats. Gastric vagal branches of the dorsal and ventral vagal trunks, which serve the proximal stomach, were electrically stimulated with paired pulses (0.3 msec duration, 300 uA, 0.5 Hz) to activate neuronal responses in the lateral hypothalamus. The latency of 70 gastric vagally-evoked unitary responses, recorded in the lateral hypothalamus, was 366 ± 39.1 msec with a conduction velocity of < 1 m/sec suggesting that the stimulus was conveyed largely by unmyelinated fibers. The majority of the vagally evoked hypothalamic unitary responses were phasic bursts containing multiple spikes. Twenty-three % of the units in the lateral hypothalamus receiving gastric vagal input showed tonic activity. The vagal effect upon these tonically active hypothalamic units was predominantly inhibitory. Distention of an intragastric balloon also activated units in the region of the hypothalamus where gastric vagally evoked responses were recorded. This study demonstrated that vagal input from the proximal stomach was capable of altering the activity of neurons in the lateral hypothalamus which other studies have implicated in control of food intake. (Supported by USPHS DK 35434).

217.17

VAGAL ALIMENTARY AFFERENT PROJECTIONS TO THE FERRET BRAIN STEM USING 2-DEOXY-GLUCOSE. B. Greenwood, J. Wood* and D. Kostreva. Depts of Pharmacology and Anesthesiology. Medical College of Wisconsin and VA Medical Center, Milwaukee, WI.

Previous studies using retrograde labelling of HRP demonstrated that in the ferret vagal afferent fibers project to the medial nucleus of the tractus solitarius (NTS) (Fitzackerly et al, 1987). The aim of this study was to investigate alimentary vagal afferent projections to the ferret brainstem by stimulating alimentary vagal afferent fibers and performing a functional mapping study with 14C-labelled 2 deoxyglucose (2DG). The central cut end of the vagal communicating branch (CB), connecting the dorsal and ventral vagal trunks just below the diaphragm was stimulated for 45 mins at 10Hz, 5mA and 0.5msec, 30sec on and 30 sec off. The right cervical vagus was cut. Prior to stimulation, a single bolus of 14C-2DG (100ug/kg i.v.) was injected and 16 serial blood samples taken for scintillation counting and blood glucose. At the end of the experiment the brain was removed and immediately frozen in isopentane at -40°C. Serial sections were cut at 20um and covered with film. After 12 days the films were developed, scanned and converted to glucose utilization. The sections were also stained with cresyl violet for histological comparisons. Electrical stimulation of vagal afferent fibers within the CB led to a significant increase in glucose utilization in the left medial NTS from 0.2mm caudal to the obex to 1.0mm rostral. Glucose utilization was also increased substantially in the left dorsal motor nucleus of the vagus 0.2mm caudal to the obex to 0.6mm rostral. Identical areas on the right side of the brain did not demonstrate significant changes in glucose utilization. Increased glucose utilization was also apparent in the inferior olive.

217.18

DENDRITIC ARCHITECTURE OF MOTONEURONS PROJECTING TO THE UPPER ALIMENTARY TRACT IN RAT: S.M. Altschuler*, X. Bao and R.R. Miselis. (SPON: J. Metzler) Depts. of Animal Biology and Pediatrics and Inst. of Neurological Sciences. Univ. of Pennsylvania, Philadelphia, PA, 19104.

The motor innervation for palatal, pharyngeal, laryngeal, and esophageal muscles involved in swallowing originates primarily within the nucleus ambiguus (NA). The visecerotopic organization of the NA in the rat has been precisely determined (Beiger and Hopkins, JCN 262:546, 1987), but little information concerning the dendritic arborizations of NA motoneurons is available. The neural tracer cholera toxin-horseradish peroxidase (CT-HRP, 0.4%), which is particularly effective at revealing dendrites of retrogradely labeled neurons, was used to determine the extra-nuclear dendritic projections of swallowing motoneurons. CT-HRP was injected into either the soft palate, pharynx, larynx or esophagus. Motoneurons innervating the soft palate, pharynx, larynx, and cervical esophagus were all found to have extensive dendrites that extended into adjacent reticular formation with a distinct pattern for each muscle group. In contrast, the dendrites of motoneurons to lower esophagus were confined to the NA. These extensive dendritic arborizations of NA motoneurons provide a wide ranging target for central afferents that modulate swallowing activity. Support: NIH GM27739, DK01747 and MacArthur Foundation.

217.19

ARE THERE SODIUM SENSORS IN THE RAT'S KIDNEY ?. M.P. Rosas-Arellano*, L.P. Solano-Flores* and A. Guevara-Rojas*. (SPON: H.U. Aguilar-Baturoni). Dept. Fisiología, Fac. Medicina, U.N.A.M. México. Apdo. Postal 70250, 04510-México, D.F.

Paradoxical antidiuresis produced by diuretic drugs suggests the existence of kidney tissue sensors which report to a central regulating mechanisms about the changes in the rate of loss or conservation of Na^+ by the organ. For each test, the left kidney of the rat was isolated and placed on a special support. It was perfused with oxigenated Locke's after its artery and vein were cannulated and the urine was collected by means of a ureteral canula. The temperature was kept at 22 °C ±1°. Filaments of the renal nerves were dissected and placed on Ag-AgCl electrodes to conventionally record the afferent impulse discharges. Spontaneous multiunitary afferent activity with a frequency of 1.1 to 4.4 impulses/second was initially recorded. An increase of the frequency from 3.3 to 6.6 impulses/second occured in many units when furosemide was added to the perfusion. On the contrary, cortisol reduced the frequency from 0.03 to 2.2 impulses/second. These responses probably are the sensor's reports about the natriuretic or antinatriuretic action of the drugs.

217.20

POSSIBLE MECHANISMS RESPONSIBLE FOR CHANGES IN Na^+ AND K^+ EXCRETION AND DIURESIS WHEN THE β-ADRENERGIC PATHWAYS OF THE SEPTAL AREA ARE STIMULATED. W.A.Saad, L.A.A.Camargo*, L. A. De Luca Jr.*, A.Renzi*, W.Abrão-Saad* and J.V.Menani*. Dep. of Physiology, Fac. of Dentistry, UNESP, Araraquara, 14.800. Dep. of Surgery, Fac. of Medicine, USP, São Paulo, Brazil.

The objective of the present investigation was to study the renal mechanisms that may be mediating the changes in Na^+, K^+ and urine volume induced by stimulation of β_2- adrenergic receptors of the medial septal area (MSA). Holtzman rats bearing a delay cannula directed toward the MSA and anesthetized with pentobarbital sodium were infused with a 3% glucose and 0.25% sucrose solution through the jugular vein so that a constant urine flow could be maintained. Urine was colleted through a catheter introduced into the bladder and mean arterial pressure(MAP) was recorded during a 20-minute control period. At the end of this time,a blood sample was collected. Isoproterenol or salbutamol was then injected centrally and the same procedure was repeated during the next 20 minutes. Injection of isoproterenol or salbutamol into the MSA induced a decrease in glomerular filtration rate (GFR),in filtered Na^+ and in MAP, and an increase in fractional Na^+ reabsorption. These results suggest that the fall in Na^+ excretion produced by activation of β_2-receptors in the MSA may be due to the fall in GFR and in filtered Na^+ and to the increase tubular Na^+ reabsorption.

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217.21

CENTRAL AND PERIPHERAL NEUROTRANSMITTER ALTERATIONS IN THE BRATTLEBORO (DI) RAT. R. Dawson, Jr. and D.R. Wallace. Dept. of Pharmacodynamics, College of Pharmacy, Univ. of Florida, Gainesville, FL 32610

Vasopressin (AVP) has been implicated as an important modulator of central and peripheral autonomic function. The present study examined the neurochemical consequences of the absence of endogenous AVP by evaluating monoamine and amino acid content in brain regions from DI rats. Catecholamine content was also determined in the heart, adrenals and kidneys of DI rats.

Three month old Long-Evans (LE) control (n=12) and homozygous DI rats (n=12) were utilized in these studies. Monoamine and amino acid levels were measured by HPLC with electrochemical detection. Norepinephrine (NE) content in DI rats was significantly elevated in the spinal cord, anterior hypothalamus, neurointermediate lobe, adrenal gland and kidney. Serotonin was significantly elevated in the spinal cord and anterior hypothalamus of DI rats. ASP, GLN, GLU and GLY were significantly elevated in the spinal cord and GLN and TAU were significantly elevated in the anterior hypothalamus of DI rats. The lack of AVP in DI rats significantly altered monoamine and amino acid neurotransmitters in the anterior hypothalamus and spinal cord. Sympathetically innervated peripheral tissues of DI rats also exhibit significant changes in NE stores. In summary, AVP exerts a significant modulatory effect on neurotransmission in brain regions involved in autonomic and cardiovascular regulation.

217.22

NUCLEUS MEDIANUS (NM) STIMULATION DEPRESSES THE ACTIVITY OF SUPRAOPTIC (SON) NEUROSECRETORY NEURONS IN THE RAT. Ralph Nissen* and Leo Renaud (SPON: A.T. Tan), McGill Univ. Centre for Research in Neuroscience, Montreal, Quebec, H3G 1A4.

NM neurons project to the SON. Since both structures participate in hydromineral homeostasis, we utilized a transpharyngeal approach in urethane anesthetized male Long Evans rats to evaluate the influence of SON vasopressin (VP) and oxytocin (OXY)-secreting neurosecretory neurons. Following 1 Hz electrical stimulation (20 µs pulse, 10-50 µA, monopolar) in NM, 54/59 SON VP neurons displayed a transient (50-250 ms) inhibition (latency range 8-15 ms) whereas 20/20 OXY cells demonstrated a brief (13-33 ms) activation followed in 5/20 cells by a depression in activity. However, at 5-10 Hz stimulation all tested OXY cells were inhibited. Chemical activation of NM neurons with 0.2 µl infusions of 2-10 µM glutamate transiently depressed spontaneous firing in 12/15 VP and 2/2 OXY cells tested. Thus, NM appears to depress a majority of SON neurosecretory cells. Supported by FCAR and MRC.

217.23

REGIONAL ALTERATIONS IN HEXOKINASE ACTIVITY WITHIN RAT BRAIN DURING DEHYDRATION AND REHYDRATION. T.L. Krukoff and D.H. Vincent*. Dept. of Anatomy & Cell Biology, Faculty of Medicine, U. of Alberta, Edmonton, Canada, T6G 2H7.

Histochemical localization and photodensitometric quantification of the metabolic enzyme, hexokinase (HK), was used to study changes in metabolic activity in rat brain during development of (5 d) and recovery from (7 d) dehydration. In water deprived (WD) rats, HK activity increased after 2 d in the subfornical organ (SFO, 22%), nucleus circularis (NC, 36%), parvo- and magnocellular divisions of paraventricular nucleus (pPVH, 17%; mPVH, 46%), and supraoptic nucleus (SON, 46%). Activity in SFO declined to control levels at 3 d, but then increased. In pPVH, mPVH, and SON, activity was elevated until the end of the experiment. In NC, activity returned to control levels when rats were given water. In salt loaded (2% NaCl in water) rats, changes were like those in WD rats up to 2 d dehydration (SFO, 25%; NC, 20%; pPVH, 16%; mPVH, 38%; SON, 50%). Activity in SFO and pPVH returned to control levels after 3 d dehydration. In mPVH, SON, and NC, activity remained elevated but declined to control levels when salt-free water was provided. Results show that metabolic activity varies during periods of de- and rehydration and that it is necessary to study changes in brain metabolism daily to more fully understand the roles of discrete brain regions in regulation of body fluids. (Supported by the Medical Research Council of Canada.)

217.24

A COMPARISON OF SPINAL SEXUAL REFLEXES IN MALE AND FEMALE RATS. K.E. McKenna and S.K. Chung, *Dept. of Physiology, Northwestern Univ. Medical School, Chicago, IL 60611*

Mature Sprague-Dawley rats were anesthetized with urethane (1.2 g/kg, S.C.) and spinalized at midthoracic levels. Recordings were made from the pudendal and cavernous nerves and from striated perineal muscles. It was determined that the coitus reflex (the neural concomitants of sexual climax) could be elicited in both sexes by mechanical stimulation of the distal urethra. This reflex consists of highly regular, rhythmic bursts of activity in both the pudendal and cavernous nerves, with a period of 1-2 seconds and a total duration of 8-30 seconds. In the male, the bursts give rise to phasic penile erections (with flips and cups) and ejaculation. In the female, there are clonic contractions of the striated sphincters and phasic contractions of the vaginal wall. Anesthesia of the of the urethral mucosa with 1% lidocaine abolished the reflex elicited by mechanical stimulation. The coitus reflex could also be elicited by electrical stimulation of the pudendal sensory branch, which contains the afferent axons innervating the distal urethra. In both sexes, section of efferent nerves or ganglionic and neuromuscular blockade did not abolish the reflex, indicating that peripheral feedback is not essential for the rhythmic behavior. The coitus reflex could not be elicited prior to spinalization, indicating that this spinal pattern generator is under descending inhibitory control. Gonadectomy (> 1 month) did not attenuate or abolish the reflex in either sex.

We conclude that sexual climax is produced by a spinal pattern generator which is very similar in both sexes; is under descending control; is insensitive to gonadal steroids; and is activated by a simple peripheral stimulus of the distal urethra.

217.25

URINARY CATECHOLAMINES IN OBESE AND NONOBESE RHESUS MONKEYS. T. Wolden-Hanson*, J.W. Kemnitz, G.A. Davis and D. Hei*. Wisconsin Regional Primate Research Center and Neurosciences Training Program, University of Wisconsin-Madison, WI 53715

A role for the sympathoadrenal system in energy homeostasis has been clearly established, and abnormal activity of the sympathetic nervous system has been implicated in the pathogenesis of obesity. We have begun to evaluate the relation of catecholaminergic function to manifestations of obesity in a nonhuman primate model. Catecholamines were measured in urine of spontaneously obese (OB, n=8) and nonobese (NOB, n=5) rhesus monkeys during successive 2-day periods of *ad libitum* feeding (baseline), food deprivation and refeeding. Total urinary dopamine (DA), norepinephrine (NE) and epinephrine (E) were measured by HPLC with electrochemical detection. Food intake of OB was not significantly different from NOB and all subjects ate more ($\bar{X}$=26%) during the refeeding phase than during the baseline period ($p<.01$).

Averaged across all three periods, OB excreted 128% more DA, 66% more NE and 67% more E than NOB (p's<.03). DA excretion during deprivation was 62% of the baseline value ($p<.001$), while E increased by 12% during deprivation and then decreased to 63% of baseline during refeeding ($p<.01$). NE excretion during refeeding was 17% greater than baseline, but this change did not achieve statistical significance. There was no statistically reliable interaction of groups with feeding conditions for DA, NE or E. In summary, OB had greater catecholamine excretion than NOB but did not differ from NOB in their response to changes in feeding condition. The basis and functional significance of the striking decrease in DA excretion during food deprivation remain to be elucidated. (Supported by NIH grants RR00167 and GM07507.)

217.26

BOMBESIN MICROINFUSION INTO THE RAT PARAVENTRICULAR NUCLEUS INCREASES BLOOD GLUCOSE, FREE FATTY ACIDS, AND CORTICOSTERONE IN RATS. M.W. Gunion[1], Y. Taché[2], M.J. Rosenthal[1]*, S. Miller[1]*, B. Butler[1]*, and B. Zibl*. [1]GRECC, Sepulveda VAMC, Sepulveda, CA 91343; [2]CURE, Wadsworth VAMC, Los Angeles, CA 90073.

Bombesin-14 (0, 15, 50, 150 ng/300 nl; 0, 9, 27, 90 pmol) was infused into the hypothalamic paraventricular (PVN) or ventromedial nucleus (VMH), lateral hypothalamus (LH), or caudate nucleus (CN) using unilateral guide cannulae previously implanted under enflurane-methoxyflurane anesthesia. Blood samples (120 ul) were taken from the tail tip 0, 15, 30, 60, 90, and 120 min postinfusion. Infusions into the PVN, but not other sites, produced significant dose-related elevations in serum glucose and corticosterone. All three doses significantly increased glucose at this site; only the highest two significantly elevated corticosterone. Free fatty acids were reliably increased by infusions into all three hypothalamic sites, but not by CN infusions. PVN infusions had larger effects on free fatty acids than did VMN infusions; both were more effective than LH infusions. These results imply that the bombesin binding sites and immunoreactive terminals previously identified in these regions may be involved in the central regulation of circulating metabolic fuel levels and the pituitary-adrenal axis. (Supported by NS20660 [MG], AM30110 & AM33061 [YT], AG04793 [MR], and Vet. Adm. research funds [MG, MR].)

217.27

INTRACRANIAL MICROINFUSION OF PANCREASTATIN ELEVATES BLOOD GLUCOSE, FREE FATTY ACIDS, AND CORTICOSTERONE IN RATS. M.J. Rosenthal[1]*, M.W. Gunion[1], K. Tatemoto[2], and J.E. Morley[1] (SPON: H. Raybould). [1]GRECC, Sepulveda VAMC, Sepulveda CA 91343; [2]Dept. Psychiatry & Behavioral Sciences, Stanford University, Stanford CA 94305.

Pancreastatin, a novel peptide recently isolated from porcine pancreas, was infused into the lateral ventricle of acutely anesthetized rats, or into the third ventricle of conscious rats with chronic intracranial cannulae that had previously been implanted under enflurane-methoxyflurane anesthesia, or subcutaneously in conscious rats. Blood samples (120 ul) were taken from the tail tip 0, 15, 30, 60, 90, and 120 min postinfusion in all three experiments. Intraventricular microinfusion of pancreastatin significantly elevated blood glucose, free fatty acid, and corticosterone concentrations in a dose-related manner. None of these effects was seen after subcutaneous injection of the same doses. Centrally administered pancreastatin appears to produce its effects on glucose and free fatty acids through actions in the brain, and either the brain, the median eminence, and/or pituitary for corticosterone. (Supported by NS20660 [MG], AG04793 [MR], DK39188 &MH23861 [KT], NS22397 [JM], and Vet. Adm. research funds [MG, MR, JM].)

217.28

CORRELATION BETWEEN PLASMA INSULIN AND ADRENAL TYROSINE HYDROXYLASE ACTIVITY IN LIFELONG DIETARILY RESTRICTED MICE. B.Davis, D. Ingram, E. Bresnahan, T. McNeill, S. Felten and R. Hamill. University of Rochester School of Medicine, Rochester, NY 14642 and Gerontology Research Center, National Institute on Aging, Baltimore, MD 21224.

Neuroendocrine regulation of blood glucose involves a complex interplay between dietary intake, the sympathoadrenal system, the endocrine pancreas and insulin-and glucagon-sensitive peripheral tissues. The present study assessed neuroendocrine changes in the lifelong dietarily restricted mouse, an animal model of increased longevity. Six week old male C57Bl/6J mice were housed 4/cage at the vivarium facility of the Gerontology Research Center, Baltimore, Maryland. Mice were randomly assigned to AL (fed ad libitum every day throughout the experiment) or DR (fed ad libitum on Mon, Wed, and Fri throughout the experiment) groups. At twenty months of age, all mice were killed after 24 hours of access to food. Trunk bood was assayed for glucose, insulin and glucagon. Adrenals were processed for tyrosine hydroxylase (TH) activity. Our results show that plasma glucose levels of AL and DR mice were comparable. However, DR mice had insulin levels which were significantly higher than in the AL group. Adrenal TH activity also was significantly higher in the DR group, and there was a significant positive correlation between plasma insulin levels and adrenal TH activity. These results suggest that lifelong dietary restriction in the mouse has profound effects on the sympathoadrenal system which may modulate hormonal regulation of metabolism in this animal model.
Supported by AG07194, AG00300, AG05445 and NS22103.

217.29

OBSERVATIONS OF EMETIC MECHANISMS IN THE FERRET (Mustela putorius furo). N.L. Strominger, A.P. Knox, A.H. Battles* and D.O. Carpenter. Dept. of Anatomy, Cell Biology and Neurobiology, Albany Medical College, Albany, NY 12208.

Recently the ferret has been used to study mechanisms of emesis. This species has a low emetic threshold to chemotherapeutic agents and radiation. To further study the suitability of the ferret as an animal model, a series of experiments was conducted to determine the ferrets' sensitivity to some commonly used emetic agents. The data were compared with that obtained from other species.

Our findings confirm observations indicating that ferrets are variably responsive to the classic emetic agent apomorphine, a D_2 agonist [Andrews et al., '86; Gylys et al. '88]. Systematic i.v. injections of apomorphine (.005-1.0 mg/kg) in 3 ferrets elicited emesis inconsistently. Dogs invariably vomit after .25 mg/kg of apomorphine; monkeys are unresponsive to high doses. Leu-enkephalin (0.01-1.0 mg/kg) injected i.v. never elicited emesis in the ferrets; it is very potent in dogs. Subcutaneous injections (1mg/kg) of apomorphine in ferrets elicited emesis in some but not all animals. Bilateral subdiaphragmatic vagotomy rendered a ferret refractory to $CuSO_4$ but did not alter sensitivity to subcutaneous apomorphine.

Data suggest that ferrets are less sensitive than dogs to i.v. emetics but more sensitive than monkeys.

217.30

PHARMACOLOGY OF THE ACETYLCHOLINE-INDUCED INCREASE IN THE SHORT-CIRCUIT CURRENT ACROSS SWINE TRACHEAL EPITHELIUM. Jerry M. Farley, J. Greg Adderholt* and Terry M. Dwyer. Dept. of Pharmacol. & Toxicol. and Dept. of Physiol. & Biophys., Univ. Miss. Med. Ctr., Jackson, MS 39216.

Mucous gland cells secrete mucus in response to stimulation of muscarinic receptors by acetylcholine (ACh). The mucus must be hydrated after release and in order to accomplish this the surface cells and possibly gland cells secrete water to permit hydration. Secretion of water should involve ion transport into the lumen of the trachea. We have examined this possibility by pharmacological modification the ACh-induced increases in short circuit current measured using the Ussing chamber technique. ACh is most likely stimulating gland cells rather than surface epithelial cells. The increase in short circuit current (I_{sc}) induced by ACh is dose-dependent. Cumulative dose-response curves peak at 10^{-4} M giving a peak I_{sc} of ~18 uA (1.26 cm^2 area). The response to a single dose of ACh (10^{-5} M) causes an increase in I_{sc} which has a fast rising phase and a more slowly developing phase. The fast rising phase can be blocked by the addition of tetraethylammonium (TEA, 10-30 mM) to the serosal solution. TEA also caused a reduction in the resting I_{sc} of 4-10 uA. TEA had no effect when added to the mucosal solution. Bay-K-8644, a calcium channel agonist, applied to the serosal surface appears to cause an increase of about 30% in the I_{sc} induced by ACh. These data are consistent with the existence of potassium channels on the serosal but not mucosal surface of the gland cells. This finding along with earlier findings suggesting that Cl^- channels exist in the mucosal membrane suggests that these cells are involved in water transport. The effects of Bay-K-8644 suggest the possible involvement of calcium channels in the increase in short circuit current. (This work was supported by the Cystic Fibrosis Foundation and the Miss. Lung Assoc.)

217.31

PENTOBARBITAL-INDUCED CHANGES IN MEDULLARY RESPIRATORY-RELATED UNIT DISCHARGE PATTERNS AND DIAPHRAGM EMG IN CHRONICALLY INSTRUMENTED GUINEA PIGS. F.-C.T. Chang, R.E. Foster and R. Olaya*. USAMRICD, APG, MD 21010-5425.

Effects of pentobarbital (35 mg/kg) on respiration were studied in guinea pigs chronically instrumented to allow concurrent recording of bulbar respiratory-related unit (RRU) activity, diaphragmatic EMG (DEMG) and ECoG. Recordings were made before and throughout the course of induction of, and recovery from, anesthesia. The most dramatic effects were seen during the development of pentobarbital-induced bradypnea. During that period, and concomitant with discrete alterations in ECoG spectral components, the temporal organization of RRU and DEMG activities underwent spectacular transformations from that of a variable wakefulness pattern (Chang and Foster, Neurosci. Abst., 2:82.7 1986) to a rhythmic pattern characterized by a) invariant periodicity; b) reduced cycle frequency (30-50% of control); c) increased burst duration and spike frequency; d) elevated threshold and diminished response magnitude to alveolar CO_2 loading (ramp, 2.5%/5-min.); and e) diminished T_I/T_E ratio in inspiratory-related units. In conclusion, this study offers, for the first time, direct evidence from functionally and structurally intact preparations that respiratory system components are either inactivated or profoundly altered by anesthesia. The significance of this study, as related to the possibility of different rhythmogenetic control mechanisms underlying sleep and wakefulness respiratory drives, will be discussed.

217.32

TIME OF NIGHT EFFECT ON RESPIRATORY SINUS ARRHYTHMIA IN VICTIMS OF THE SUDDEN INFANT DEATH SYNDROME (SIDS). V.L. Schechtman, R.M. Harper, K.A. Kluge*, A.J. Wilson* and D.P. Southall*. Brain Res. Inst. and Dept. of Anatomy, UCLA, Los Angeles, CA 90024; Dept. of Medical Physics & Clinical Engineering, Sheffield University, Sheffield, England; Cardiothoracic Inst., Brompton Hospital, London.

Respiratory sinus arrhythmia (RSA), variation in heart rate from respiratory sources, is known to be diminished in infants who later succumb to SIDS. Since infants show distinct circadian periodicities in cardiac rate and variability measures after the first month of life, we determined if the reduced RSA in SIDS victims is present throughout the night, or only at particular points during the night. Extent of RSA during quiet sleep (QS), rapid eye movement sleep (REM), and waking (AW) was determined during three time periods (7-11 pm, 11 pm - 3 am, and 3-7 am) in 7 infants 40 to 65 days of age who subsequently died of SIDS and in 21 age-matched control infants. During REM, RSA was diminished in the SIDS victims in all three time periods. During AW, the SIDS victims showed diminished RSA only in the first two periods of the night; from 3-7 am there was no significant difference in extent of RSA. These results confirm previous findings of a disparity in cardiac measures between the 7 pm - 3 am period and the final four hours of the night in SIDS victims, suggesting the possibility of circadian influences operating to differentiate infants at risk for SIDS from other infants. Supported by HD 22695.

217.33

INTERACTIONS BETWEEN VERAPAMIL INJECTION TIME AND MORPHINE EFFECTS ON PHYSIOLOGICAL PARAMETERS IN RATS. F. Ford-Rice*, A. Della Puppa*, F. Snyder*, E. Cone* and E.D. London (SPON: W. Pickworth). Neuropharmacology Laboratory, NIDA ARC, Baltimore, MD 21224.

Verapamil, a calcium channel antagonist, has been shown to facilitate morphine-induced analgesia (Benedek, G. and Szikszay, M., Pharmacol. Res. Comm., 16:1009, 1984) while antagonizing morphine-induced respiratory depression in rats (Szikszay, M. et al., J. Pharmacol. Exp. Ther., 238:192, 1986). Respiratory and cardiovascular effects of s.c. doses of verapamil injected simultaneously with and 10, 30 and 60 minutes before morphine were assessed in 64 partially restrained, awake Fischer-344 rats. Morphine alone did not significantly affect mean arterial blood pressure, but did produce hypoxia, hypercapnia, acidosis and marked tachycardia. Morphine, however, when given together with verapamil facilitated verapamil-induced hypotension. Verapamil alone did not affect arterial blood gases, pH, or cardiac rhythm, but verapamil in combination with morphine increased morphine-induced acidosis and greatly attenuated morphine-induced tachycardia. Furthermore, verapamil suppressed morphine-induced hypercapnia only when injected simultaneously with morphine. The present findings support speculation that calcium ions and calcium channel blockade influence opioid activities, but further studies are needed to establish the relevance of regulation of opioid activity by calcium antagonists.

OPIATES, ENDORPHINS AND ENKEPHALINS: ANATOMY AND CHEMISTRY I

218.1

INTERACTIONS OF OPIOID PEPTIDE AND CATECHOLOAMINE NEURONS IN THE RAT HYPOTHALAMUS. M.D. Fitzsimmons, J.A. Olschowka, and G.E. Hoffman[1]. Dept. of Neurobio. and Anat., Univ. of Rochester, Roch NY 14642 [1]Dept of Physio., U. of Pittsburgh, Pgh PA 15261

A great deal of evidence suggests that opiate agonists exert their effect on prolactin secretion by attenuating an inhibitory influence of dopamine neurons in the hypothalamus. Our goal in these experiments was to see if endogenous opioid peptide-containing neurons interact directly with hypothalamic dopamine neurons which regulate prolactin release. Using both light and electron microscopic double-labeling techniques, we have found evidence for direct synaptic interaction between these systems.

The tissue, obtained from Sprague-Dawley rats, was processed by two consecutive immunocytochemical procedures (ABC-Vector Labs). In the first iteration, the primary antibody was directed against a representative of one of the three major opioid peptide families: ACTH (proopiomelanocortin or POMC), Dyn A 1-17 (prodynophin), or mEnk (proenkephalin). The first reaction is distinguished from the second by adding nickel sulfate which produced a blue-black reaction product. The second primary antibody was directed against tyrosine hydroxylase, a marker for catecholamine neurons. Thus, the opioid peptide neurons were labeled blue-black and the dopamine neurons were labeled brown. Some sections, specially prepared for electron microscopy, were cut to 1-2 μm, photographed, reembedded, cut to 70-100 nm, and observed under an electron microscope. The color photographs were used as a reference to determine the chemical nature of electron dense structures.

Light microscopic analysis suggests that neurons from all three opioid peptide families contact dopamine neurons in the hypothalamus (both A12 and A14). Qualitatively, A14 neurons appear to be more frequently contacted than A12. It also appears that prodynorphin and proenkephalin neurons interact more frequently with dopamine neurons than do POMC neurons. Quantitative studies are now underway. Electron microscopic observations have confirmed that apparent contacts correspond to synapses. Supported by NS 23591.

218.2

SUBPOPULATIONS OF HYPOTHALAMIC ENKEPHALIN NEURONS ARE DIFFERENTLY REGULATED BY ADRENERGIC STIMULI. S.R. George, L. Roldan* and D.A. Haas. Departments of Medicine and Pharmacology, University of Toronto, Toronto, ONT M5S 1A8, Canada.

Enkephalin neurons are present in many areas of the hypothalamus and likely subserve a variety of functions there. We have examined the effects of adrenergic manipulation in male rats on Met-enkephalin immunoreactivity in hypothalamus and in neurointermediate pituitary. Met-enkephalin was quantified by sensitive radioimmunoassay. Depletion of hypothalamic catecholamines by reserpine resulted in an increase of Met-enkephalin concentrations in neurointermediate pituitary ($p<0.001$). Treatment with the alpha-adrenergic agonist methoxamine resulted in depletion of Met-enkephalin from neurointermediate pituitary ($p<0.01$) and an augmentation of Met-enkephalin in hypothalamus ($p<0.05$). This increase was shown to be confined to the median eminence ($p<0.01$). Selective depletion of brain noradrenalin/adrenalin by 6-hydroxydopamine, with protection of dopaminergic and serotonergic neurons by benztropine and fluoxetine respectively resulted in a reduction of Met-enkephalin in median eminence only ($p<0.05$). These results suggest that alpha-adrenergic mechanisms have a role in attenuating Met-enkephalin levels in neurointermediate pituitary, and in augmenting Met-enkephalin levels in median eminence. Whether these effects result through alterations of synthesis and/or release of the enkephalins remain to be shown.

218.3

NICOTINE ALTERS MET-ENKEPHALIN NEURON ACTIVITY IN DISCRETE AUTONOMIC NUCLEI. G.R. Van Loon, K. Pierzchala*, L. Marson and M. Palkovits*. VA Medical Center and Univ. of Kentucky, Lexington KY 40511 and Semmelweis Univ., Budapest, Hungary.

Nicotine acts in CNS to regulate autonomic outflow, however the pathways and mechanisms of its action are poorly understood. Endogenous opioids present in autonomic nuclei modulate sympathoadrenal and cardiovascular function. Recent studies suggest an interaction between brain nicotinic receptors and endogenous opioid peptides. We examined the effects of a single systemic injection of nicotine on levels of native and cryptic Met-enkephalin (MENK) in discrete regions of hypothalamus, brain stem and spinal cord. Nicotine decreased native MENK in caudal ventrolateral medulla, intermediolateral column of spinal cord and central amygdala, which may suggest an increase in release of MENK. A decrease of cryptic with no change in native MENK was observed in central grey, indicating increased processing of the larger peptides to the native form. No changes were found in hypothalamic nuclei or NTS in either native or cryptic MENK. These data suggest that nicotinic receptors modulate autonomic function by altering the release of native MENK and/or the synthesis and processing of proenkephalin A.

218.4

STRIATAL CONCENTRATION OF THE TRIPEPTIDE, TYR-GLY-GLY, PROVIDES AN INDEX OF ENKEPHALIN RELEASE IN VIVO. A.A. Houdi* and G.R. Van Loon. VA Medical Center and Department of Medicine, University of Kentucky, Lexington, KY 40511.

Enkephalins function putatively as neurotransmitters or neuromodulators in brain. Although considerable is known regarding their localization, mechanisms of biosynthesis, and changes in regional concentration, methods of assessing their turnover rates have not been generally available. Recently, Schwartz, Llorens-Cortes and colleagues have described the use of steady-state levels of Tyr-Gly-Gly (YGG), an extraneuronal metabolite of proenkephalin A-derived peptides, as an index of changes in enkephalin release in vivo. In the present study using a sensitive RIA with antiserum raised in our laboratory, we found regional variation in YGG levels in rat brain, highest in striatum and lowest in cerebral cortex. Inhibition of YGG synthesis by icv treatment with the enkephalinase inhibitor, thiorphan, produced a 50 per cent reduction in rat striatal YGG, whereas aminopeptidase inhibition with bestatin produced a 4-fold increase in striatal YGG. To confirm the use of this index under conditions of altered neuronal activity, we examined the effects on YGG of chronic treatment with haloperidol which is known to increase levels of Met-enkephalin and proenkephalin A mRNA. Haloperidol increased striatal YGG by 45 per cent, an effect consistent with increased enkephalin release.

218.5

VARIATION IN ß-ENDORPHIN AND CHOLECYSTOKININ FOLLOWING ARCUATE NUCLEUS LESION OR DEAFFERENTATION. G.J. Lavigne[1,2] and G.P. Mueller[1]. Dept. Physiol., USUHS, Bethesda, MD 20814[1]; Méd. dent. et CRSN, Univ. Montréal, H3C 3J7, Canada[2].

Recent evidence from several laboratories indicates that opioids and cholecystokinin octapeptide (CCK-8) function as natural antagonists in their effects on nociception and other behavior. Little is known, however, about the biochemical mechanisms which underlie these observations. In this study we assessed in rats the influence of brain ß-endorphin (ß-END) denervation on the content of immunoreactive (i) ß-END in the hypothalamus (HYP) and of iCCK in the subcollicular-periaquaductal gray (sc-PAG). Electrolytic lesion (EL, 3 mA, 15 sec) of the HYP arcuate nucleus, the origin of a major proportion of brain ß-END neurons, resulted in a significant reduction ($p<0.01$) in the HYP iß-END by 7 days (from mean control value of 20.1±1.6 (n=10) to 13.5±1.0 fm/mg (n=20)), while sc-PAG iCCK was significatively increased ($p<0.025$ from 63.5±3.4 to 77.8±3.4 fm/mg). However, levels of iCCK in sensory cortex and striatum, sites not well innervated by arcuate ß-END neurons, were unaffected by the lesion. In a different experiment, the same observations were replicated at 7 days; however, nonsignificant trends were observed at 14 and 21 days. HYP deafferentation (Halasz knife) produced a similar pattern of responses, though the magnitude of the effects was greater when compared to those of EL. Although the exact mechanisms through which ß-END and CCK may interact remains to be determined, the present findings provide evidence for a biochemical relationship between these two peptide systems. (G.J.L. supported by the FRSQ, Québec, Canada.)

218.6

EFFECT OF HYPERPROLACTINEMIA ON IMMUNOREACTIVE BETA-ENDORPHIN CONCENTRATIONS IN DISCRETE HYPOTHALAMIC REGIONS. L.P. Kapcala and P.M. Wise. Depts. of Medicine and Physiology, U. Maryland, Baltimore, MD 21201

Hyperprolactinemia (hyperPRL) inhibits pulsatile LH secretion. Previous work has suggested that enhanced opioid tone may be a mechanism whereby hyperPRL inhibits gonadotropin secretion. Therefore, we tested whether hyperPRL alters ß-endorphin concentrations in discrete hypothalamic regions. Adult rats were ovariectomized (day 0). Rats were treated with ovine PRL (4 mg/kg, sc, every 8h) or vehicle on days 4-6. Rats were decapitated on day 6. Extracts from discrete hypothalamic regions (medial preoptic nucleus [MPN], paraventricular nucleus [PVN], median eminence [ME] and arcuate nucleus [AN]) were assayed for IR-ß-endorphin.

Mean ± S.E. IR BetaEndorphin (pmole/mg protein)

	MPN	AN	PVN	ME
Control	11.7±1.1	4.7±0.5	3.1±0.6	7.1±0.7
oPRL	6.3±0.7*	5.5±0.3	6.2±1.0*	5.6±0.7

*Significantly different from control ($p < 0.01$)

Conclusion: changes in hypothalamic β-endorphin concentrations may play a role in hyperPRL-induced suppression of pulsatile LH release. (NIH NS23317 [LPK]; HD15955 & AG02224 [PMW])

218.7

BETA-ENDORPHIN IMMUNOREACTIVE CELLS IN RATS PRENATALLY EXPOSED TO ETHANOL. R.A. Baker and W.J. Shoemaker. Center for Neurological Sciences and Dept. of Psychiatry, Univ. of Conn Hlth. Center, Farmington, CT 06032.

Previous results have shown significantly increased levels of immunoreactive Beta-Endorphin (B-End) in offspring of rats prenatally exposed to ethanol (Shoemaker et al. 1983). Using pregnant rats fed either an alcohol containing diet, an isocaloric pair-fed control diet or standard chow, we confirmed the increase in B-End levels using RIA. In order to determine whether the alcohol induced increase in peptide level results in a concomitant change in the number of B-End containing cells, we have examined the number of B-End cells in the mediobasal hypothalamus (MBH) with immunocytochemistry. Plotting the number of immunoreactive B-End cells in 40 micron sections, our preliminary evidence indicates a 40% increase in the number of immunoreactive cells: counting every 5th section, we find 8415 cells in control, vs. 11880 cells in prenatal ethanol exposed animals. In rats prenatally exposed to ethanol, mapping of positively stained cells indicates that the rostro-caudal extent of stained cells is similar to control animals, but the major difference is that the ethanol exposed offspring has an increase in stained cells in the dorso-lateral aspect of the MBH.
Supported by NIAAA grant #06927.

218.8

STIMULATION OF β-ENDORPHIN RELEASE FROM RAT HYPOTHALAMUS *IN VITRO*. D.M. Bronstein and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Cell bodies of neurons containing β-endorphin (βE) and other pro-opiomelanocortin (POMC)-derived peptides are localized primarily in the arcuate nucleus of the mediobasal hypothalamus and project to a variety of brain regions. Little is known about the neural systems that regulate POMC neurons in the arcuate or which neurotransmitters affect βE release from these cells. The goal of this study was to examine the effects of various chemicals on the release of βE-immunoreactive (βE-ir) peptides from hypothalamic tissue *in vitro*. Hypothalamic blocks (ca. 100 mg) were dissected from adult male rats, cut into 4-6 pieces, and incubated in a Krebs-Ringer-Bicarbonate (KRB) buffer at 37°C in O_2/CO_2 (95:5%). Following a 45 min equilibration period, tissue was transferred every 10-15 min to normal KRB media or to KRB containing one of several putative neurotransmitters or secretagogues. After incubation, media were immediately frozen on dry ice for subsequent determination of βE-ir content. When hypothalami were incubated with high concentrations of K^+ (55 mM), 2-3 fold more βE-ir was detected in the media compared to that found in normal KRB media. Corticotropin releasing factor (CRF), 5-hydroxytryptamine (5-HT) and vasopressin each produced dose-dependent (10^{-8}, 10^{-7}, 10^{-6} M) increases in βE-ir release into the media. Dopamine (10^{-6} M) inhibited basal release of βE-ir to virtually non-detectable levels and completely blocked the effects of 55 mM K^+. It is interesting that the same chemicals which stimulate βE-ir release from hypothalamic tissue also are potent secretagogues of βE from corticotrophs of the anterior pituitary gland.

218.9

INCREASES IN PRODYNORPHIN PEPTIDES FOLLOWING CHRONIC MORPHINE TREATMENT. K.A. Trujillo, D.M. Bronstein and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI, 48109.

The discovery of endogenous opioid peptides and receptors led to early speculations that opiate tolerance and dependence might result from changes in endogenous opioid systems. It was thought that chronic treatment with opioids would lead to a down-regulation of these systems, resulting in decreases in opioid peptides or receptors. Such speculations have recieved little experimental support; chronic treatment with opiate drugs has been found to have little effect on pro-opiomelanocortin or proenkephalin peptides, or on opioid receptors.

In the present studies, we examined the effects of chronic morphine on prodynorphin (PRODYN) peptides in forebrain and midbrain regions of the rat. Animals were implanted subcutaneously with morphine or placebo pellets under ether anesthesia and sacrificed 1, 3 or 7 days after the start of treatment. Dorsal striatum (caudate-putamen), ventral striatum (nucleus accumbens and olfactory tubercle), substantia nigra and ventral tegmental area were obtained and radioimmunoassayed for Dyn A 1-17, Dyn A 1-8, Dyn B, and alpha neo-endorphin. Morphine caused an increase in all four PRODYN peptides at 7 days, averaging 60-70% above placebo in the dorsal striatum, and 20-30% above placebo in ventral striatum. Assays on midbrain tissue have yet to be completed, however preliminary data suggests no consistent changes. These results suggest that PRODYN peptides may be involved in some of the long-term consequences of opiate treatment, including tolerance and dependence. It remains to be determined if the observed changes result from increased synthesis or decreased release of these peptides.

218.10

THE ANTIESTROGEN TAMOXIFEN ALTERS THE PITUITARY CONTENT OF DYNORPHINS IN THE FEMALE RAT. S.Spampinato and S. Ferri*. Inst. of Pharmacol., Univ. of Bologna, Bologna, Italy.

In the anterior pituitary(AP), immunoreactive dynorphin (ir-dyn) has been detected within gonadotroph cells and is regulated by ovarian factors(Spampinato et al., Life Sci., 38:403, 1986). In the neurointermediate lobe (NIL), dynorphins are colocalized with vasopressin. In this study, we have employed the antiestrogen tamoxifen (TAM) to obtain further insight into how estrogens regulate the pituitary content of ir-dyn A and ir-dyn B (evaluated by RIA). In intact adult female rats, TAM (50 µg/day, sc injected for 7 days) caused an increase of AP ir-dyn A(0.29±0.05 vs 0.15±0.03 fmol/µg prot, $p<0.01$) and ir-dyn B(0.31±0.06 vs 0.14±0.01, $p<0.01$). Estradiol benzoate(EB, administered for 7 days via sc implants) fully prevented this increment and by itself caused a significant reduction of both peptides. As regards the NIL, the levels of ir-dyn A and ir-dyn B were not changed in rats exposed for 7 days to TAM or EB. Interestingly, a 14-day treatment with TAM markedly reduced either ir-dyn A(2.81±0.47 vs 7.54±1.17, $p<0.01$) than ir-dyn B(5.14±0.32 vs 9.83±0.8, $p<0.01$). Although this effect was not observed in ovariectomized rats, it was not prevented by EB. This study shows that TAM alters AP dynorphins acting as antiestrogen. More complex mechanisms underlie the effects elicited on NIL.

218.11

RELEASE OF ENDOGENOUS OPIOIDS DISPLACES [^{3}H]-DIPRENORPHINE FROM RAT HIPPOCAMPUS. J. F. Neumaier, J. N. Wiemann*, and C. Chavkin, Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195.

Release of endogenous dynorphin and enkephalin peptides was detected by displacement of the opioid antagonist, [^{3}H]-diprenorphine ([^{3}H]-DPN), from rat hippocampal slices. Freshly dissected slices (500 μm) were equilibrated in oxygenated Kreb's bicarbonate buffer in individual culture wells. All wells contained peptidase inhibitors (bestatin, thiorphan, and captopril). Slices were incubated with 1 nM [^{3}H]-DPN for 30 min and then homogenized and counted or frozen for autoradiographic processing. Non-specific binding was determined in the presence of 10 μM naloxone. Depolarization by 50 mM KCl significantly reduced specific [^{3}H]-DPN binding by 30%. 10 μM veratrine induced a similar displacement. The displacement of binding by both KCl and veratrine was calcium dependent and blocked by 10 mM Mg^{++}. The magnitude of the KCl-induced displacement was diminished by ommission of the peptidase inhibitors. KCl-induced displacement of [^{3}H]-DPN binding was transient; addition of KCl 15 min prior to adding [^{3}H]-DPN caused maximal competition. None of the experimental compounds reduced [^{3}H]-DPN binding in membrane homogenate assays. Preliminary autoradiography and densitometric analysis showed that KCl depolarization reduced [^{3}H]-DPN binding in CA3 and the dentate gyrus. These results suggest that depolarization leads to a calcium-dependent release of a peptidase-sensitive material that displaces specific [^{3}H]-DPN binding (presumably dynorphin and enkephalin peptides). Supported by NS-23483 and GM-07266.

218.12

DEPOLARIZATION DISPLACES [^{3}H]-DITOLYLGUANIDINE FROM HALOPERIDOL-SENSITIVE SIGMA RECEPTORS IN RAT HIPPOCAMPUS. C. Chavkin and J. F. Neumaier. (SPON: L. Halpern) Dept. of Pharmacol., Univ. of Washington, Seattle, WA 98195.

We examined the effects of ionic conditions and tissue depolarization on sigma receptors by studying [^{3}H]-propyl-3-(3-hydroxyphenyl)-piperidine ([^{3}H]-3-PPP) and [^{3}H]-ditolylguanidine ([^{3}H]-DTG) binding. In rat brain homogenates, binding of 1 nM [^{3}H]-3-PPP was reduced by NaCl with an IC_{50} of 8 mM, whereas [^{3}H]-DTG binding was insensitive to NaCl. LiCl, choline chloride, and KCl did not reduce binding significantly at concentrations below 100 mM. $CaCl_2$ or $MgCl_2$ (10 mM) reduced binding of both radioligands by 30%. Previous physiologic studies demonstrated agonist properties of 3-PPP and antagonist properties of DTG; our current results suggest that sodium sensitivity in binding assays may differentiate agonists from antagonists at the sigma receptor as it does for the μ and ∂ opioid receptors.

Freshly dissected hippocampal slices (500 μm) were equilibrated in oxygenated Kreb's bicarbonate buffer in individual culture wells containing peptidase inhibitors (bestatin, thiorphan, and captopril), incubated in 3 nM [^{3}H]-DTG for 30 min, and then homogenized and counted. Nonspecific binding was defined by 10 μM haloperidol. Depolarization by 10 μM veratridine or 50 mM KCl reduced specific [^{3}H]-DTG binding by 65%. Removal of $CaCl_2$ and the addition of 10 mM $MgCl_2$ blocked the depolarization-induced displacement by 50%. 10 μM tetrodotoxin completely blocked the veratridine effect. The magnitude of the depolarization-induced displacement was diminished by ommission of the peptidase inhibitors. Depolarization either releases an endogenous sigma ligand or directly affects properties of the sigma receptor (e.g. internalization). The calcium-dependence and peptidase-sensitivity of the [^{3}H]-DTG displacement supports the former interpretation. Supported by NS-23483 and GM-07266.

218.13

HIPPOCAMPAL TRANSPLANTS EXPRESS ENKEPHALIN BEFORE DYNORPHIN IMMUNOREACTIVITY. J.F. McGinty, P. Tandon and H.A. Tilson. Dept. Anatomy & Cell Biology., East Carolina U. Sch. of Med., Greenville, NC 27858 and Lab of Integrative & Molecular Neuroscience, NIEHS, NIH, Research Triangle Park, NC 27709.

Impaired acquisition of a spatial working memory task after colchicine-induced destruction of dentate granule cells (Walsh et al. Brain Res. 398:23, 1986) is ameliorated by fetal (E17-18) hippocampal cell suspension grafts (Tandon et al. Brain Res. submitted). In this study, we investigated the histochemical organization of the mossy fiber system in similar hippocampal grafts after placement in the dorsal or ventral hippocampus of rats treated 3 weeks previously with an intradentate colchicine or artificial cerebrospinal fluid (ACSF) injection. Four, 6, or 11 weeks after grafting, the rats were perfused; serial sections through the transplant were cut and Nissl-stained, Timm-stained (for zinc), or immunoperoxidase-stained with anti-dynorphin A 1-8 (DYN) or met-enkephalin (ENK) antiserum. There was no difference in the degree of transplant survival in colchicine or ACSF-treated rats. In 1 month old transplants, Timm-stained and ENK-immunoreactive fibers had formed clusters around presumptive pyramidal cells; however, no DYN immunoreactivity (IR) was present in adjacent transplant sections. In 6 and 11 week old transplants, ENK-IR and DYN-IR were present in fiber clusters presumed to be mossy fibers. To support the conclusion that DYN-IR and ENK-IR were present in grafted mossy fibers, in situ hybridization histochemistry is now being used to identify dentate granule cells in the grafts.

218.14

CORTICOTROPIN RELEASING-HORMONE (CRH) IN OLD WORLD NONHUMAN PRIMATE HYPOTHALAMUS, PLACENTA AND LATE PREGNANCY PLASMA. A.N.Margioris*, M.Grino* and G.P.Chrousos(SPON: D.Symmes). Developmental Endocrinology Branch, NICHD, NIH, Bethesda, Md, 120892.

CRH is synthesized and secreted by human placenta. We have found that the CRH gene is also expressed in nonhuman primate placentae. In this study we examined the post-translational processing of CRH in Rhesus monkey (Macaca mulatta) placenta and hypothalamus. In gel chromatography of hypothalamic extracts, all IR-CRH eluted in one peak with a Kav corresponding to that of hCRH. Gel filtration of placental extracts showed that the bulk of IR-CRH eluted in two peaks. The first peak coeluted with the high mol wt IR-CRH present in human placental extracts and the second exhibited a Kav between that of the high mol wt CRH and synthetic hCRH. Ion exchange chromatography showed that the IR-CRH present in monkey placental extracts consisted of at least three different forms. IR-CRH was also present in the plasma of pregnant rhesus monkeys the mean concentration of which was 31.3±4.6 pg/mL (n=7); on gel chromatography, the bulk of plasma IR-CRH coeluted with hCRH. In conclusion: The IR-CRH present in nonhuman primate placentae consisted of multiple mol wt forms, while the bulk of IR-CRH in the plasma of pregnant primates and in the hypothalamus of nonpregnant monkeys had the mol wt of synthetic hCRH. These findings suggest that the post-trasnlational modifications of nonhuman primate placental CRH differ from those in hypothalamus but are similar to those described in human placenta.

218.15

EXPRESSION OF PREPROENKEPHALIN mRNA BY CULTURED ASTROCYTES AND NEURONS. M.H. Vilijn[2*], P.J.-J. Vaysse[2*], R.S. Zukin[2] and J.A. Kessler[1,2], (SPON: P.G. Model) Departments of Neurology[1] and Neuroscience[2], Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, USA.

Astrocytes are the most numerous cell type in the brain and appear to subserve multiple functions. The present study reports that cultured neonatal astrocytes from rat hypothalamus, striatum, frontal cortex, hippocampus and cerebellum contain significant levels of preproenkephalin mRNA (PPE mRNA). Cultured cells were identified as astrocytes on the basis of morphology, expression of immunoreactivity for glial fibrillary acidic protein (GFAP) and absence of immunoreactivity for fibronectin, for galactocerebroside or for the monoclonal antibody A_2B_5. PPE mRNA levels determined by Northern blot analysis were significant in astrocytes cultured from all of the brain areas. Levels of PPE mRNA were significantly higher in hypothalamic astrocytes compared to astrocytes derived from other brain regions and were comparable to levels present in cultured embryonic hypothalamic and striatal neurons. Prodynorphin mRNA was not detectable in cultured astrocytes, although cultured hypothalamic neurons contained substantial levels of this mRNA. Thus, glia do not express all opioid peptide genes during development. These observations suggest that expression of the PPE gene by astrocytes may play a role in development of the brain.

218.16

THE LOCALIZATION OF PRODYNORPHIN AND PROENKEPHALIN mRNAs IN THE RAT ADRENAL GLAND BY *IN SITU* HYBRIDIZATION. R. Day, M. K.-H. Schafer*, J. Douglass*, M.R. Ortega*, S.J. Watson and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109. Vollum Institute for Advanced Biomedical Research, The Oregon Health Sciences University, Portland, Oregon, 97201.

Pro-enkephalin (PE) and pro-dynorphin (PD) are both known to exist in the adrenal gland. The better studied of the two, PE, has been localized by immunocytochemistry and *in situ* hybridization in various species. In the rat adrenal, low levels of peptides and mRNA have made similar studies difficult. Less is known about PD in the rat adrenal. Previous studies have demonstrated high levels of PD mRNA by Northern analysis in the rat adrenal (Civelli et al., 1985, *Proc. Natl. Acad. Sci. USA*, 82, 4291). Our study aims to answer a few basic question about these two mRNAs. How are they distributed in the rat adrenal? Are they co-localized or are there distinct cells which synthesize and process their precursors? Our approach is to use high specific activity ^{35}S labelled cRNA and cDNA probes to PE and PD to visualize their mRNAs by *in situ* hybridization. Our results clearly show a patchy distribution of PE mRNA in the chromaffin cells of the adrenal medulla. PD mRNA, however, is localized mainly in the adrenal cortex. Low levels of PD mRNA are also observed in cells of the medulla. It is not yet known whether these medullary cells are co-localized with PE mRNA. Further studies are underway to elucidate this point.

218.17

THE DISTRIBUTION OF PRODYNORPHIN mRNA THROUGHOUT THE RAT BRAIN: A SEMI-QUANTITATIVE MAPPING STUDY. M.K.-H. Schafer*, J.P. Herman, R. Day, J. Douglass*, H. Loats, H. Akil and S.J. Watson (SPON: H. Akil). Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109, Vollum Institute for Advanced Biomedical Research, The Oregon Health Sciences University, Portland, OR 97201 and LOATS Associates Inc., Westminster, MD 21157.

^{35}S labeled cRNA probes of high specific activity were generated from prodynorphin cDNA subclones. Prodynorphin mRNA was localized in serial frozen sections throughout the rat brain by in situ hybridization. Autoradiograms were analyzed by dark field microscopy and prodynorphin positive neurons mapped according to the coordinate system of Paxinos and Watson. Semi-quantitative analyses of different prodynorphin containing regions were performed using a LOATS image analysis system. We will report the detailed distribution of prodynorphin mRNA in the rat brain and compare it with proenkephalin mRNA in certain brain areas. Furthermore, a quantitative comparison of prodynorphin mRNA content between different neuronal cell types will be made.

218.18

LOCALIZATION AND REGULATION OF RAT KIDNEY ENKEPHALINASE: AN *IN SITU* HYBRIDIZATION ANALYSIS. S. Burke*, L. Hersh, S. Unnithan*, R. Chipkin, M.K.-H. Schafer*, A. Mansour and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI, University of Texas Health Sci. Center, Dallas, TX and Shering Corp., Bloomfield, NJ.

Enkephalins can be hydrolyzed into inactive fragments by a variety of peptidases. One of these enzymes, Enkephalinase, hydrolyses the Gly^3Phe^4 amide bond. Enkephalinase has been purified and cDNA clones isolated by Malfroy for both rat brain and kidney. The analysis of these cDNAs indicates that the Enkephalinase enzyme is identical in kidney and brain.

In this study we report the use of a 1.6 Kb clone isolated from a rat kidney lambda gt11 library for localization and regulation of mRNA in rat kidney. Kidney was chosen for initial studies, as Enkephalinase is 100 times more abundant in kidney than in brain. By both immunocytochemistry and *in situ* hybridization we have found the enzyme and message to be mainly localized in the distal tubules. We are studying three types of pharmaceutical manipulations on the rat: Gentamicin (for a general tubular insult), Naltrexone and a Shering compound (SCH 32615; an Enkephalinase inhibitor). Data to date suggests that naltrexone has little or no effect on mRNA levels. Gentamicin treated rats exhibit a 45% increase in mRNA levels over normal saline controls. Studies with the Shering Enkephalinase inhibitor are in progress.

218.19

LOCALIZED LESION OF NEURONS WITH N-METHYL-D-ASPARTATE (NMDA): SUPPORT FOR A NEURONAL LOCALIZATION OF ENDOPEPTIDASE 24.11 (ENKEPHALINASE) IN FOREBRAIN. S. A. Back, M. E. Hargreaves*, J. E. Swett, and C. Gorenstein, Department of Pharmacology, University of California, Irvine CA 92717.

We have previously observed cell bodies in enkephalin-positive regions of the CNS to stain for the enzyme enkephalinase (endopeptidase 24.11, E.C. 3.4.24.11). The sites of enzymatic activity were visualized by a fluorescent histochemical method using nitrosalicyaldehyde to localize a reaction product resulting from sequential enzymatic cleavage of the synthetic peptide substrate, glutaryl-alanyl-alanyl-phenylalanyl-4-methoxy-2-naphthylamide by enkephalinase and exogenous aminopeptidase M. In the present study we sought further support for a neuronal localization of the enzyme. Enkephalinase staining was examined in the CNS of male rats which were either untreated, which received 250 ug of colchicine i.c.v, or which received NMDA 5-7 days prior to sacrifice. Injections of 10 ug of NMDA were made into the nucleus accumbens, the medial caudate putamen, or the globus pallidus. The cellular response to injury was assessed by Nissl stain and by ethidium bromide fluorescent counterstain.

We did not observe a marked difference in the regional distribution of enkephalinase in untreated versus colchicine-treated animals in regions including the nucleus accumbens, ventral pallidum, caudate putamen, globus pallidus, nucleus basalis, and the substantia nigra, compacta and reticulata. There were extensive populations of enkephalinase-positive cell bodies in these regions in untreated animals. Enriched staining in proximal processes and decreased neuropil staining were the major differences observed in response to colchicine. NMDA altered both the amount and distribution of enkephalinase reaction product in the injected regions. The overall intensity of staining was markedly reduced and was largely unassociated with any cellular elements. By contrast, it was often possible to observe normal cellular enkephalinase staining at the periphery of the lesioned areas. The Nissl stain and ethidium bromide counterstain confirmed a marked decrease in neuronal cell bodies and a corresponding increase in glial cells particularly at the injection site. These cellular changes were focal in nature, and no indications of distant morphological damage or decreased enzyme staining in any other CNS regions were detected.

The size and morphology of enkephalinase-stained cell bodies, the lack of alteration in enzyme distribution in response to colchicine, and the pronounced decrease in cellular enkephalinase staining in repsonse to NMDA-induced injury, support the presence of extensive populations of enkephalinase-positive neurons in the forebrain.

218.20

MEMBRANE ASSOCIATED PUROMYCIN SENSITIVE RAT BRAIN ENKEPHALIN DEGRADING AMINOPEPTIDASE: ARTIFACT OR REAL?. S. H. Dyer*, K. Orth+*, C.R. Moomaw+*, C.A. Slaughter+* and L.B. Hersh. Depart. of Biochemistry, Univ. of Texas Southwestern Medical Ctr. and Howard Hughes Medical Inst. Dallas, Texas 75235

An enkephalin degrading puromycin sensitive aminopeptidase has been shown to exist in soluble and membrane forms in rat brain. The kinetic properties and molecular weight of these enzyme forms have been found to be the same. In this study the physical properties of the two enzymes were compared. The enzymes exhibit the same subunit and native molecular weight indicating they are monomers of identical size. An antiserum generated against the soluble enzyme exhibits identical cross-reactivity with the membrane enzyme and both enzymes exhibit the same pI and the same mobility on native gels. Identical tryptic maps and N-terminal sequence for the two enzyme forms were obtained.

The membrane enzyme is not released by salt and does not exhibit the latency expected of vesiculated proteins. These results suggest the membrane enzyme may associate with the membrane by non-covalent interactions with integral membrane proteins.

218.21

ISOLATION OF PRO-DYNORPHIN-RELATED END PRODUCTS FROM THE BRAIN OF THE AMPHIBIAN, XENOPUS LAEVIS. R.M. Dores and C.A. Sei. Dept. of Biological Sciences, Univ. of Denver, Denver, CO 80208.

Methanol/HCl extracts of the brain of the amphibian, Xenopus laevis were analyzed for the presence of pro-dynorphin-related end products. Radioimmunoassay analysis coupled with Sephadex G-50 column chromatography and reverse phase HPLC revealed the presence of immunoreactive alpha-neo-endorphin, dynorphin A(1-17) and dynorphin A(1-8). Following HPLC analysis, these peptides eluted with the same retention times as the synthetic mammalian standards. These extracts were also screened for the presence of enkephalin-related peptides. Both met-enkephalin and leu-enkephalin were detected following HPLC analysis. This observation was unexpected since Xenopus pro-enkephalin does not contain a leucine enkephalin sequence (Martens and Herbert, Nature 310:251, 1984). These results would indicate that in this species some pro-dynorphin-related peptides are processed to yield leucine enkephalin as an end product. This study was supported by NIH grant HD23100.

218.22

FURTHER CHARACTERIZATION OF AN ENDOGENOUS LIGAND ("SIGMAPHIN") FOR *SIGMA* RECEPTORS IN THE BRAIN. T.-P. Su and D. B. Vaupel*. Neuropharmacology Lab., Addiction Research Center, NIDA, Baltimore, MD 21224.

An endogenous ligand for *sigma* receptors was partially purified from guinea-pig brain extracts using molecular sizing and ion-exchange chromatography. Two systems were used simultaneously to monitor *sigma* activity during purification: a *sigma* receptor binding assay and a relatively specific bioassay using the guinea-pig vas deferens (Vaupel, D.B. and Su, T.-P., Eur. J. Pharmacol., 139:125, 1987). The "sigmaphin" thus identified had an apparent molecular weight of about 485 daltons. Like prototypic *sigma* drugs, sigmaphin potentiated electrically-evoked twitches of the guinea-pig vas deferens in a dose-responsive manner, and the potentiation was inhibited by the putative *sigma* antagonist, haloperidol. The activity of sigmaphin was not affected by pronase treatment or 6N HCl hydrolysis. EDTA did not inactivate sigmaphin. Sigmaphin was resistant to chloroform extraction under acidic, neutral and alkaline conditions. No stainable spot could be detected on TLC plates containing sigmaphin using either ninhydrin, fast blue B or iodoplatinate spray. It is concluded that sigmaphin is nonpeptidic, small, chemically polar and probably contains no nitrogen. Further purification and structural determination of sigmaphin would facilitate our understanding of the possible physiological roles of *sigma* receptors.

218.23

ISOLATION AND IDENTIFICATION OF THEBAINE FROM OVINE BRAIN. H. Kodaira, C.A. Lisek*, I. Jardine*, A. Arimura# and S. Spector. (SPON: R.F. Margolskee). Roche Inst. Molec. Biol., Nutley, NJ 07110. *Mayo Clinic, Rochester Minn. 55905. #Tulane Univ., Herbert Cntr., Belle Chasse, LA 70037

Morphine and codeine have been identified in various mammalian tissues. The increase of these alkaloids following the administration of thebaine or salutaridine and the conversion of reticuline to salutaridine were demonstrated in mammals. Recently we reported on the enzymatic conversion by mammalian tissues of thebaine to morphine and its cofactor requirements (Proc. Natl. Acad. Sci. U.S.A.,85:1267,1988); the pathway was similar to that which exists in the opium poppy. Thus, the enzymes necessary for the biosynthesis of morphine or its intermediates exist in mammalian tissue. Here we report the isolation of thebaine from ovine brain. The intermediate was purified to homogeneity by HPLC with the combination of a radioimmunoassay for thebaine. The immunoreactive material behaved identically to standard thebaine in two HPLC systems, then was confirmed by gas chromatography and mass spectrometry. This is the first identification of the intermediate thebaine in mammalian tissue. The presence of thebaine as well as codeine and morphine in ovine brain provides strong evidence that morphine and codeine in mammalian tissues are of endogenous origin and actually biosynthesized from the precursor.

STAINING AND TRACING TECHNIQUES I

219.1

HISTOCHEMICAL DEMONSTRATION OF ACETYLCHOLINESTERASE IN PLASTIC SECTIONS. M.R.Donald, K.S.Jodelis*, and L.R.Williams, CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

Improved morphological preservation and resolution of AChE-expressing structures in rat brain were achieved by processing 10 μm glycol methacrylate plastic sections according to Hedreen et al. (1985). Several modifications were made to the basic methodology reported by van Straaten et al. (1986). Optimal parameters of tissue preparation included: fixation in 4% paraformaldehyde, 0.1% glutaraldehyde with 7% sucrose in 0.1M phosphate buffer, pH 7.2; rinsing with 50 mM NH_4Cl in phosphate buffer containing 2% sucrose; acetone dehydration; vacuum infiltration with LKB Historesin; polymerization at 4°C; overnight incubation in the Hedreen et al., AChE reaction mixture at 37°C using iso-OMPA as the inhibitor of non-specific esterase; and intensification with 2% $(NH_4)_2S$. As well as providing excellent visualization of axonal processes, the method also enabled visualization of cholinergic neurons in serial sections through the basal forebrain using the pharmacohistochemical method of Butcher (1983).

219.2

DOUBLE-LABEL ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY USING TETRAMETHYLBENZIDINE (TMB) AS A CHROMAGEN. Y.Tsai*, R.B. Norgren, Jr.* and M.N. Lehman (SPON: R.R. Cardell). Dept. Anat. & Cell Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH 45267.

A technique developed by Olucha (*J. Neurosci. Meth., 13*:131-138) allows one to use TMB as a chromagen for HRP histochemistry at close to a physiological pH. Although initially developed for tract tracing, we have found that, with some modifications, it can be used reliably for EM immunocytochemistry. We investigated the possibility of using this technique in conjunction with a diaminobenzidine (DAB) protocol to demonstrate the presence of two antigens at the EM level. Adult Suffolk ewes were perfused with 4% paraformaldehyde and 0.2% glutaraldehyde. Vibratome sections were processed for demonstration of the first antigen (TH or NPY) using an avidin-biotin-HRP procedure (Vectastain) with DAB as the chromagen. Sections were then processed for the second antigen (LHRH) as above with the exception that the chromagen used was a TMB-ammonium heptamolybdate (TMB-AHM) solution (pH 6.0). After a 20 min. pre-incubation, hydrogen peroxide was added until blue-green reaction product was observed. Sections were quickly washed in Tris buffer (pH 7.3) and immediately put in a 0.5% DAB-cobalt solution in order to make the TMB-AHM reaction product electron dense and stable at a higher pH. As soon as reaction product turned turned blue-black, sections were transferred to phosphate buffer. Areas of interest were microdissected, osmicated, dehydrated, and embedded in epon. LHRH cell bodies and processes labelled with TMB-AHM could be easily identified by the presence of crystalline rods, and were distinct from granular DAB labelled elements. Good ultrastructural preservation allowed identification of NPY synapses onto LHRH neurons. [Supported by NIH HD21068 to M.N.L.]

219.3

DOUBLE-LABEL CYTOCHEMICAL-AUTORADIOGRAPHIC METHOD FOR ELECTRONMICROSCOPIC (EM) CO-LOCALIZING ACETYLCHOLINE-STERASE (AChE) AND ACETYLCHOLINE RECEPTORS (AChRs) IN THE SAME INNERVATED CULTURED HUMAN MUSCLE FIBER (Inn-CHMF). R.B. Alvarez,* V. Askanas, P. Alhissin,* W.K. Engel. USC Neuromuscular Center, Los Angeles, CA 90017.

In adult human muscle fibers cultured aneurally in monolayer, AChE stain is negative and AChRs are weakly and evenly distributed plasmalemmaly. In CHMFs innervated by fetal rat spinal cord, AChE and AChRs accumulate at newly-formed neuromuscular junctions (NMJs). Without stains for AChE or AChRs, NMJs on Inn-CHMFs are not easily identified by light-microscopy (LM). We have developed a new method that enables LM identification of NMJs on Inn-CHMFs by AChE reaction and subsequent EM studies of AChE and AChRs on the same section, as follows: 1) living cultures are incubated in ^{125}I-alpha-bungarotoxin; 2) after fixation, cultures are stained for AChE; 3) after short osmification and embedding, NMJs are identified by LM, marked and core-drilled out; 4) thin- sections containing AChE-stained NMJs are EM-photographed, then dipped in Ilford L-4 emulsion, and processed for EM-autoradiography (EM-AR); 5) since most of the AChE reaction is lost during EM-AR processing, easily visible grains representing AChRs can be EM-photographed on the same NMJs. This method enables comparative studies of AChE and AChR co-localization at various stages of NMJ formation in tissue-culture.

219.4

IMMUNOHISTOCHEMICAL LABELING OF OPTIC NERVE TISSUE PRINTS ON NITROCELLULOSE PAPER. L.L.Y. Chun* and B.A. Barres (SPON: A. Ames), Department of Neurology and Howard Hughes Medical Institute, Massachusetts General Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA.

Analysis of neural structure can be impeded by the high density of processes and the often poor access of antibodies to cell *surfaces* in fixed tissue sections. We have developed a simple immunohistochemical technique that allows excellent visualization of neural cell bodies, along with fine details of their connectivity.

Pieces of tissue (optic nerve or retina) were briefly incubated in a papain solution and gently blotted on wet nitrocellulose paper. The adherent tissue, which included cells and their processes, was then fixed with 85% methanol or 2% paraformaldehyde. A variety of immunohistochemical methods were used to visualize the antigenic determinants. Staining methods compatible with the technique included standard counterstains, indirect immunofluorescence and immunoenzymatic methods of higher sensitivity utilizing peroxidase/DAB/Ni, alkaline phosphatase (black substrate only), and intensification of DAB with silver. With the immunoenzymatic techniques the final result was visualized by dehydration of the tissue in graded t-butyl alcohol and clearing with xylene (Other solvents dissolved either DAB or the nitrocellulose.). The clearing process also made the nitrocellulose paper transparent for transillumination, and thus compatible with DIC optics. Related techniques have been reported by Seshi (1986) and Cassab and Varner (1987).

We have used this technique to analyze aspects of optic nerve structure. All of the optic nerve glial cell types identified by Raff *et al.* (1983) have been observed as well as the relationships of their processes to axons and to each other. We have also immunohistochemically labelled whole layers of retina obtained by the technique of Shiosaka *et al.* (1984). This technique may be directly applicable to other CNS regions.

219.5

SPECTROPHOTOMETRIC DETERMINATION OF FLUOROCHROME-TO-PROTEIN RATIOS IN IgG CONJUGATED WITH THE BLUE FLUOROCHROME AMINOMETHYLCOUMARIN ACETIC ACID (AMCA). S.Tallaksen-Greene, M.W. Wessendorf, and R. Elde. Department of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455

IgG conjugated with the blue fluorochrome 7-amino-4-methylcoumarin-3-acetic acid (AMCA) has been successfully employed for fluorescence immunocytochemistry. However, AMCA conjugates of IgG sometimes precipitate out of solution. This tendency seems to be related to amount of AMCA-N-hydroxysuccinimide used to conjugate the IgG, suggesting that over-conjugation of the IgG is responsible. To study this phenomenon a simple means of determining the ratio of fluorochrome to protein (f/p ratio) in AMCA conjugates would be useful.

Tryptophan residues in protein cause it to absorb light at 280 nm. Similarly, free AMCA absorbs strongly at 355 nm. Beer's law states that the absorbance of light by a solute in a solution equals its concentration, times the extinction coefficient (E) for the substance at the wavelength employed. The extinction coefficient at 280 nm and 355 nm for free AMCA in pH 7.2 PBS was measured spectrophotometrically at concentrations of 65 μM, 22 μM, and 6.5 μM. The mean value determined for the E_{280} was 3,271. The mean value for the E_{355} was 15,333. Previous reports indicate that the E_{280} for IgG is 210,800 (Handbook of Biochemistry), and that the E_{355} for IgG is negligible. These values suggest the following formula for the f/p ratio of AMCA conjugates of IgG:

$$f/p = 13.7 \times \frac{A_{355}}{A_{280} - (.213) \times A_{355}}$$

These studies were supported by NS22665 and DA02148.

219.6

m-PHENYLENEDIAMINE: A NOVEL FLUORESCENT NISSL-LIKE STAIN FOR NEUROANATOMY. B.Quinn & E. Weber. Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

Although fluorescent techniques are widely used in neuroanatomy, the high specificity of these methods in identifying isolated neurons may impair visualization of surrounding neuronal architecture. Several compounds with distinct spectra have been proposed as fluorescent counterstains (1); and Oriol & Mancilla-Jimenez (2) have reported that p-phenylenediamine, sometimes used as an anti-fading agent for fluorescein, could be used in an oxidized state as a brown nuclear counterstain. We report that an isomer of this compound, m-phenylene-diamine (m-PhD), can provide a Nissl-like counterstain with characteristics different from fluorescent counterstains previously described. 20 mg of m-PhD (Sigma) are dissolved in 900 ul of H_2O, 20 ul of HAc, and 100 ul of glycerol, and heated for 10 min at 50-60° C, developing a light tan color. 50 ul of this solution are applied to slide-mounted sections, rinsed, and sections mounted under Tris- or PO4-buffered glycerol, pH 8. Alternatively, the m-PhD solution is diluted 1:2 in buffered glycerol and used as a coverslipping medium. Either method produced yellow-green staining of neuronal perikarya well visualized with routine fluorescein filters. An excellent counterstain effect was obtained for retrograde tract tracing using 6% rhodamine B isothiocyanate (Sigma) or rhoadamine-labeled antibodies in immunohistochemistry. This m-PhD technique has also been utilized as an effective counterstain to aid intracellular labeling in an *in vitro* slice preparation (see Walker, Graybiel, Baughman, & Arbuthnott, this meeting.)

(1) Schmued, Swanson, & Sawchenko, J Histochem Cytochem 30:123 (1982). (2) Oriol & Mancilla-Jimenez, J Immunol Meth 62:185 (1983). Supported by NIMH Grant MH40303 to EW and a MacArthur Foundation predoctoral fellowship to BQ.

219.7

AB5 MONOCLONAL ANTIBODY- A SPECIFIC MARKER FOR 'PROJECTION NEURONS' IN THE CENTRAL NERVOUS SYSTEM. K.R. Fry and D.M.-K. Lam. CENTER FOR BIOTECHNOLOGY, BAYLOR COLLEGE OF MEDICINE, THE WOODLANDS, TX. 77381.

The AB5 monoclonal antibody (Fry et al, Brain Res. 338:360, 1985) has been determined to be a specific marker for retinal ganglion cells in several mammalian species. The present study was undertaken to determine whether the AB5 antibody also labels neurons in other regions of the central nervous system. An adult cat was anesthetized and perfused with 4% paraformaldehyde. Frozen sections of the brain and spinal cord were immunostained with the AB5 monoclonal antibody and examined at the light microscopic level. Several neuronal cell types were observed to be labelled. In the spinal cord, only the spinal motor neurons and some cells located in the intermediate gray matter were labelled. In the cerebellum, Purkinje cells and neurons of the deep cerebellar nuclei were labelled. The AB5 antibody labelled neurons in most of the major brainstem nuclei. Some AB5-labelled neurons were observed in the diencephalon and in the cerebral cortex, where pyramidal cells were labelled. In all cases, the neurons labelled by the AB5 antibody were neurons which project to other locations within the nervous system, ie. 'projection' neurons. The possible role that this antigen may play in long distance signal transduction remains to be determined. Supported by NIH grants EY06469 (KRF) and EY02608 (DMKL) and USPHS Grant No. RR-05425 (KRF).

219.8

FRAGMENT C OF TETANUS TOXIN IS A NON-TOXIC LABEL FOR LIVING NEURONS. E.A. Neale, J. Koh* and W.H. Habig*. Lab. Devel. Neurobiol., NICHD, NIH and Div. Bact. Prod., FDA, Bethesda, MD 20852

Tetanus toxin has been used with radioautography and immunohistochemistry as a neuron-specific marker. A complex of the binding portion of the toxin molecule, Fragment C, with a non-neutralizing monoclonal antibody specific for this fragment, labels the surface of mouse CNS neurons in culture more intensely than tetanus toxin and does not cause the electrophysiologic effects of intact toxin. The complex does not interfere with early morphologic development and can be detected on the cell surface with a fluorescent secondary antiserum. Intense, diffuse fluorescence is observed over the soma and processes of neurons labeled two hours after plating. Twenty-four hours after labeling, fluorescence is less bright and punctate, and redistributed out to the tips of newly extended neurites. Forty-eight hours after labeling, fluorescence no longer can be detected on the neuronal surface. Fixation and detergent treatment prior to secondary antibody reveal small amounts of internalized complex. Newly dissociated cells, labeled in suspension, show a similar redistribution of label over twenty-four hours. The complex persists longer on the surface of older neurons. The Fragment C / monoclonal antibody complex should prove useful as a neuronal probe that can be applied to living cultures.

219.9

RETROGRADE LABELING OF EMBRYONIC MAMMALIAN MOTONEURONS WITH A CARBOCYANINE DYE. E. F. Salazar-Grueso, and R. P. Roos*, Dept. of Neurology, Univ. of Chicago, Chicago, IL 60637.

Several laboratories have reported isolation and purification of avian and mammalian motoneurons by retrograde labeling with fluorescent conjugated lectins (FCL) and subsequent fluorescence activated cell sorting (FACS). Limitations of this approach include: rapid photobleaching of FCL, leakage of FCL and the relative transience of fluorescence in cultures of FCL-labelled cells. Carbocyanine dyes (CD) are hydrophobic fluorochromes that have none of these limitations, and fluorescence persists within labeled cells for weeks (Honig & Hume Soc. Neurosci. 11:98). We have been able to label mammalian embryonic motoneurons with the CD, 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO). Limbs of E12 mouse embryos were injected with DiO in ethanol. After 12-18 hours in a oxygenated balanced salt solution at room temperature, embryos were processed for cryostat sectioning. Fluorescent cells in the spinal cord were confined to the ventral horn. The identity of these cells has been determined to be motoneurons in other experiments employing lectin conjugated horseradish peroxidase as the chromophobe. Future studies of long-term cultures of FACS isolated DiO labeled motoneurons will make immunohistochemical, neurophysiologic, and developmental studies possible. (Supported by a grant from the MDA.)

219.10

IDENTIFIED CHOLINERGIC BASALOCORTICAL NEURONS IN TISSUE CULTURE. A.A. Khan and R.W. Baughman. Dept of Neurobiolgy, Harvard Medical School, Boston MA 021115.

The role of cholinergic input from the basal forebrain to cerebral cortex is difficult to study in vivo. Monolayer tissue culture provides a more favorable preparation for investigating the synaptic pharmacology of this system, but it is necessary first to establish a method to identify reliably the cholinergic basalocortical cells. We have achieved this by combining retrograde labelling with immunohistochemical staining for choline acetyltransferase (ChAT). The fluorescent dye 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (di-I) was injected into primary and secondary visual cortex of 6-7 day-old Long/Evans rat pups. After two days to allow for retrograde transport of the dye to the basalocortical cells, the basal forebrain was dissociated enzymatically (Huettner and Baughman '86) and plated in monolayer tissue culture on feeder layers of glia from the same area. In order to enhance survival of the cholinergic basal forebrain neurons, 7S nerve growth factor was added to the culture medium (Eagle's MEM with 5% rat serum). At times from a few days up to 72 days, large multipolar cells were easily recognizable in the cultures. The majority of these cells were ChAT-positive, indicating that they were cholinergic. Many di-I labelled cells also were present in the cultures. Of the large (> 20 um) di-I labelled cells, over 80% were ChAT-positive. The majority of the ChAT-negative, di-I labelled cells were smaller in size (< 20 um). These results indicate that retrograde labelling from cortex with di-I provides a reliable marker for cholinergic basalocortical cells in tissue culture. Supported by NIH EY03502 and ADRDA II-86-099.

219.11

GREEN FLUORESCENT LATEX MICROSPHERES: A NEW RETROGRADE TRACER. L.C. Katz and D.M. Iarovici*. (Lab. of Neurobiology, The Rockefeller University, NY, NY and Yale Univ. School of Med., New Haven, CT).

Fluorescent latex microspheres ("beads") (Katz et al. Nature 310:498,1984) have many advantages over other fluorescent retrograde tracers, including minimal diffusion from injection sites, low cytotoxicity, resistance to fading, and compatibility with all other retrograde tracers and with immunocytochemistry. The currently available "red" beads fluoresce under rhodamine excitation. We have now developed "green beads," which are invisible under rhodamine excitation, but fluoresce a bright green under fluorescein illumination. Except for the fluorochrome, the behavior of the two colors is identical. Green bead injections into rat or cat cortex label cells in local, cortico-cortical, and cortico-thalamic pathways with the distinctive granular cytoplasmic fluorescence characteristic of beads. In double-labelling experiments, small injections (<50 nl) of green and red beads separated by less than 500 μm were completely distinct. Double labelled cells were easily distinguished, since the cytoplasmic granules containing the beads fluoresced different colors. Even weak double labelling is detectable, as only a few grains of each label can easily be distinguished within a labelled cell. This is a clear advantage over other commonly employed double labels, with which weak labelling is difficult to distinguish from other fluorochromes or from autofluorescence. When a mixture of red and green beads was injected, the labels were co-localized . Thus cells do not differentiate between the two labels. However, the green beads seem to label cells somewhat less intensely than the red ones. Under the electron microscope, the retrogradely transportable fraction of both green and red beads consists mainly of 30-60 nm diameter particles. The properties of green and red beads should be extremely useful in understanding local circuits, in situations in which multiple tracers are to be combined with immunocytochemistry, and in developmental studies where long term label retention is important. Supported by the L.P. Markey Charitable Trust (to L.C.K.).

219.12

SELECTIVE UPTAKE OF LATEX NANOSPHERES CONTAINING ACTIVE CHROMOPHORES INTO SUBPOPULATIONS OF CULTURED RAT NEOCORTICAL NEURONS. **J.D.Macklis and R.D.Madison. Departments of Neurology and Neuropathology, Harvard Medical School, and Neuroscience, Children's Hospital, Boston, MA 02115**

A novel latex nanosphere delivery system (LNDS) is developed for selective delivery of fluorescent dyes, photoactive agents, and drugs to subpopulations of neurons. Chromophores are incorporated within latex nanospheres of defined size (100-300 nm diameter) and surface properties (charge, protein conjugate). These nanospheres are nontoxic, become concentrated within cell bodies indefinitely, appear to withstand processing for electron microscopy, and allow double or triple labeling studies with ultrastructural analysis on the same cells.

We screened several LNDS samples for uptake into cultured rat neocortical neurons. Neurons were cultured by standard methods and exposed to LNDS samples with various surface properties and proteins. We compared uptake results with those for cultures exposed to commercially available Rhodamine latex microspheres. Uptake of commercially available microspheres is concentration dependent, but nonspecific; neurons, glia, and fibroblasts all accumulate these beads avidly. Some samples of the LNDS undergo low levels of nonspecific uptake that is concentration dependent. Other LNDS samples exhibit selective uptake into neuronal subpopulations, dependant on the surface properties and proteins. These preliminary studies do not attempt to define target subpopulations for the different LNDS samples. We will use such culture studies to guide our future transport studies in vivo. **Supported by a grant from the Whitaker Foundation.**

219.13

RETROGRADE LABELING OF HYPOTHALAMIC PROJECTIONS TO NUCLEUS TRACTUS SOLITARIUS IN THE RAT USING RHODAMINE-LABELED LATEX MICROSPHERES. R. A. Cava*, D. Melancon*, J. A. Weyhenmeyer and D. C. Kuo. College of Medicine, Univ. of Ill., Urbana, IL 61801.

The paraventricular nucleus (PVN) of the hypothalamus has been shown to send projections to the nucleus tractus solitarius (NTS) and vagal motor nucleus (DMX). These descending projections to brainstem autonomic regions have been implicated in the regulation of cardiovascular activity. In the present study we retrogradely labeled hypothalamic neurons by microinjecting (1.5-3.0 μl) rhodamine-coated microspheres (0.02-0.2 μm) into the dorsomedial medulla at the level of the obex. Medial injections resulted in a large number of labeled cells that were clustered in the PVN and scattered throughout the lateral hypothalamus. Injections placed more laterally resulted in a significant decrease in the number of labeled neurons. Adjusted cell counts indicated a larger projection than previously reported using other fluorescent markers. Staining was bilateral with a slight ipsilateral predominance. Further, staining was often visible in dendritic arbors. These findings suggest that rhodamine-coated latex microspheres are superior to other fluorescent dyes for retrograde labeling due to their stronger intensity and resistance to fading. Supported by grants from NSF and AHA-IL.

219.14

SELECTIVE SUPPRESSION OF ENDOGENOUS PEROXIDASE ACTIVITY: APPLICATION FOR ENHANCING APPEARANCE OF HRP-LABELED NEURONS *IN VITRO*. C.B. Metz*, S.P. Schneider and R.E.W. Fyffe* (SPON: E.R. Perl). Department of Physiology, Univ. of N. Carolina, Chapel Hill, NC 27514.

The use of HRP for staining neurons in brain slices is complicated by peroxidase activity in red blood cells (rbc's) distributed throughout the tissue. We have developed a simple procedure that suppresses selectively the peroxidase activity of rbc's in aldehyde fixed sections of the mammalian CNS while enhancing the staining density and uniformity of HRP-injected neurons. These effects are accomplished by washing the sections in ethanol solutions prior to histochemical processing. The procedure is compatible with standard methods for demonstrating HRP; with diaminobenzidine (DAB) as chromogen our protocol is as follows:

1) Incubate sections in a series of 50%, 70%, 50% ethanol solutions for 10, 15 and 10 min, respectively.

2) Wash sections in phosphate buffer for 10 min. Incubate for 30 min in a solution containing 25 mg nickel ammonium sulphate, 20 mg DAB and 7μl of 30% hydrogen peroxide in 100 ml of TRIS-HCl buffer (0.05 M) at pH 7.6.

3) Mount sections, counterstain and coverslip.

Supported by grants NS 25547, NS 14899 and NS 10321 from the NINCDS.

219.15

ENHANCEMENT OF HORSERADISH PEROXIDASE UPTAKE AND/OR TRANSPORT AND ENZYME ACTIVITY BY A RADIOCONTRAST MEDIUM. C.N.R. Henderson and S. Saporta. Dept. of Anat., Coll. of Med., Univ. of South Florida, Tampa, FL 33612

Bilateral, symmetrical injections (0.016 μl each) were made in the somatosensory cortex of five rats. The left cortex received 2% WGA-HRP in deionized water (DI) and the right received 2% WGA-HRP in MD 40 (a 1:0.9 dilution of MD-76 with DI). Twenty-four hours later, each animal was deeply anesthetized and perfused transcardially with saline followed immediately by cold 1% paraformaldehyde-2% glutaraldehyde in 0.1 F phosphate buffer (pH 7.4). Frozen 40 μm coronal sections through the diencephalon were reacted with tetramethyl benzidine (TMB) to demonstrate the presence of HRP.

The amount of anterograde label was consistently greater in the ventrobasal nucleus ipsilateral to the side of the WGA-HRP/MD40 injection, though there was not a clear enhancement of retrograde label. The relative enzymatic activity of the two solutions, as measured by spectrophotometric assay (Worthington Manual, 1972), revealed greater activity in the WGA-HRP/MD40 solution. Control experiments have indicated that the increased amount of anterograde label is probably due to increased uptake and/or transport of WGA-HRP rather than simply an enhancement of HRP enzymatic activity.
Supported by BRSG SO7 RR05749

219.16

TRANSNEURONAL TRANSPORT OF HERPES SIMPLEX VIRUS IN THE RAT BRAIN. J.H. McLean, M.T. Shipley and D.I. Bernstein*. Dept. of Anatomy and Cell Biol., Univ. of Cincinnati Coll. of Med., Cinti., OH., 45267-0521 and The Gamble Inst., Cinti., OH.

At last year's meeting, we presented evidence that herpes simplex virus type 1 (HSV1) is transported transneuronally in a retrograde direction in the rat olfactory system. Here, we examined the generality of transneuronal label by HSV1 in several additional neural systems. Anaesthetized rats were injected with 50-200nl of 10^5 to 10^9 PFU/ml of HSV1 (McIntyre strain) into the visual cortex, septum, somatosensory cortex or the olfactory bulb. Sites of HSV1 incorporation in the brain were observed by immunocytochemistry.

In all systems studied, multineuronal connections were demonstrated and the number of synapses crossed by the virus was determined, based on known circuitry of the involved systems. For example, in the visual system the virus crossed 2-3 synapses to reach bipolar cells in the retina. In all systems studied the transport was mainly retrograde but, occasionally, label could be attributed to anterograde transport of the virus. Generally, with short survival periods of 2-3 days postinoculation, only neurons incorporated the virus. With longer survival both neurons and glia became infected. Preliminary results also indicate that, at least for serotonergic neurons, the neurotransmitter can also be identified immunocytochemically in infected cells. Because the virus is amplified at each level of synaptic transfer, the strength of the label does not diminish even in neurons located many synapses removed from the injection site and in many neurons the label resembles Golgi impregnation. Supported by NINCDS PO1 23348-01 & AHAF-ADR.

219.17

ANTI-RICIN ANTIBODY PROTECTS FROM SYSTEMIC TOXICITY WITHOUT AFFECTING SUICIDE TRANSPORT. R.G. Wiley and T.N. Oeltmann*. Lab of Experimental Neurology, VAMC, Nashville, TN 37212.

Subepineural injection of the toxic lectin, ricin, is a useful way to selectively destroy neurons projecting through the injected nerve. However, the ricin dose necessary to reliably destroy all neurons projecting through a large, heavily myelinated nerve often causes systemic toxicity and/or death. Systemically administered lactose or anti-ricin antibodies can protect against systemic ricin poisoning. In the present study, we sought to determine if commercially available anti-ricin antibodies given at the time of subepineural ricin injection could protect rats from systemic ricin toxicity without interfering with the suicide transport action of ricin. 24 anesthetized, adult, male Sprague-Dawley or Long Evans hooded rats received subepineural pressure microinjections of ricin into one sciatic nerve (2.7-13.5 ug) or one vagus nerve (0.05-4 ug). Half of the rats received 0.1-0.25 mg of anti-ricin antibody (Vector Labs) subcutaneously within 5 mins of the ricin injection. Animals were observed daily and long term survivors were subsequently reanesthetized, perfused transcardially with aldehyde fixative and examined histologically for evidence of destruction of neurons projecting through the injected nerves. All rats with sciatic nerve injections died unless given antibody. Antibody protected 3 of 4 rats given 6.5 ug and 2 of 2 given 2.7 ug of ricin into the sciatic nerve. In all sciatic experiments, there was extensive destruction of neurons in the ipsilateral L5 and L6 dorsal root ganglia and in the ipsilateral ventral horn of the spinal cord. Antibody did not protect rats injected with 4 ug of ricin into the vagus but this may have been due to the excessively large brainstem lesion in these animals. 4 rats were injected with 0.05 ug of ricin (2 also received antibody) into one vagus nerve to assess the effect of antibody on suicide transport of less than maximally effective ricin doses. In all cases, the subtotal vagal lesions were similar whether or not antibody was given concurrently. These results are interpreted as showing that anti-ricin antibody can protect animals against systemic ricin toxicity without altering the suicide transport activity of the toxin. This strategy should facilitate experiments using ricin to ablate large nerves. (This work supported by the Veterans Administration.)

219.18

THE USE OF PHA-L FOR TRACING CONNECTIONS IN THE IMMATURE RAT BRAIN. J. Weber * and D. G. Amaral. The Salk Institute, P. O. Box 85800, San Diego, CA. 92138.

We have attempted to use the lectin anterograde tracer phaseolus vulgaris leucoagglutinin (PHA-L) to study the development of connections in the rat hippocampal formation. A variety of parameters were varied to establish an optimal injection procedure and most of the studies were conducted in animals approximately 24 hours of age. In all cases, a 2.5% solution of PHA-L in phosphate buffered saline (pH 7.2) was injected either by iontophoresis (2-9 uamp, positive current, 7 sec. duty cycle) or by air pressure (5-20 nl) through glass micropipettes; survival times ranged from 6 hours to 3 days.

The associational and commissural connections of the dentate gyrus were clearly labeled in the one-day-old animals as were hippocampal subcortical projections to the septal and mamillary nuclei. As in the adult, selective and intense anterograde labeling of projections was only achieved with iontophoretic injections and currents of approximately 7 uamp proved most successful. In several cases, apparent growth cones could be visualized at the tips of labeled axons. Transport also occurred with the pressure injections though labeled fibers had a fragmented appearance and there was substantial retrograde labeling. Labeling of the contralateral hippocampal formation was achieved with as little as 6 hours survival. While PHA-L is potentially a valuable tracer for developmental studies, our rate of successful injections (≈ 30%) was far lower than in mature animals.

219.19

REGIONAL VARIATION IN PROTEIN LEVELS IN RAT BRAIN TISSUE SECTIONS . J.A. MILLER, Merrell Dow Research Institute, Cincinnati, OH, 45215.

Using computerized densitometry the variation in protein levels in rat brain sections (10 um) was examined. The technique uses the dye Coomassie Blue (2.5% w/v in 20 mM HEPES buffer, pH 7.4) to stain protein. The neocortical layers were heterogeneous in their protein concentrations (Table 1). Other brain areas, such as thalamus, hippocampus and cerebellum also showed a distribution of protein concentrations. The highest levels of protein were those seen in layers 2 through 5, corresponding to the regions which contain the greatest density of dendrites. Conversely, layers rich in axonal fibers, layers 1 and 6, are relatively deficient in protein. These variations in tissue protein levels should be considered in receptor autoradiography when measuring receptor density.

Table 1. Protein in rat neocortical sections (n = 3).
Protein concentration (ng protein/mm2) ± SEM

Cortical Layer	Region of Neocortex: Frontal	Frontoparietal	Striate
1	142 ± 9	148 ± 23	139 ± 13
2	381 ± 92	428 ± 99	349 ± 38
3	300 ± 25	282 ± 58	346 ± 50
4	295 ± 33	275 ± 40	311 ± 41
5	246 ± 38	199 ± 16	276 ± 52
6	193 ± 37	143 ± 18	230 ± 37

219.20

HIGH RESOLUTION SCANNING AND ANALYSIS FROM MICROSCOPE SLIDES. J.S. McCasland and T.A. Woolsey (SPON: S. Highstein). Division of Experimental Neurology & Neurosurgery and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

We have developed an automated analysis system for generating high-resolution maps from tissue sections on slides. The method can be used with various combinations of emulsion autoradiography, immunohistochemical stains and other specific markers. Data are collected directly from microscope slides by means of a computer-controlled microscope, image processor, and an integrated software package (J. Voyvodic, Soc. Neurosci. Abstr., 12:390, 1986). Macro image processing functions tailored to specific applications can be user developed and written. The data are reconstructed as a 2-dimensional matrix for analysis by RS/1® software. The software generates density "maps" of grains, stains, etc. (for laser printer or color output) and allows statistical analysis of features within defined anatomical contexts. These maps can be combined or compared for features in the same section and different sections. The method is extremely flexible, and new image processing strategies can be developed and incorporated as needed to extract information of interest. (Supported by NIH 2 P01 NS17763 and McDonnell Center for Studies of Higher Brain Function.)

STAINING AND TRACING TECHNIQUES II

220.1

THREE DIMENSIONAL STRUCTURE OF SELECTIVELY STAINED NEURONS: CONFOCAL LIGHT AND HIGH-VOLTAGE ELECTRON MICROSCOPY. J.N. Turner, D.N. Collins, D.O. Carpenter, J.W. Swann K. Smith, M. Siemens, and D. Szarowski (SPON: M. Evans) Wadsworth Center for Laboratories and Research, New York State Dept. of Health, Albany, NY 12201

Three dimensional (3-D) morphology is critical for correlating structure and function in the CNS. We have developed methods to apply scanning laser confocal light microscopy (SLCM) and high-voltage electron microscopy (HVEM) to 3-D imaging of individual cells and cell components deep (several hundred microns) within thick slices. Unlike conventional light microscopy, SLCM images are not degraded by other labeled structures or unlabeled tissue. HVEM images samples microns thick at high resolution. Our correlation of these images directly compares microanatomy and ultrastructure. Hippocampus and pyriform cortex were imaged using flourescent dyes and gold toned Golgi samples. Cells studied physiologically via intracellular or field microelectrodes were selectively stained for SLCM with lucifer yellow or hydroethidine. The former was used for 3-D imaging of neurons. Photooxidation and osmium provide a correlative stain for HVEM. Hydroethidine was useful for microanatomy and nuclear morphology. SLCM imaging methods were developed for gold toned Golgi samples and correlated to HVEM and conventional electron microscopy. Support: PHS grants RR01219 and RR02984 DRR; NS13809.

220.2

SHAPE CHARACTERISTICS OF NEURONS

W.B.MARKS, T.G.SMITH, G.D.LANGE, W.H.SHERIFF* AND E.A.NEALE
Labs of Neural Control and Neurophysiology and Instrumentation and Computer Section, NINCDS, and Lab. of Developmental Biology, NICHD, NIH, Bethesda, MD 20892.

Mandelbrot (The Fractal Geometry of Nature, W.H. Freeman & Co, NY, 1982) has pointed out that the length L of the borders of natural objects often increases as the length E of the measuring tool decreases, and that the form of the relationship L(E) can be used to characterize the surface or the shape of the object. We have implemented several methods for measuring the outlines of two dimensional objects, using a computer controlled image processor. We have applied these methods to constructed images whose border length satisfies $L(E) = \lambda E^S$, where $-1 < S < 0$ ("fractal" objects), and to the outlines of stained cultured neurons. The methods differ in their sensitivity to branching, their speed, and their generalization to the measurement of surfaces, but they are all forms of smoothing followed by measuring. We give examples of the use of these methods to characterize form, including the measurement of the "fractal dimension" $D = 1 - S$. For objects such as our computer constructed figures, made by processes that act similarly at a range of length scales, the log-log plot of L vs E is straight for several octaves of change in E. For neurons, which are presumably not "self similar" in this way, the plots were also surprisingly straight, so that the roughness or complexity of the shape of neurons can often be characterized by the number D.

220.3

N-DIMENSIONAL CLUSTERS OF NEURONAL SOMATA: A STATISTICAL ANALYSIS. R.D. Rose and R.C. Grimson*. VA Med. Ctr. and Dept. Pharmacol., U. Pitt. Med. Sch., Pgh PA 15240 and Depts. Neurobiol. & Behavior and Community & Preventive Med., SUNY, Stony Brook NY 11794

Neuronal somata often appear physically clustered in histological sections. But are such clusters real or only apparent? We describe a simple and versatile test based on the number of associations between neurons in apparent clusters (those clusters one expects to see due to chance) and the actual number of associations one observes in the microscope. Comparison of these numbers using Chebychev's inequality results in highly conservative answer.

Very briefly, if one considers potential clustering within a labeled subset of cells that is scattered (in N dimensions) among non-labeled cells, the number of expected associations between labeled cells due to chance alone is $\approx yn^2/2x$; where y is the average number of associations per cell, n is the number of labeled cells, and x is the total number of cells. The number of observed associations is compared to this expected value.

Typically, in 1D $y \leq 2$, in 2D $y \leq 6$, in 3D $y \leq 12$, etc. In tissue, n and x can be determined accurately directly (2D) or using recursive translation (3D, Rose and Rohrlich JCN 1988). Furthermore, since our analysis is not dimension limited, higher order associations can also be examined.

Supported by NIMH MH08323 (RDR), NIH NS14899 & NS16996 (to L. Mendell), and the Veteran's Administration.

220.4

THE M.I.T. "NEUROTRACE" SYSTEM FOR MICROCOMPUTER-AIDED MICROSCOPY. A.Passera*, S.Fulks*, G.E.Schneider, S.Ayres*, S.Jhaveri* and R.S.Erzurumlu. Dept. Brain & Cognitive Sciences, M.I.T., Cambridge, MA, 02139.(Spon.: V.Domesick)

The Neurotrace System has been developed at MIT for use with color graphics-equipped models of the IBM PC, AT, model 80 (and compatibles) and also for the NEC APC3 microcomputer. The graphics and math capabilities of these machines are used by programs for 3-D reconstruction and quantitative analysis of anatomical material. Memory requirements are 640K or more of RAM.

Two modules are currently in use: "AXON" can be used for single- or serial-section 3-D reconstructions of individual axons or cells from Golgi-impregnated tissue or HRP, PHA-L (or equivalent) labelled material. "CELLS" allows a reconstruction, in 2 or 3 dimensions, of the size and/or spatial distribution of cells, axon terminals or other structures. Both software packages have been optimized for ease of data entry, and of viewing and plotting the resulting pictures, by computer-naive users. Plotter files can be used by a customized CAD system for combining drawings and for adding lettering, for creation of publishable figures.

The creative use of various symbols and colors allows representation of various classes of axons, axon branches, cells or terminals in the same drawing. Results can be viewed and plotted at any magnification and orientation. Illustrative examples from our work on the visual system will be presented: optic tract axons traced from ventral thalamus to midbrain, trajectories of groups of retinal axons traced from the embryonic eye through the entire optic nerve, spatial distributions and sizes of retinal ganglion cells backfilled from specific brainstem structures, 3-D reconstructions of the distribution of axon terminal types in the rodent LGBd with accompanying bouton size histograms. [Supported by the Whitaker Foundation, and the InterActions Co., Cambridge, MA]

220.5

NEUROLUCIDA: A PC BASED 3-DIMENSIONAL IMAGING SYSTEM FOR VISUAL AND VIDEO NEURON TRACING. J.R. Glaser and E.M. Glaser. University of Vermont College of Medicine, Burlington, VT 05405.

Image combining microscopy is a method for 3-d tracing of neurons. It uses computer graphic displays that are superimposed by camera lucida on optical images. Computer controlled stage movement in 3 dimensions allows the analysis of large regions of tissue with one half micron spatial precision for position and distance. We have refined this method into a more powerful PC-based version that also works with video images. The new version is based upon Neurolucida (copyright), DOS compatible 'C' software that runs on XT, AT and PS2 type PC computers. While the system works best with a 3-axis computer controlled stage, operation with a manually controlled stage that monitors 3-d position has also been demonstrated. Neurolucida utilizes EGA, VGA, or 'standard' (512x480 pixels) graphics. In the video mode the overlay graphics and the microscopic image are video combined. This permits full use of the tracing features of the original optical system. A frame grabber (Matrox) permits working with stored sequences of dynamic images as well as weakly fluorescent images. An image editor permits immediate correction of an erroneous graphics image by comparing it with the original image. Neurolucida can generate dynamic rotations of stored stick figure images about any selected axis.

220.6

THE USE OF LASER ACTIVATED MICROPROBE MASS ANALYSIS (LAMMA) FOR STUDY OF TRACE METALS IN RAT BRAIN. P. Szerdahelyi*, W. Markesbery*, W.D. Ehmann* and E.J. Kasarskis, Depts. Neurol., Chem., and Path. VA Hosp and Univ. Kentucky, Lexington, KY 40536.

Trace metals, especially zinc and copper, are essential for development of the CNS and the maintenance of synaptic and metabolic processes. A wide variety of analytical and histochemical methods has been developed to anatomically localize and determine the levels of these metals in brain regions. However these methods typically detect only a single element and are plagued by low sensitivity and/or spatial resolution.

The LAMMA technique resolves metal ions in a time-of-flight mass spectrometer which are produced by ionization of a microscopic region of an individual cell. Samples of brain were embedded in Epon and the 0.3 µm thick sections were stained with toluidine blue before analysis with the LAMMA-500 instrument (Leybold, Köln). The high detection efficiency (typically 10^{-18}-10^{-20}g), multielement capability, and high spatial resolution of this technique allowed a semiquantitative analysis of zinc bound to the hippocampal mossy fiber zone (at m/z=64 and 66). Similarly, copper was localized to the Bergman glial cells of the cerebellum (m/z=63 and 65).

220.7

RAPID FIXATION OF IN VITRO BRAIN SLICES USING MICROWAVE IRRADIATION. F.E. Jensen and K.M. Harris, Dept. Neurosci., Children's Hospital, Boston MA 02115.

We have evaluated the use of microwave (MW) irradiation to enhance immersion fixation of rat hippocampal slices. This method produces perfusion quality fixation within seconds of immersion. 400u thick slices were prepared from adult male rats and maintained in vitro for 90 min. Slices were then transferred on nets into mixed aldehydes within a MW oven (700W,2450MHz), irradiated, and then washed immediately in buffer and processed for LM and EM. In order to determine optimal conditions, parameters varied included MW time (7-30sec) producing tissue temperatures of 34-92C, and concentration of glutaraldehyde in the fixative (2.5%,4%, or 6%: paraformaldehyde 2%). These MW fixed slices were compared to within animal control slices immersed in the same concentration of aldehydes for 11 sec to 16 hours. Electron micrographs were taken of area CA1 pyramidal cell bodies and dendrites at 100-125u depth from the slice surface and compared between MW,control slices,and perfusion fixed material. Slices irradiated for 11sec (40-50C) while immersed in fixative containing 6% glutaraldehyde yielded fixation quality comparable to perfusion. Post fixation in aldehydes after MW did not enhance quality. MW fixation to higher temperatures caused disruption of dendritic microtubules. Concentrations of 2.5% and 4% glutaraldehyde resulted in inferior quality at all temperatures of MW fixation. Control slices immersed without MW irradiation revealed markedly inferior fixation. MW enhanced fixation markedly reduces the occurence of dentritic and mitochondrial swelling and other necrotic changes which occur with conventional immersion fixation and should provide more accurate ultrastructural data related to dynamic electrophysiological properties recorded in slice tissue.

221.1

[³H]-BMY 7378: A SELECTIVE, HIGH AFFINITY 5-HT$_{1A}$ LIGAND IN RAT HIPPOCAMPAL AND CORTICAL MEMBRANES. F.D. Yocca[1], L. Oneto[1] and S. Maayani.[2] CNS Research, Bristol-Myers Co., Box 5100, Wallingford, CT, 06492 and Depts. of Anesthesiology and Pharmacology, Mt. Sinai School of Medicine, New York, NY, 10029.

The 5-HT$_{1A}$ receptor and its related binding sites are distributed nonuniformly throughout mammalian brain. Additionally, 5-HT-sensitive [³H]-8-OH-DPAT binding sites are homogenous in hippocampus yet heterogenous in in cortex, as evidenced in membranes from rat brain with respect to the K_d and IC_{50} values of several drugs. Previously, we reported that BMY 7378 demonstrated high affinity, selectivity and low efficacy at 5-HT$_{1A}$ receptors linked negatively to adenyl cyclase (Yocca et al., 1987). We have tritiated BMY 7378 and used it to label apparent homogenous populations of [³H]-5-HT and [³H]-8-OH-DPAT sensitive sites in cortical and hippocampal membranes. The pharmacology of the [³H]-8-OH-DPAT and [³H]-BMY-7378 sites in hippocampus was indistinguishable with respect to IC_{50} values of 5-HT$_{1A}$ agonist and antagonists, GppNHp sensitivity and with respect to the inhibition of forskolin-stimulated adenyl cyclase activity. Similarly, the pharmacology of the 5-HT-sensitive [³H]-BMY 7378 sites in cortical and hippocampal membranes in rat was indistinguishable. These data suggest that [³H]-BMY 7378 selectively labels 5-HT$_{1A}$ sites in brain regions other than hippocampus. (Supported in part by USPH GM 34852).

221.2

EVIDENCE FOR THE EXISTENCE OF PUTATIVE 5-HT$_{1D}$ RECEPTOR BINDING SITES IN HUMAN BRAIN. J. Martial*, M. Dalpé*, C. deMontigny and R. Quirion. (Spon: B. Suranyi-Cadotte) Douglas Hosp. Res. Ctr. and McGill Univ., Montreal, Quebec, Canada H4H 1R3

Multiple serotonin (5-HT) receptor sub-types are present in mammalian brain tissue. In human brain, the existence of 5-HT$_{1A}$ and 5-HT$_{1C}$ receptor sites have been clearly demonstrated while 5-HT$_{1B}$ sites are most likely absent. Very little information is currently available on the presence of 5-HT$_{1D}$ binding sites in human brain. Brain membranes were prepared using control postmortem human brain cortical tissues. Membrane binding assays were usually performed at 25°C for 30 min in 50 mM Tris.HCl containing 10µ M pargyline, 4 mM $CaCl_2$, 0.1% ascorbic acid and various concentrations of [³H]5-HT in presence of saturating concentrations of 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ blockers to inhibit binding of the ligand to those sites. Under these conditions, [³H]5-HT apparently binds to a remaining single class of high affinity sites in human brain cortex. The ligand selectivity pattern demonstrates that 5-HT > mianserin > 8-OH-DPAT > mesulergine = propranolol in competing for [³H]5-HT sites. This is similar to the ligand selectivity pattern reported by Heuring and Peroutka (J. Neurosci. 7, 894-903, 1987) for the putative 5-HT$_{1D}$ sites present in bovine brain. Thus, it would appear that a similar sub-type of 5-HT$_1$ sites is also found in human brain cortex.

221.3

EFFECT OF GEPIRONE ON RAT CORTICAL AND HIPPOCAMPAL SEROTONIN SYNTHESIS. J. Torrente,* E. Ryan,* and F.D. Yocca. (Spon: M.S. Eison).CNS Research, Bristol-Myers Company, Wallingford, CT, 06492-7660.

Gepirone (G) is a novel anxiolytic/antidepressant candidate which demonstrates selective agonist activity at central 5-HT$_{1A}$ receptors.Evidence for this includes: 1) G displaces [³H]8-OH-DPAT from 5-HT-sensitive binding sites in rat and guinea pig hippocampal homogenates (Yocca et al., 1986); 2) G elicits a partial agonist action on 5-HT$_{1A}$ receptor coupled adenylate cyclase in similar preparations (Yocca and Maayani, 1985; Yocca et al., 1986); 3) G induces the 5-HT behavioral syndrome in rats (Eison et al., 1986); and 4) G inhibits the firing of serotonin containing dorsal raphe neurons after systemic administration (Gehlbach and VanderMaelen, 1985). Acute administration of gepirone (either s.c. or p.o.) decreases serotonin synthesis in both cortex and hippocampus as measured by accumulation of L-5HTP after administration of the decarboxylase inhibitor NSD-1015. Furthermore, at a low dose (1 mg/kg, s.c.; 5 mg/kg,p.o.) gepirone selectively inhibits 5-HT synthesis in rat cortex. This effect is probably not mediated by synthesis controlling terminal 5-HT$_{1B}$ receptors (Galloway et al., 1987) since gepirone lacks affinity for 5-HT$_{1B}$ binding sites, and may be due to a preferential action of G at 5-HT$_{1A}$ somatic auto receptors on 5-HT neurons projecting to the cortex.

221.4

ADENOSINE A_1 AND 5-HT$_{1A}$ RECEPTORS SHARE A COMMON PERTUSSIS TOXIN (PT)-SENSITIVE, G_i-LIKE PROTEIN IN RAT HIPPOCAMPUS: J.M. Zgombick[1], S.G. Beck[1], and S. Maayani[1,2] Depts. of Pharmacology[1] and Anesthesiology[2], Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Adenosine A_1 and 5-HT$_{1A}$ receptors in rat hippocampal preparations share common features including inhibition of forskolin-stimulated adenylate cyclase (FSAC), hyperpolarizations, and high density of their related binding sites. Biochemical and electrophysiological studies were initiated to test the possible sharing of effector mechanisms. Maximal inhibition of FSAC produced by PIA (40%) was greater than 5-HT (30%), but both agonists (1 uM) stimulated [^{35}S]-GTPγS binding approximately 15% above basal. PIA (10 uM) and 5-HT (15 uM) elicted hyperpolarizing responses (7-8 mV) within the same cell which were mediated through enhanced K^+ conductance. The combination of maximal 5-HT and PIA concentrations did not potentiate either the biochemical or electrophysiological responses from that observed with PIA alone. Central administration of PT produced similar reductions in 5-HT- and PIA-mediated inhibition of FSAC and hyperpolarizations. We propose that adenosine A_1 and 5-HT$_{1A}$ receptors may coexist on the same hippocampal pyramidal cells and share a common PT-sensitive, G_i-like transducing protein. [Supported by USPH Grants GM 34852, MH 41917 and Training Grant DA-07135].

221.5

EFFECTS OF INTRANIGRAL SEROTONIN AGONISTS ON STRIATAL DOPAMINE METABOLISM. **S.L. Rubinstein* and K. Gale (SPON: R. McGee). Dept. of Pharmacol. Georgetown Univ. Med. Ctr., Washington, DC 20007.**

Intranigral microinfusion of either serotonin (5-HT, 45 nmoles) or the 5-HT$_{1B}$ selective agonist 1-(m-trifluoromethylphenyl)-piperazine (TFMPP, 10 nmoles) caused an elevation in dopamine (DA) metabolism (30% increase in DOPAC and HVA) in the homolateral striatum of awake, unrestrained rats sacrificed 60 min after microinfusion (Soc. Neurosci. Abstr. 12; 872, 1986). We present here the effects on DA metabolism of the 5-HT$_{1A}$ selective agonist 8-hydroxy-dipropylaminotetralin HBr (8-OH-DPAT) and the 5-HT uptake inhibitor fluoxetine HCl. 8-OH-DPAT (10 nmoles) microinfused into one substantia nigra (SN) did not significantly alter striatal levels of DOPAC, HVA or DA in rats sacrificed 60 min after microinfusion. This suggests that nigrostriatal DA neurons are not influenced by 5-HT$_{1A}$ receptor stimulation in the SN. Fluoxetine HCl (15 nmoles) microinfused into one SN significantly elevated homolateral striatal DOPAC and HVA to 40% above the corresponding values in control rats receiving a saline microinfusion in the SN. This effect of fluoxetine was not reduced by prior depletion of 5-HT via pretreatment with p-chlorophenylalanine (a tryptophan hydroxylase inhibitor), suggesting that the effect of fluoxetine is not dependent upon presynaptic stores of 5-HT in SN.
Supported by ADAMHA grants MH32359, DA 02206, NRSA (to SLR) MH09477 and RSDA (to KG) 00497.

221.6

A NON-5-HT$_{1A}$, 5-HT$_1$-"LIKE" RECEPTOR MEDIATES INHIBITION OF ADENYLYL CYCLASE IN RAT SUBSTANTIA NIGRA AND NUCLEUS ACCUMBENS. C.D. Mahle, J.M. Zgombick, F.D. Yocca, and S. Maayani. Depts. Anesthesiology and Pharmacology, Mt. Sinai School of Medicine, CUNY, NY, NY 10029 and CNS Research, Bristol Meyers Co., Wallingford, CT 06492.

At least five distinct subtypes of functional 5-HT receptors have been suggested. The spiperone and BMY 7378 sensitive 5-HT$_{1A}$ receptor is negatively linked to adenylyl cyclase (AC). The 5-HT$_{1A}$ and a second 5-HT receptor that are negatively linked to AC were examined in membrane preparations from rat hippocampus, substantia nigra (SN), and nucleus accumbens (NA) using 5-HT, 5-carboxyamidotryptamine (5-CT), spiperone and (+/-) pindolol. 5-CT was a more potent agonist than 5-HT in eliciting the inhibition of AC linked to both the 5-HT$_{1A}$ and the non-5-HT$_{1A}$ receptor.

	E_{max} (%)	EC_{50} (nM):	5-CT	5HT
HIPPOCAMPUS	30		5	50
SUBSTANTIA NIGRA	20		10	50
NUCLEUS ACCUMBENS	10		15	50

While spiperone and pindolol antagonized the response to 5-HT and 5-CT in the hippocampus, (5-HT$_{1A}$ receptor), in SN and NA the 5-HT and 5-CT effect was not antagonized. Futhermore, the forskolin stimulation of AC activity in these regions was insensitive to 8-OH-DPAT, mesulergine, BMY 7378, ketanserin, or ICS 205-930. In SN, 5-HT displaced [³H]-5-HT in a competitive manner (IC_{50} = 10 nM) 5-CT displaced [³H]-5-HT with a slope less than unity (IC_{50} = 7 nM). It is proposed that in the regions examined, (SN and NA) 5-HT and 5-CT inhibit the AC activity via a non 5-HT$_{1A}$ receptor. (USPH grant GM 34852).

221.7

AUTORECEPTOR CONTROL OF SEROTONIN (5HT) SYNTHESIS IN BRAIN SLICES: REVERSAL OF 5HT-1B EFFECTS BY 5TH1-A AGONISTS, M.P. Galloway, C. McIntyre*, E.A.Novak*, B.N. Mathews*, Wayne State Univ. Sch. Med. & Lafayette Clinic, Detroit, MI

We have previously shown that 5HT synthesis in vivo is inhibited by 5HT agonists regardless of their 5HT-1A/1B character. However, using brain slices in vitro, we found that 5HT synthesis is attenuated exclusively by 5HT-1B agonists, 5HT-1A agonists are without effect on 5HT synthesis in isolated nerve terminals. In light of the possible opposing biochemical roles exerted by each receptor subtype, we have studied the combined interaction between 1A and 1B agonists at the 5HT synthesis modulating autoreceptor. In depolarized cortical slices (K^+=30 mM), 5HT synthesis is inhibited concentration dependently by the 5HT-1B agonists TFMPP, mCPP, and RU-24969. In the presence of the 5HT-1A agonist 8-OHDPAT (10 uM), the inhibition afforded by the 5HT-1B agonists (1uM) is almost completely reversed. The effect of 8-OHDPAT was dose dependent in the presence of a fixed concentration of 1B agonist. Related monohydroxylated aminotetralins such as (+)-7- or 5-OH-DPAT (potent dopamine autoreceptor agonists), had little ability to reverse TFMPP thus demonstrating receptor specificity. Although putative inhibition of kinase C by polymixin B (100 uM) partially prevented (70%) the K^+ activation of 5HT synthesis, it did not potentiate TFMPP effects. Apparently, 8-OHDPAT can either exert 5HT-1B antagonist activity or 1A and 1B receptors interact in an opposing fashion.

Support: USPHS MH-41227, DA-4120, State of Michigan.

221.8

SEROTONIN AUTORECEPTORS IN GUINEA PIG CORTEX SLICES RESEMBLE THE 5-HT_{1d} BINDING SITE. J.Schipper and M.T.M.Tulp*. Dept. of Pharmacology, Duphar B.V., P.O.Box 2, 1380 AA, Weesp, The Netherlands

Serotonin (5-HT) release is modulated by presynaptic receptors. In the rat, these 5-HT autoreceptors have been characterized as 5-HT_{1b} binding sites. In pigs and monkeys, the 5-HT autoreceptors have different characteristics (Schipper et al. Soc. Neurosci Abstr 1987, 13: 345). In this study, the pharmacology of the 5-HT autoreceptors in guinea pig brain slices was further evaluated with regard to subtypes of 5-HT binding sites.

Slices of parietal cortex were loaded with [^{3}H]-5-HT, superfused with Krebs-Ringer buffer containing 10 µM fluvoxamine (5-HT uptake inhibitor) and release of 5-HT was induced by 20 mM K^+. The K^+-induced release of [^{3}H]-5-HT was almost completely abolished in the presence of 1 µM 5-HT (pD_2=8.5). Release was also inhibited by the 5-HT agonists RU24969 (pD_2=7.9) TFMPP (pD_2=6.8), 8-OH-DPAT (pD_2=7.1) and 5-methoxytryptamine (pD_2=7.9). The effect of 5-HT could be antagonized by methiotepine (pA_2=8.3), metergoline (pA_2=7.4) and yohimbine (pA_2=7.4). Cyanopindolol (pA_2<6.5), mianserine (pA_2<6) MDL 72222 (pA_2<6) and ketanserine (pA_2<6) were inactive.

In comparison to receptor binding data, these findings indicate that the terminal autoreceptor of the guinea pig does not match with the 5-HT_3, 5-HT_2, 5-HT_{1a}, 5-HT_{1b}, 5-HT_{1c} binding sites. The best correlation ifs found with the 5-HT_{1d} binding sites.

221.9

TRYPTOPHAN HYDROXYLASE ACTIVITY IN BRAIN SLICES AND ITS REGULATION BY SEROTONIN (5-HT) AUTORECEPTORS. L.B.Bosch* and J.Schipper (SPON: L.D.Bradford). Dept. of Pharmacology, Duphar B.V., P.O.Box 2, 1380 AA, Weesp, The Netherlands

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the 5-HT biosynthesis. In contrast to modulation of 5-HT release, relatively little is known about the regulation of TPH activity by 5-HT autoreceptors. Therefore we studied the role of 5-HT autoreceptors on TPH activity in brain slices under conditions at which 5-HT release is modulated. Raphe and cortex slices from rat brains were incubated for 60 min at 37°C in an oxygenated Krebs-ringer buffer containing 10µM NSD 1015 and 100µM l-tryptophan. After homogenation in the medium, the total amount of 5-HTP was determined with HPLC. K^+ (20mM)-induced 5-HT release was measured by [^{3}H] overflow from [^{3}H]-5-HT preloaded cortex slices.

K^+ stimulated 5-HT release is inhibited by 5-HT (pD_2=7.8) and other agonists such as TFMPP (pD_2=7.7) and Eltoprazine (pD_2=7.6). Under similar conditions, the TPH activity is not influenced by these 5-HT agonists. Incubation under depolarizing conditions (56 mM K^+) increased TPH activity to 140%. Low Ca^{2+} (0.1 mM) prevented this increase, whereas 5-HT agonists were inactive. The results suggest that TPH activity is not directly influenced by a 5-HT autoreceptor. This is different from the situation with DA autoreceptors which modulate tyrosine hydroxylase activity. The K^+-induced increase of TPH activity suggests that the biosynthesis of 5-HT is mainly under a depolarization induced control.

221.10

SEROTONIN STIMULATION OF CYCLIC NUCLEOTIDE PRODUCTION AND CYCLIC NUCLEOTIDE MODULATION OF PHOSPHOINOSITIDE TURNOVER IN PORCINE CHOROID PLEXUS. M.J. Kaufman, B.J. Hoffman and P.R. Hartig. Dept. of Env. Health Sci., The Johns Hopkins Medical Institutions, Balto., MD, 21205.

Maximal production of inositol monophosphate (IP) by the choroid plexus (CP) 5HT_{1c} receptor occurs at 1uM 5HT with an EC_{50} of 46nM. At higher 5HT concentrations (10uM and 100uM) there is a decrease in IP production (25 and 33%). We now report that activation of this receptor leads to accumulation of cGMP. The EC_{50} for this response is similar to that for IP production. Maximal formation of cGMP is several fold above basal levels. We hypothesize that the IP decrease seen at high 5HT levels may be caused by 5HT-stimulated cyclic nucleotide production. Therefore we tested other compounds that are known to elevate CP cyclic nucleotide levels to determine whether they could influence 1uM 5HT-stimulated IP production. Incubation of CP with cAMP elevating agents (2.5uM Isoproterenol, 100uM dBcAMP) did not alter IP production, however, compounds which increase cGMP levels (100uM dBcGMP, 500uM Carbamylcholine, 100uM Sodium Nitroprusside) inhibited IP production by 14-44%. Since cholinergic and atrial natriuretic peptide stimulation of CP have been shown by others to elevate cGMP, these inputs into CP may modulate 5HT function in this tissue. Further, cGMP increases produced by 5HT may negatively modulate the IP elevations produced by 5HT in CP.

221.11

THE BOVINE HIPPOCAMPAL 5-HT_{1A} RECEPTOR -G-PROTEIN INTERACTIONS ARE CONSERVED IN DETERGENT SOLUTION. T.B.Kline*[1], Y.Gold,*[2] L.R.Meyerson,[2] R.Iyengar*[1] (1)Department of Pharmacology, Mt. Sinai School of Medicine, CUNY, NY,NY10029 and (2)Medical Research Division,American Cyanamid Co., Ramapo College, Mahwah, NJ 07430.

Studies on the bovine hippocampal 5-HT_{1A} receptor, solubilized in 50 mM TRIS/10 mM CHAPS/200 mM NaCl, in the absence of agonist, provide evidence for the tight coupling of the receptor with its associated guanine-nucleotide-binding (G)protein(s). Scatchard analysis of [^{3}H] 8-OH DPAT binding shows a single site with K_d = 1.9 nM, B_{max} = 42 fmol/mg protein. The rank order of affinity of WB4101, 5-HT, and 5-$CONH_2$T indicates that a 5-HT_{1A} receptor has been solubilized. The K_{act} values for guanine nucleotides for the converstion of the receptor from a high to a low affinity agonist state are 50-300 nM. Agonist (5-HT, 8-OH DPAT, 5-$CONH_2$T) stimulated [^{35}S]GTPγS binding to G proteins is observed in detergent extracts. The K_{act} of 8-OH DPAT for this allosteric effect (2 nM) indicates that this effect occurs as a consequence of agonist binding to the 5-HT_{1A} receptor. Although guanine-nucleotide sensitivity for other receptors in detergent solutions has been demonstrated, many (although not all) receptors require preexposure to agonist in order to retain the G-protein coupling. In contrast, the bovine hippocampal 5-HT_{1A} receptor-G-protein complex is apparently formed prior to agonist occupancy.(Supported by NIDA grant DA-01875 and NIH grants DK-38761 and CA 44998).

221.12

GUANYL NUCLEOTIDE SENSITIVE SEROTONIN 5HT_{1D} RECEPTORS IN HUMAN CORTEX AND CAUDATE. K.HERRICK-DAVIS* and M.Titeler (SPON: M. Miller) Dept. Pharmacol. Toxicol. Albany Medical College, Albany, NY 12208.

Radioligand binding studies in mammalian brain have revealed the presence of multiple serotonin receptors: 5HT_{1A}, 5HT_{1B}, 5HT_{1C}, 5HT_2 and 5HT_3. Recently, evidence has been provided for a new subtype of serotonin receptor, 5HT_{1D}, in bovine, porcine and rat brain. The pharmacology of this site is unique in that it displays high affinity for 5CT, yohimbine, methysergide and ergotamine.

Radioligand binding studies were performed to characterize 5HT_{1D} receptors in human prefrontal cortex and caudate homogenates. 3H-5HT binding, in the presence of pharmacologically blocked 5HT_{1A}, 5HT_{1B}, 5HT_{1C} and 5HT_2 receptors, was specific, saturable, reversible and of high affinity. In both brain regions the majority of 5HT_1 sites were of the 5HT_{1D} subtype. 5HT_{1D} sites in human brain have a similar pharmacology to 5HT_{1D} sites in rat, bovine and porcine brain. Guanyl nucleotides modulated the binding of 3H-5HT to 5HT_{1D} sites, while adenyl nucleotides had no effect. These findings are supportive of the presence of serotonin 5HT_{1D} receptors in human prefrontal cortex and caudate which appear to be coupled to a GTP binding protein.

221.13

DETECTION AND CHARACTERIZATION OF THE NOVEL 5HT1E RECEPTOR IN HUMAN AND OTHER MAMMALIAN BRAIN TISSUES. M. TITELER and K. HERRICK-DAVIS*. Dept. of Pharmacol. Toxicol., Albany Medical College, Albany, NY 12208.

Radioligand binding studies utilizing 3H-5HT and brain tissue homogenates have previously revealed the presence of four $5HT_1$ receptor subtypes. In human cortical tissue homogenates, in the presence of pharmacologically blocked $5HT_{1A}$, $5HT_{1C}$ and $5HT_{1D}$ receptors (there are no detectable $5HT_{1B}$ receptors in human brain tissue), a saturable, high affinity binding site for 3H-5HT was detected. This binding site (termed "$5HT_{1E}$" in keeping with the currently popular 5HT nomenclature system) was pharmacologically distinguished from any of the other six 5HT receptor subtypes by displaying low affinity for ketanserin (a high affinity $5HT_2$ drug), ICS-205-930 (a high affinity $5HT_3$ drug), 8-OH-DPAT (a high affinity $5HT_{1A}$ drug), TFMPP (a high affinity $5HT_{1B}$ drug), mesulergine (a high affinity $5HT_{1C}$ drug) and 5CT (a high affinity $5HT_{1D}$ drug). Preliminary studies indicate this site is present in human, bovine and porcine brain tissue and is sensitive to guanyl nucleotides and insensitive to adenyl nucleotides (indicating a $5HT_{1E}$/GTP binding protein interaction). Detailed radioligand characterization of this novel $5HT_{1E}$ receptor in mammalian brain tissue will be presented.

221.14

HALLUCINOGEN INTERACTIONS WITH RADIOLABELLED HUMAN $5HT_2$ RECEPTORS. S. LEONHARDT*, R.A. LYON, M. TITELER, B.SAZDOT*, J. BARABAN, and R.A. GLENNON*.(SPON: M. DENTINGER). Dept. Pharmacol. Toxicol., Albany Medical College, Albany, New York 11208. Dept. Neurosciences, Johns Hopkins Univ. Med. Ctr., Baltimore, Maryland 21205. Dept. Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia 23298

It has been hypothesized that stimulation of brain $5HT_2$ receptors is the mechanism-of-action of LSD and similarly acting hallucinogens. Therefore studies to determine the affinities of the hallucinogens in binding to human cortical $5HT_2$ receptors were undertaken in order to determine whether the affinities for the human $5HT_2$ receptor would match the affinities for the rat $5HT_2$ receptor and would correlate closely with the hallucinogenic potencies in humans. Preliminary data appear to indicate that the hallucinogens interact with the human and rat brain $5HT_2$ receptors with similar affinities, supporting the hypothesis that brain $5HT_2$ stimulation produces the psychoactive properties of these drugs. Autoradiographical studies designed to investigate the interactions of hallucinogens with radiolabelled human $5HT_2$ receptors in slide-mounted tissue sections are being conducted and will be presented.

221.15

SEROTONIN NEURONAL GROWTH FACTOR RELEASED BY ASTROGLIAL 5-HT_1 RECEPTORS. P.M. Whitaker-Azmitia, L. Noreika*, E. Azmitia, Department of Psychiatry SUNY, Stony Brook, NY 11794

We have shown the presence of high affinity serotonin receptors on astroglial cells. The number of sites (B_{max}) is greatest in immature astrocytes, an observation which led us to the hypothesis that these receptors may play a role in brain development.

To test this hypothesis, we treated immature astroglial cultures with 500 nM 5-HT, 8-OH-DPAT, TFMPP or mCPP and collected the media after 4 and 24 hours incubation. The astroglial-conditioned media was then added to cultures of fetal rat serotonergic neurons. Growth of the neurons was assessed by the amount of specific uptake of 50 nM ^{3}H-serotonin in twenty minutes.

Greatest stimulation of growth (139% increase) was observed in cultures containing media from astrocytes treated with 5-HT for 24 hours. The selective 5-HT_{1a} agonist 8-OH-DPAT also stimulated growth (134%). The 5-HT_{1b} agonists, TFMPP and mCPP showed the least effect (115% and 114% respectively).

Media from astroglial cultures, incubated for only 4 hours with the receptor agonists, did not show growth-promoting factors. This suggests that the factor is not a readily available constituent of astroglial cells (for example, glucose), but rather requires time to be produced.

221.16

ESTROGEN ENHANCES SEROTONIN-1A ELECTROPHYSIOLOGICAL RESPONSES IN FEMALE RAT HIPPOCAMPAL SLICES. W.P. Clarke, S.G. Beck and J. Goldfarb. Department of Pharmacology, Mount Sinai School of Medicine, CUNY, New York, NY 10029-6574.

The gonadal steroids are known to modulate several aspects of CNS serotonergic neurotransmission: synthesis, content, turnover and uptake of serotonin (5-HT) as well as 5-HT receptor density. In this study we investigated the effects of estrogen on electrophysiological responses to 5-HT in rat hippocampal slices.

Female Sprague-Dawley rats were anesthetized with ether and ovariectomized. At least two weeks later, rats were treated for 4 to 6 days with estrogen (either injections of 10 µg estradiol benzoate/day sc or implants of silastic capsules [5 mm x .058 in id] containing estradiol, sc). Chronic estrogen treatment enhanced the 5-HT_{1A} induced decrease in CA1 population spike amplitude. The concentration response curves to 5-carboxyamidotryptamine, a 5-HT_{1A} agonist, were shifted to the left approximately 5-fold as compared with control. Chronic estrogen enhanced the 5-HT_{1A} mediated hyperpolarization of CA1 pyramidal cells without affecting 5-HT's reduction of the afterhyperpolarization. The mechanism(s) by which estrogen enhances the sensitivity of 5-HT_{1A} responses is under investigation. Supported by grants DA 01875 and MH 41917.

221.17

SEROTONERGIC PHENYLPIPERAZINES: HYPOTHERMIA OR HYPERTHERMIA RELATED TO AFFINITY FOR 5HT-1A or 5HT-1B BINDING SITES. G.E. Martin, M.K. Scott, W.J. Baldy, R.P. Shank and J.R. Mathiasen. Janssen Research Foundation, Department of Biological Research, Spring House, PA USA.

Previously, we have reported that selected phenylpiperazines (PP) block conditioned avoidance responding in the rat, although they exhibit no affinity for dopamine D-2 receptors. (Martin, et al., Abst. Neuroscience 1987, 13:800). Herein the effect of PP on the rectal temperature of the rat is reported. In general, following administration of ortho-substituted PP, a fall in the rectal temperature of the rat was observed. When substitutions were placed in the meta-position of the phenyl ring, however, hyperthermia was produced concomitant with a shift in selectivity of the PP in binding assays from the 5HT-1A to the 5HT-1B binding site. Hyperthermia evoked by 5HT-1B selective agonists was abolished by pretreatment with the serotonin antagonist metergoline. Metergoline, on the other hand, failed to reduce hypothermia produced by 5-HT-1A agonists. Conversely, the 5HT-1A selective antagonist, (-) propranolol did reverse some of the 5HT-1A agonist induced hypothermia without blocking 5HT-1B-induced rises in rectal temperature. The data suggest activation of 5HT-1A and 5HT-1B binding sites produce opposite effects on the rat's rectal temperature, and reveal meta vs ortho substitution on the phenyl ring is more likely to render a 5-HT-1B selective agent than a similar substitution in the ortho position.

221.18

HALLUCINOGENS DIRECTLY ACTIVATE SEROTONIN 5HT-1c RECEPTORS IN CHOROID PLEXUS. K.D. Burris* and E. Sanders-Bush. Dept. of Pharmacology and Psychiatry, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

Central serotonin (5HT) receptors, in particular the 5HT-2 subtype, are believed to be involved in mediating the actions of hallucinogens. Because of similarities between the 5HT-2 and 5HT-1c receptors, the effects of hallucinogens at 5HT-1c receptors were examined in an in-vitro assay of receptor function. Agonist-induced phosphoinositide (PI) hydrolysis was determined in rat choroid plexus by measuring ^{3}H-inositol monophosphate formation in the presence of LiCl. 1-(2,5-dimethoxy-4-phenyl)-2-aminopropane (R,S-DOM) increased PI hydrolysis with an EC_{50} of 0.6 µM and maximum effect that was 70% that produced by 5HT. The R(-) isomer of DOM was 10-fold more potent than the S(+) isomer. D-lysergic acid diethylamide (+ LSD) potently stimulated PI hydrolysis with an EC_{50} of 50 nM and maximum effect 25% of that produced by 5HT. The potent 5HT-1c receptor antagonist, mianserin, was more efficacious than the less potent 5HT-1c antagonist, spiperone, at blocking the effect of both R,S-DOM and (+) LSD. Lisuride and (-) LSD, both nonhallucinogenic congeners of (+) LSD, were pure antagonists of 5HT-1c mediated PI hydrolysis with IC_{50} values of approximately 100 nM and 100 µM respectively. This study demonstrates for the first time that hallucinogens are agonists at 5HT-1c receptors. (Supported by DA05181, MH34007 and GM07628.)

221.19

SEROTONINERGIC MODULATION OF LOCAL INHIBITION IN HIPPOCAMPAL SLICE: MEDIATION BY 5-HT$_{1A}$ RECEPTOR.

S. Springfield, C. Ayala and D. Delma, Dept. of Biology, City College of City Univ. of N.Y., New York, NY 10031.

In previous studies, we demonstrated that serotonin (5-HT) attenuates local inhibition in the hippocampus. We report here that this effect may be mediated through the 5-HT$_{1A}$ receptor.

Rat hippocampal slices were constantly superfused with a bicarbonate-buffered balanced salt solution equilibrated with 95% O_2/5% CO_2 and maintained at 32° C. Evoked population spikes were recorded with a single barreled glass micropipette from the somal layer of CA1 pyramidal neurons. To activate inhibitory interneurons, pairs of stimuli were delivered to either the stratum radiatum or the alveus at 1/30 Hz. When stimuli were presented at short interstimulus intervals (10-40 msec), a reduction in the second population spike occurred. This reduction has been attributed to the action of GABA-mediated inhibition superimposed upon orthodromic excitation.

Superfusion of 0.1 uM 8-hydroxy-2(di-n-propylamine)-tetralin (8-OH-DPAT), a 5-HT$_{1A}$ receptor agonist, mimics the action of 5-HT, while superfusion of 0.1 uM spiroxatrine, a 5-HT$_{1A}$ antagonist, blocks the action of 5-HT on local inhibition. These studies demonstrate that the effect of 5-HT on local inhibition may be mediated through the 5-HT$_{1A}$ receptor. (Supported by NSF grant BNS 86-06419, PSC-CUNY award and MBRS program).

221.20

INHIBITION OF PURKINJE CELL SPONTANEOUS DISCHARGE BY THE 5HT$_{1A}$ SPECIFIC AGONIST 8-OH-DPAT IN THE IN VITRO CEREBELLAR SLICE IS MG^{++} SENSITIVE.

E.J. Darrow, H.K. Strahlendorf, and J.C. Strahlendorf. Depts. of Physiology and Neurology, Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX.

We have previously shown (Darrow, et al., Soc. Neurosci. Abst. 17: 1652, 1987) that the 5HT$_{1A}$ specific agonist 8-OH-DPAT produces inhibition of Purkinje cell (PC) spontaneous discharge, in the in vitro cerebellar slice preparation. Recent studies by Norman, et al. (Mol. Pharm., 28: 487, 1985) using radioligand binding studies, and by Daval, et al. (J. of Neurosci. 6: 3474, 1986) using autoradiography have shown agonist binding to the 5HT$_{1A}$ receptor subtype is [Mg^{++}] dependent. Our studies examine the effects of low [Mg^{++}], in the perfusion medium, on the inhibition of PC spontaneous discharge by microiontophoretic application of 8-OH-DPAT. In the cerebellar slice preparation, we have seen that lowering the [Mg^{++}] from 1.15 mM to 0.56 mM significantly decreases the inhibition of PC spontaneous firing produced by 8-OH-DPAT. This study gives physiological support to the findings of Norman, et al. and Daval, et al. in that the decreased inhibition caused by lowered [Mg^{++}] appears to be due to a decrease in the binding affinity of 8-OH-DPAT to the 5HT$_{1A}$ receptor subtype. (Research supported in part by the Tarbox Parkinson's Disease Institute, TTUHSC.)

221.21

DIRECT EFFECTS OF SUPERFUSED SEROTONIN ON NEURONS OF DEEP CEREBELLAR NUCLEI.

P.A. Cumming*, J.C. Strahlendorf and H.K. Strahlendorf (SPON: R. Norman). Depts. Physiology and Neurology, Texas Tech University Health Sciences Center, Lubbock,TX 79430.

Based on anatomical studies demonstrating a serotonergic innervation to both the cerebellar cortex and deep cerebellar nuclei (Chan-Palay, V., In: Cerebellar Dentate Nucleus: Organization, Cytology and Transmitters, 1977), as well as our studies characterizing the actions of serotonin (5-HT) on Purkinje cells (Strahlendorf, J.C., et. al., *Exp. Brain Research*, 56: 50, 1984), this study examines the actions of 5-HT on the neurons of the dentate/interpositus nuclei in an in-vitro brain slice. Using an interface chamber with the temperature controlled at 34° C., horizontal slices of Sprague-Dawley rats (350 µm sections) were superfused with various concentrations of 5-HT. Dentate/interpositus cells display a regular firing pattern with a frequency range between 15-80 Hz and an average of 47 Hz (n=19). Seventy-one percent of the cells responded to 5-HT with a decrease in the discharge rate, while 29% exhibited an increase. 5-HT at 10^{-7}, 10^{-6},10^{-5} M produced a dose related decrease in spontaneous firing rate of 34, 47, and 52%, respectively. At these same concentrations of 5-HT, increases of 16, 13, and 33%, respectively, were seen in the cells displaying excitation. The cells within the deep cerebellar nuclei appear to be more sensitive than Purkinje cells in the cerebellar cortex by approximately three orders of magnitude. Collectively, our previous studies and these results further identify a neurotransmitter/modulator role of 5-HT on cerebellar neurophysiology at both the cortical and nuclear levels. Supported by NS19296 (JCS & HKS).

221.22

THE CONTRACTILE EFFECT OF 5-HYDROXYTRYPTAMINE (5-HT) ON CEREBRAL BLOOD VESSELS IS MEDIATED BY HETEROGENEOUS 5-HT RECEPTORS.

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We tested a range of 5-HT agonists and antagonists on the in vitro reactivity of the cat middle cerebral artery and compared their vascular potency with affinity values for the various 5-HT receptor subtypes. 5-Carboxamidotryptamine and RU 24969 (agonists at 5-HT$_{1A-1B}$ subtypes) were, respectively, significantly more potent and as potent as 5-HT itself in eliciting vasoconstriction. α-Methyl-5-HT (5-HT$_{1C-2}$ agonist) and 2-methyl-5-HT(5-HT$_3$ agonist) were 20 and 60 times less potent than 5-HT. All these agonists induced similar maximal responses (E_{AM} varied from 0.83 to 1.20g; that of 5-HT was 1.16±0.09g and the EC_{50} value for 5-HT was 7.9±0.8 x 10^{-8}M). The most selective 5-HT$_{1A}$ agonist, 8-OH-DPAT, was the least potent but induced a significantly more intense vasoconstriction (E_{AM} = 1.84±0.12g; $p<0.01$). Amongst the antagonists tested, 5-HT$_{1A-1B}$ 5-HT$_{1C}$ and 5-HT$_3$ selective drugs such as propranolol-cyanopindolol, mesulergine and MDL 72222 were inactive. In contrast, the 5-HT$_2$ receptor antagonists exerted a non-competitive inhibition of the 5-HT-induced constriction. The order of potency was pizotifen > ritanserin ≥ dihydroergotamine > cyproheptadine ≥ methiothepin > ketanserin ≥ methypergide. The pD_2' values (negative logarithm of the molar concentration of antagonist needed to decrease E_{AM} response by 50%) varied from 8.74±0.14 (pizotifen) to 6.88±0.26 (methypergide).

A 5-HT$_2$ and a 5-HT$_{1B}$-like receptors appear to be involved in this cerebrovascular response to 5-HT. The vascular 5-HT$_{1B}$-like site shares pharmacological similarities with the 5-HT$_{1D}$ subtype recently described.

221.23

ALTERATIONS IN SEROTONIN (5-HT) BINDING SITES AFTER 5,7DIHYDROXYTRYPTAMINE (5,7DHT) TREATMENT IN THE RAT SPINAL CORD.

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These studies attempted to confirm the presynaptic distribution of the 5-HT$_{1B}$ binding sites presumed to modulate transmitter release in the rat spinal cord. Experimental rats received desipramine (DMI) (25mg/kg, i.p.) prior to the intrathecal (i.t.) injection of the 5-HT neurotoxin, 5,7DHT (100µg). Paired controls also received DMI and the vehicle (i.t.). At 3,7,10 and 14 days after 5,7DHT, competition binding assays were performed using 2nM ^{3}H-5-HT and Trifluoromethylphenyl piperazine, a drug which interacts with 5-HT$_{1B}$ and 5-HT$_{1A}$ binding sites. Binding capacities and affinities were determined using LIGAND (Munson and Rodbard Anal. Biochem. 106:220,1980).

At three days, 5,7DHT caused a significant decrease (35%) in 5-HT$_{1B}$ binding sites with the affinity of those remaining being higher. The decrease in binding correlated (r=0.71) with the degree of 5-HT depletion. At later time periods, no significant changes were observed in the 5-HT$_{1B}$ binding site perhaps because of adaptive changes and/or gliosis. By day 14 an increase (30%) in the binding capacity of the 5-HT$_{1A}$ site was also observed. These results confirm the presynaptic distribution of the 5-HT$_{1B}$ binding site.

221.24

INHIBITION OF ISOLATION-INDUCED AGGRESSION BY SELECTIVE SEROTONERGIC AGENTS.

S. M. White, R. F. Kucharik* and J. A. Moyer. Wyeth-Ayerst Research, Department of Experimental Therapeutics, Princeton, NJ 08543.

The inhibition of isolation-induced aggressive behavior in male mice has been proposed as a preclinical screen for anxiolytic activity. Benzodiazepines and, more recently, several serotonergic (5-HT1A) partial agonists have been found to reduce aggressive behavior.

CF-1 male mice were individually housed for 3 weeks and then trained to attack a group-housed intruder mouse. Drugs were administered IP 60 min prior to the test and total fighting time during the 3 min test was measured. Debilitation was evaluated by the rotorod/ataxia test.

The 5-HT1A partial agonists, buspirone and SM-3997, were equipotent to diazepam in antagonizing aggression, while ipsapirone and chlordiazepoxide were less potent. Selective 5-HT1A agonists (8-OHDPAT and Wy-48,723) and 5-HT1A antagonists (BMY-7378 and Wy-47,846) had greater potency in inhibiting aggressive behavior than any of the benzodiazepines tested. O-methoxyphenyl-piperazine (oMPP) exhibits 5-HT1A partial agonist/antagonist activity and may be an active metabolite of enciprazine, a non-benzodiazepine anxiolytic. Both were found to inhibit aggressive behavior as potently as lorazepam. Both 5-HT1A partial agonists and antagonists inhibit aggression at non-sedative doses, suggesting a pre/postsynaptic 5-HT or a non-serotonergic mechanism of action.

221.25

5-HT SYNDROME: DIFFERENTIAL EFFECTS OF 5-HT1A SELECTIVE COMPOUNDS. **R. F. Kucharik*, S. M. White, T. H. Andree, and J. A. Moyer. Wyeth-Ayerst Research, Department of Experimental Therapeutics, Princeton, NJ 08543**

The agonist and antagonist properties of serotonin (5-HT) selective compounds were elucidated through their ability to elicit or block the 5-HT syndrome in rats, a post-synaptic 5-HT1A mediated response. Agonist activity was evaluated by quantifying the behavioral response using a four-point ranked intensity scale on each of the six symptoms that characterize the 5-HT syndrome. Antagonist activity was assessed by administering compounds 15 min prior to a subsequent 5-methoxy-N,N-dimethyltryptamine (MeODMT) challenge.

MeODMT, 8-OH-DPAT, Wy-48,723, buspirone and gepirone dose-dependently increased 5-HT syndrome scores. BMY 7378, Wy-47,846, buspirone and gepirone inhibited the syndrome produced by MeODMT. A single test dose of enciprazine was unable to block or elicit the 5-HT syndrome, but its putative metabolite o-methoxyphenylpiperazine (oMPP) produced a profile similar to that of buspirone and gepirone. Binding studies on Wy-48,723, Wy-48,846, and oMPP have demonstrated a high affinity for the 5-HT1A site, whereas enciprazine had low affinity. In the 5-HT syndrome MeODMT, 8-OHDPAT and Wy-48,723 appear to be full agonists. Buspirone, gepirone and oMPP are partial agonists/antagonists, while BMY-7378 and Wy-47,846 may be considered antagonists.

221.26

5HT1A AGONISTS: ASSOCIATION BETWEEN HYPOTHERMIA AND BRAIN FUNCTION AS MEASURED BY THE AUDITORY-EVOKED BRAINSTEM RESPONSE. **R. R. Notvest, L. Sutherland, and J. T. Haskins. Department of Experimental Therapeutics, Wyeth-Ayerst Research Inc., Princeton, NJ 08543-8000**

In a preliminary study we found that 5HT1A agonists increased all peak latencies of the auditory-evoked brainstem response (ABR). Other studies have shown that 5HT1A agonists induce hypothermia (G. M. Goodwin, et al., 1985) and, independently, hypothermia increases the peak latencies of the ABR (Rossi and Britt, 1984). This study more fully examined the effect of 5HT1A agonists on both the ABR and body temperature (BT).

Male SD rats, previously implanted with cortical electrodes, were restrained and placed in sound attenuated chambers. Following baseline recordings, rats received 5 mg/kg ip of either buspirone (n=6), 8OH-DPAT (n=6), Wy-48,723 (n=4) or vehicle (n=6). ABRs and BT were recorded at regular intervals for 4 hrs.

All three 5HT1A agonists significantly decreased BT and increased ABR latency (quantified as a change in Peak IV latency), with the order of potency being Wy-48,723 $\geq$ 8OH-DPAT > buspirone ($p<0.05$). Changes in BT and ABR latency were correlated. The results suggest that 5TH1A-induced changes in the ABR are non-specific effects due to hypothermia.

221.27

PRECLINICAL NEUROPHARMACOLOGICAL PROFILE OF WY-48,723, A POTENT SEROTONIN (5-HT_{1A}) AGONIST. T.H. Andree, J.A. Moyer, G. Stack, E.A. Muth and J.T. Haskins. Wyeth-Ayerst Research, CNS Subdivision, Philadelphia, PA 19101

Wy-48,723 (decahydro-3-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-1,5-methano-6,7,9-metheno-2H-pentaleno-[1,2-d]azepine-2,4(3H)-dione, hydrochloride hemihydrate) is a substituted arylpiperazine synthesized for potential anxiolytic/antipsychotic activity. In vitro receptor binding assays reveal high affinity for 5-HT_{1A} sites (Ki=0.3nM) with lower affinity for D_2 (Ki=56nM) and 5-HT_2 (Ki=272nM) binding sites. Wy-48,723 produced less than 40% inhibition of $alpha_1$ (36%), $alpha_2$ (0%), beta (4%) and D_1 (0%) radioligand binding at 1µM drug concentration. Both i.p. and p.o. administration of Wy-48,723 (10 mg/kg) inhibited the ex vivo binding of ^{3}H-8-OHDPAT in hippocampal membranes. Electrophysiologically, Wy-48,723 and buspirone inhibited the firing of dorsal raphe neurons (IC_{50} values=0.008 and 0.03 mg/kg, i.v., respectively), suggesting agonistic activity. In behavioral studies, Wy-48,723 was active in inhibiting apomorphine-induced climbing (ED_{50}=0.4 mg/kg, i,p.) but not stereotyped behavior (inactive at 40 mg/kg). Like buspirone, Wy-48,723 inhibited conditioned avoidance behavior following i.p. administration (AB_{50}s <10 mg/kg), but was inactive in the Geller-Seifter conflict model. In summary, Wy-48,723 has an interesting profile suggesting potential antipsychotic/anxiolytic activity.

221.28

BUSPIRONE PARTIALLY GENERALIZES TO THE STIMULUS EFFECTS OF DPAT USING THE DISCRIMINATED AVERSION PROCEDURE. J.M. Marcoccia* and I. Lucki (SPON: M. Bauer). Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

Rats were trained to discriminate the stimulus effects of the 5-HT_{1A} agonist DPAT (0.4 mg/kg IP) using a new discriminated aversion procedure (Lucki et al., Soc. Neurosci. Abstr., 13:344, 1987). Rats demonstrated the stimulus effects of DPAT with a 2-bottle choice test by a reversal of their ordinary preference for saccharin over tap water. The ability of 3 anxiolytic drugs with high affinity for the 5-HT_{1A} receptor, buspirone, ipsapirone, and gepirone, to substitute for the DPAT stimulus was examined with this method.

Administration of DPAT produced a dose-dependent reduction of the preference for saccharin over tap water in conditioned rats, but did not alter saccharin preference in untrained controls. Buspirone only partially substituted for the effects of DPAT. Gepirone completely substituted for effects of DPAT, whereas the effect of ipsapirone was intermediate between that of gepirone and buspirone. The results suggest that the discriminated aversion method can detect the effects of full and partial 5-HT_{1A}.

This research was supported by USPHS grants MH 36262 and GM 34781.

221.29

BMY 7378 BLOCKS BEHAVIORAL RESPONSES PRODUCED BY THE 5-HT_{1A} AGONIST 8-OH-2-(di-n-propylamino)tetralin (DPAT). I. Lucki, D.Z. Press* and J.M. Marcoccia*. Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA 19104.

BMY 7378 was suggested to be a weak partial agonist at the 5-HT_{1A} receptor because of its high binding affinity and low response efficacy in vitro (Yocca et al., Eur. J. Pharmacol., 137:293, 1987). Because of this profile, BMY 7378 was examined at responses produced by systemic administration of the 5-HT_{1A} agonist DPAT in rats.

BMY 7378 alone did not produce signs of the 5-HT behavioral syndrome but pretreatment with BMY 7378 prevented DPAT from producing the syndrome. BMY 7378 alone produced a small (-0.6°C) reduction in body temperature, but pretreatment attenuated the larger (-2.0°C) hypothermic effect of DPAT.

The stimulus effects of DPAT (0.4 mg/kg) were studied using a new discriminated aversion method (Lucki et al., Soc. Neurosci. Abstr., 13:344, 1987). BMY 7378 administered alone produced a small but significant substitution for the stimulus effect of DPAT. However, pretreatment with 1.0 mg/kg BMY 7378 prevented discrimination of the DPAT training stimulus.

When administered in combination with the 5-HT_{1A} agonist DPAT, BMY 7378 prevented many of its behavioral actions. These behavioral results support the characterization of BMY 7378 as a weak partial agonist at the 5-HT_{1A} receptor.

This research was supported by USPHS grants MH 36262 and GM 34781.

221.30

THE DEVELOPMENT OF TOLERANCE FOLLOWING CHRONIC ADMINISTRATION OF 5-METHOXYDIMETHYLTRYPTAMINE. K. A. Brown and M. A. Blackshear. Department of Biological Sciences, Tennessee State University, Nashville, TN 37209-1561.

It has been recently reported that tolerance develops to the induction of serotonin (5-HT) mediated behaviors following chronic administration of 5-HT agonists. The present study examines the effects of repeated administration of 5-methoxydimethyltryptamine (5-MeODMT), a putative selective 5-HT_1A agonist, on three parameters of 5-HT receptor function; food intake, body temperature, and 5-HT binding sites.

Male Sprague-Dawley rats received a single dose (6 mg/kg) or 7 daily doses (3 mg/kg, twice daily) of 5-MeODMT. Body temperature and food intake were measured 30 minutes after acute treatment, or 30 minutes after a challenge dose of 5-MeODMT was given at 48 hours after termination of treatment. Serotonin-2 binding sites were measured at 48 hours after acute or chronic administration of 5-MeODMT.

Food consumption was markedly reduced after a single injection of 5-MeODMT (11 ± .4 saline controls vs 3 ± .74 gms 5-MeODMT), but was unchanged after repeated doses (11.4 ± .4 saline vs 9.2 ± .4 gms 5-MeODMT).

Similarly, body temperature was decrease after acute 5-MeODMT (36.3 ± .11° C controls vs 34.0 ± .3°C 5-MeODMT), but was unaltered following chronic treatment (36.4 ± .2°C control vs 36.0 ± .3°C 5-MeODMT). [^{3}H]-ketanserin binding to 5-HT2 sites in the frontal cortex was not altered by acute or chronic 5-MeODMT treatment. These results suggest that food intake may be mediated by 5-HT_1A receptors and provides supporting evidence of the development of tolerance after chronic stimulation of central 5-HT receptors. (Supported by NIH - ROMI grant# G12RR03033).

221.31

COMPARISON OF THE NOVEL 5-HT_{1A} RECEPTOR RELATED ANXIOLYTICS BUSPIRONE, GEPIRONE, IPSAPIRONE, SM 3997, THEIR METABOLITE 1-PYRIMIDINYLPIPERAZINE AND DIAZEPAM IN ANIMAL MODELS OF ANXIETY.

J.Traber*, T.Schuurman* and U.Benz* (SPON. W.Daunicht), Neurobiology Department, Tropon Pharmaceuticals, Neurather Ring 1, D-5000 Köln 80, F.R.G.

The second generation anxiolytics buspirone, gepirone, ipsapirone and SM 3997, which potently and selectively interact with central 5-HT_{1A} receptors, their common metabolite 1-pyrimidinylpiperazine (1-PP) and diazepam were compared in various rat models of anxiety and in motor function tests. The former included the shock suppressed drinking (SSD), the Geller-Seifter conflict, the conditioned emotional response (CER), the social interaction (SI) and the ultrasonic vocalization (USV) test. All five anxiolytics released punished behavior in the SSD and Geller-Seifter test in the potency rank order diazepem > gepirone = ipsapirone > buspirone > SM 3997 and disinhibited lever pressing in the CER. In the USV paradigm, in which ultrasonic vocalization was elicited by electric footshock, all test compounds mentioned decreased the duration of 22 kHz vocalization. The duration of social interaction between rats in a brightly illuminated test arena was increased by all compounds except SM 3997 and 1-PP. Interestingly, under low light, less stressful conditions only ipsapirone but not the other substances showed prosocial effects. 1-PP was active in a few of the anxiety models its potency being lower than that of the parent compounds. Motor functions in mice were stronger affected by diazepam than by the 5-HT_{1A} receptor related anxiolytics.

The results indicate differences in the efficacy and potency as well as in their activity profile of the serotonergic anxiolytics.

221.32

FUNCTIONAL INTERACTION OF THE NOVEL 5-HT_{1A} RECEPTOR ANXIOLYTICS WITH 5-HT_{1A}- and α-ADRENERGIC RECEPTORS

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There is substantial evidence that the 5-HT_{1A} receptor subtype in the brain is involved in mechanisms underlying anxiety. The aim of the present study was to compare on a biochemical level the novel 5-HT_{1A} receptor related anxiolytics buspirone, gepirone, ipsapirone and SM 3997 as well as 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in respect to their functional interaction with 5-HT_{1A} and α-adrenergic receptors. These compounds bound with high affinity to 5-HT_{1A} receptors in the rank order 8-OH-DPAT > ipsapirone SM 3997 > buspirone > gepirone. 8-OH-DPAT and SM 3997 acted as full agonists on these receptors as measured by inhibition of forscolin-stimulated adenylate cyclase in rat hippocampus. The intrinsic activity of buspirone, gepirone and ipsapirone was considerably lower than that of the full agonists. At higher concentrations ipsapirone, but only marginally buspirone could antagonize the agonist induced inhibition of enzyme activity. Ipsapirone bound with higher affinity than the other 5-HT_{1A} receptor related anxiolytics or 8-OH-DPAT to central α_1- and α_2-adrenergic receptors. Functional in vitro studies revealed ipsapirone to possess antagonistic or agonistic properties on α_1- or α_2-adrenergic receptors, respectively.

The present results indicate differences of the novel anxiolytics in their intrinsic activities on 5-HT_{1A} receptors and in their affinity to α-adrenergic receptors, which might be relevant for their pharmacological profile.

221.33

PHARMACOLOGICAL DIFFERENTIATION OF $5HT_1$ AND $5HT_2$ COMPONENTS OF A SEROTONERGIC SYNDROME IN MICE

B. Dubinsky, R. Ernest and D. A. Shriver. Research Laboratories, Ortho Pharm. Corp., Raritan, NJ 08869.

Administration of 5-hydroxytryptophan (90 mg/kg, iv) to mice 1 hr after serotonin (5-HT) uptake inhibition with zimelidine (12 mg/kg, sc) produces head twitch (TWITCH), body tremors (TREMOR) and abduction of the hindlimbs (ABDUCT). TWITCH and TREMOR are probably mediated by both $5\text{-}HT_1$ and $5\text{-}HT_2$ receptor systems. However, ABDUCT is thought to be mediated by $5\text{-}HT_1$ receptors in the spinal cord. Compounds with relatively selective affinity for $5\text{-}HT_2$ receptors, such as, ritanserin (RIT), pirenperone (PIREN) or cyproheptadine (CYPRO) potently blocked TWITCH (ED_{50}=0.3, 0.3 or 1.0 mg/kg, respectively); and, with the exception of CYPRO, inhibited TREMOR. However, ABDUCT was not inhibited by RIT (ED_{50}>20 mg/kg), PIREN (ED_{50}>3 mg/kg) or CYPRO (ED_{50}>10 mg/kg). In contrast, metergoline, a compound less selective for $5\text{-}HT_2$ vs. $5\text{-}HT_1$ sites, blocked or inhibited all three components of the syndrome at similar doses; TWITCH (ED_{50}=3 mg/kg), TREMOR (ED_{50}=3 mg/kg) and ABDUCT (ED_{50}=12 mg/kg). In conclusion, the syndrome is influenced by various oral treatments in a manner consistent with their putative effect on 5-HT receptors; thus, this method represents a model for the detection of compounds having differential affinities for $5\text{-}HT_1$ or $5\text{-}HT_2$ receptors in mice.

SEROTONIN, HISTAMINE AND OTHER BIOGENIC AMINES V

222.1

FENFLURAMINE NEUROTOXICITY: SELECTIVE DEGENERATION AND RECOVERY OF BRAIN SEROTONIN NEURONS.

J.F. Contrera, G. Battaglia, R. Zaczek and E.B. De Souza Neuroscience Branch, Addiction Research Center, NIDA, Baltimore, MD 21224 and FDA, Rockville, MD 20857.

Fenfluramine, a widely prescribed anorectic agent, has been reported to suppress brain serotonin (5-HT) systems. The present study investigates the neurotoxic effects of fenfluramine (1-24 mg/kg s.c., b.i.d. for 4 days) on rat brain 5-HT neurons with respect to dose-dependence, neuroanatomical specificity and timecourse of recovery. Neurotoxicity was assessed by decreases in 5-HT and 5-HIAA content and decreases in ^{3}H-paroxetine-labeled 5-HT uptake sites. Fenfluramine caused dose-dependent reductions in all serotonergic markers in the cerebral cortex, with maximal decreases (80%) in 5-HT, 5-HIAA, and 5-HT uptake sites observed at 12 and 24 mg/kg. Marked decreases in all 5-HT parameters were also observed in olfactory bulb, striatum, hippocampus, hypothalamus, midbrain, pons-medulla and cerebellum. Recovery of 5-HT uptake sites in cerebral cortex and hippocampus occurred over a protracted period of time. Following 75-80% reductions in 5-HT uptake sites at 18 hours after short-term treatment with fenfluramine, the initial rate of recovery was faster in cortex than in hippocampus; a 20% deficit was present in both brain regions as long as 8 months after drug administration. These data demonstrate that fenfluramine elicits potent, widespread and long-lasting neurodegenerative effects on brain 5-HT neurons.

222.2

FENFLURAMINE SELECTIVELY DESTROYS SEROTONIN TERMINALS IN BRAIN: IMMUNOCYTOCHEMICAL EVIDENCE.

Nathan M. Appel and Errol B. De Souza. Neuroscience Branch, National Institute on Drug Abuse, Addiction Research Center, Baltimore, MD 21224.

Fenfluramine is an amphetamine derivative which is used as an appetite suppressant. In rats fenfluramine treatment decreases brain serotonin (5HT) levels and inhibits high affinity uptake of 5HT. Recent studies in our laboratory have shown that fenfluramine decreases ^{3}H-paroxetine-labeled 5HT uptake sites in cerebral cortex and cerebellum. In view of these data we used immunohistochemistry to assess whether these neurochemical changes were associated with destruction of 5HT-containing neurons.

Male Sprague-Dawley rats were injected with fenfluramine HCl 24 mg/kg sc b.i.d. for 4 days. Twelve-18 h following the final injection rats were treated with tranylcypromine, anesthetized and perfused with buffered paraformaldehyde. Ten and 35 μm brain sections were processed for fluorescence and avidin-biotin-peroxidase complex immunohistochemistry, respectively, using antisera to 5HT or tyrosine hydroxylase (TH).

Fenfluramine treatment caused a profound reduction in fine-caliber 5HT-immunoreactive fibers and terminals visible in frontal, parietal and striate cortex. In cerebellum there was almost total depletion of 5HT-like immunoreactivity in both molecular and granular layers. Remaining 5HT-immunoreactive fibers showed cytopathologic features characteristic of degenerating neurons (i.e. thick, swollen and fragmented appearance). On the other hand 5HT-immunoreactive cell bodies in midbrain nuclei appeared to be spared. TH-like immunoreactivity seemed to be unaffected.

These data suggest that fenfluramine is neurotoxic and that this neurotoxicity is in part expressed by massive destruction of 5HT-containing fibers and terminals.

222.3

RITALIN AND PEMOLINE DO NOT CAUSE MONOAMINE TERMINAL DEGENERATION. R. Zaczek, G. Battaglia, J.F. Contrera and E.B. De Souza (SPON: N. Bernick). NIDA, Addiction Res. Ctr., Baltimore, MD 21224 and FDA, Rockville, MD 20857.

Several amphetamine-like stimulants such as methamphetamine (METH), fenfluramine and methylene-dioxymethamphetamine have been shown to cause monoamine terminal degeneration. We assessed the possible neurotoxic properties of pemoline (PEM)(20 and 60mg/kg) and Ritalin (RIT)(7 and 21mg/kg), two psychostimulants used clinically to treat attention deficit disorder. METH (5 and 15mg/kg) was included in the survey to validate the procedure.

Rats were injected s.c. with drug or vehicle twice daily for 4 days. One day after the last injection the rats were sacrificed, cerebral cortex and striatum were dissectd and analyzed for biogenic amine markers. Cortex was analyzed for norepinephrine (NE), NE transport sites, serotonin (5-HT) and 5-HT transport sites. Striatum was analyzed for dopamine (DA), DA transport sites, 5-HT and 5-HT transport sites.

While METH at the high dose caused significant depletions in all markers measured, neither PEM nor RIT elicited any decrease in monoamine markers.

We conclude that repeated injection of PEM or RIT does not cause a pattern of monoamine terminal damage similar to that observed after repeated injection of METH. Thus, use of PEM or RIT may carry less risk than the use of other amphetamine-like stimulants.

222.4

INCORPORATION OF ^{3}H-METHYLENEDIOXYAMPHETAMINE (MDA) INTO RAT BRAIN SYNAPTOSOMES. Steven Culp*, Robert Zaczek and Errol B. De Souza. Neuroscience Br., NIDA Addiction Res. Ctr., Baltimore, MD 21224.

MDA and its N-methyl derivative MDMA have been proposed as adjuncts to psychotherapy. Recently MDMA has surfaced as an illicit drug of choice. Several studies have demonstrated specific neurodegeneration caused by repeated injection of either MDA or MDMA in rats and monkeys. While attempts to demonstrate specific interactions of ^{3}H-MDMA with brain membranes have been unsuccessful, possible interactions of ^{3}H-MDA have not been studied. We have found that ^{3}H-MDA was incorporated into rat brain synaptosomes; this incorporation could be divided into three pools. ^{3}H-MDA associated with a saturable-non-specific pool which remained after synaptic membranes had been boiled for 15 min. Non-linear curve fitting analysis of ^{3}H-MDA saturation isotherms revealed specific high (Kd = .57μM) and low (Kd = 51μM) affinity components. The low affinity component was abolished by preincubation in hypoosmolar media indicating that it may represent sequestration. Eliminating the low affinity component left a single population of high affinity binding (Kd = 1.2μM). Pharmacologic profiles revealed subtle differences between the high and low affinity sites. While the inhibition profile for the low affinity site was: paroxetine = desipramine > 2,5 methoxy-4-methamphetamine (DOM) = MDA; the profile for the hgh affinity component was paroxetine > desipramine = DOM > MDA. Of the endogenous neurotransmitters used in the inhibition studies, 100uM serotonin inhibited 1.0uM ^{3}H-MDA incorporation by 26%, while neither dopamine nor norepinephrine were effective inhibitors at 100uM. Preliminary studies of ^{3}H-MDMA incorporation into rat brain synaptosomes indicate the presence of a site similar to the low affinity component of ^{3}H-MDA incorporation.

222.5

NEUROANATOMICAL SPECIFICITY OF MDA- AND MDMA-INDUCED DEGENERATION OF SEROTONIN NEURONS IN RAT BRAIN. G. Battaglia, J. Sharkey*, M.J. Kuhar and E.B. De Souza (SPON: A.C. Church). Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD 21224.

It has been demonstrated that the "designer" drugs MDA (3,4-methylenedioxyamphetamine) and MDMA (3,4-methylene-dioxymethamphetamine) cause long-lasting destruction of serotonin (5-HT) terminals in rat brain. We have used in vitro autoradiography of ^{3}H-paroxetine-labeled 5-HT uptake sites to assess whether specific 5-HT pathways may be differentially affected by these drugs. Following treatment with MDA or MDMA (20 mg/kg, b.i.d. for 4 days), marked decreases in 5-HT uptake sites were observed in a number of brain regions known to receive projections of 5-HT neurons. These regions included cerebral cortex, caudate nucleus, hippocampus, nucleus accumbens, olfactory tubercle, superior and inferior colliculi, geniculate nuclei and some thalamic nuclei. Other 5-HT projection areas such as the septal nuclei were not substantially affected. Additional brain areas that were less sensitive to the neurotoxic effects of MDA and MDMA were those containing 5-HT cell bodies (e.g. dorsal and median raphe and pontine nuclei) and 5-HT axons of passage (e.g. indusium griseum and lateral hypothalamus). These data suggest that MDA and MDMA cause preferential degeneration of 5-HT terminals whereas 5-HT perikarya and axons of passage are less affected.

222.6

MDMA SELECTIVELY DESTROYS BRAIN SEROTONIN TERMINALS IN RHESUS MONKEYS. J.N. Johannessen, T.R. Insel, G. Battaglia, M.J. Kuhar and E.B. De Souza. Lab. Clin. Sci., NIMH, Poolesville, MD 20837 and Neurosci. Branch, NIDA Addiction Research Center, Baltimore, MD 21224.

The potential neurotoxic hazard of 3,4-methylenedioxy-methamphetamine (MDMA, "Ecstasy") in humans was assessed by examining the effects of repeated systemic administration of MDMA (2.5 or 10 mg/kg, b.i.d. for 4 days) on selected neurochemical and behavioral measures in rhesus monkeys. Following either dose of MDMA, greater than 50% decreases in CSF concentrations of the serotonin (5-HT) metabolite 5-hydroxyindoleacetic acid (5-HIAA) were noted. By contrast, CSF concentrations of other monoamine metabolites showed little change. Eighteen hours after the last dose of MDMA, concentrations of 5-HT and 5-HIAA, but not dopamine or norepinephrine, were reduced by at least 70% in cerebral cortex, caudate and putamen. Furthermore, at the high dose of MDMA selective destruction of brain 5-HT terminals (decreases in 5-HT uptake sites) was observed; no significant decreases were noted in the densities of dopamine and norepinephrine uptake sites. Behavioral changes following MDMA were consistent with reduced 5-HT transmission in brain. We are currently investigating long-term changes in CSF monoamine metabolite concentrations following MDMA administration. These data demonstrating potent effects of MDMA on various brain 5-HT parameters in rhesus monkeys suggest that high doses may selectively destroy 5-HT terminals in humans.

222.7

[3H]PAROXETINE BINDING IS ASSOCIATED WITH SEROTONIN (5-HT) UPTAKE IN BLOOD PLATELETS OF HEALTHY CONTROLS. **L.J. Iny, M.J. Meaney, P. Desjardins*, R. Quirion & B.E. Suranyi-Cadotte. Douglas Hospital Research Centre, Dept. of Psychiatry, McGill Univ., Verdun, Que. H4H 1R3**

Altered 5-HT uptake is associated with the pathophysiology of various disorders. Recently, paroxetine was found to be a potent, selective inhibitor of 5-HT uptake, and specific sites for [3H]paroxetine have been associated with the 5-HT carrier in rat brain and platelets. Platelet [3H]paroxetine binding could thus be a useful index of changes in the 5-HT uptake system.

We investigated binding and uptake in parallel in blood platelets of healthy volunteers. A single class of high affinity [3H]paroxetine binding sites was observed, with a Bmax of 1416 ± 111 fmol/mg prot. The Vmax of [14C]5-HT uptake was 142 ± 22 fmol/105 platelets/2 min. A significant positive relationship between the two measures was found ($p<.05$). The results provide support for an association between [3H]paroxetine binding and the 5-HT carrier, and also suggest that platelet [3H]paroxetine binding may be a useful marker of the 5-HT uptake site.

222.8

USE OF TETRABENAZINE AS A TOOL TO DISTINGUISH DIRECT FROM INDIRECT 5-HT AGONIST EFFECTS ON 5-HT NEURON ACTIVITY IN VITRO. G.C. Rigdon and C.M. Wang. Dept. Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

5-HT reuptake blockers inhibit the firing of 5-HT neurons in vitro (SFN, 13:1648, 1987). This experiment was designed to determine if this inhibition was the result of direct agonist effects. Single unit recordings were obtained from slices of rat midbrain. The effects of increasing concentrations of the 5-HT uptake blockers, imipramine and fluoxetine and the direct agonist, 8-OHDPAT, on the firing rates of these dorsal raphe neurons were investigated in brain slices treated with and without (TBZ), a compound which depletes neuronal 5-HT. Brain slices were exposed to TBZ (10 uM) for 20-30 min prior to testing and 1 uM during testing. The concentration-response curves of both imipramine and fluoxetine were shifted to the right as was predicted. The IC50 values in untreated brain slices were; imipramine-2.7 ± 0.8 uM, fluoxetine-4.2 ± 2.1, and the IC50 values in TBZ treated slices were; imipramine-7.3 ± .7 uM, fluoxetine->>10 uM (50% inhibition not observed). TBZ treatment did not decrease the potency of the direct agonist, 8-OHDPAT; untreated slice IC50=1.8 ± 1.4 nM, TBZ slice IC50=0.9 ± 0.3 nM. This evidence supports the hypothesis that the inhibition of 5-HT neuron firing by antidepressant drugs in vitro is the not the result of a direct agonist effect.

222.9

EFFECTS OF HIGH-DOSE METHAMPHETAMINE (MA) ADMINISTRATION ON SEROTONIN UPTAKE SITES IN RAT BRAIN MEASURED BY QUANTITATIVE AUTORADIOGRAPHY. **G.B. Kovachich, C.E. Aronson* & D.J. Brunswick, VA Med. Ctr. & Univ. of Pa School of Medicine; Phila., PA 19104.**

High doses of MA cause long-term neurotoxic effects on serotonergic neurons in brain. In the present study the effect of MA on serotonin uptake sites in rat brain was examined by quantitative autoradiography using ^{3}H-cyanoimipramine (0.3nM) (Kovachich et al., Brain Res. in press). Rats were treated with either MA (15 mg free base/Kg N=6) or saline (N=6) given subcutaneously every 6 hrs for 5 doses and were killed 7 or 30 days following the last dose. MA caused large reductions in ^{3}H-cyanoimipramine binding in several brain regions examined both at 7 and 30 days after MA. Seven days after MA, greatest reductions were seen in the superior colliculus, central gray area and entorhinal cortex (53-61%). Reductions of 28-38% were seen in caudate putamen, parietal cortex, hypothalamus and hippocampus. In contrast, no significant effect was noted in the substantia nigra or dorsal raphe n.. Thirty days after MA, greatest reductions were seen in superior colliculus and entorhinal cortex (50-57%). Reductions of 30-36% were seen in parietal cortex, hippocampus and central gray area. Small reductions occurred in substantia nigra (19%) and dorsal raphe n. (14%). No significant reductions were seen in caudate putamen or hypothalamus. These results show that MA causes localized reductions in ^{3}H-cyanoimipramine binding in terminal areas of rat brain. The dorsal raphe n., a serotonin cell body area, is more resistant to the neurotoxic effect of MA. **(Supported by Research Funds from the Veterans Administration).**

222.10

IN VIVO LABELING OF SEROTONIN UPTAKE SITES WITH ^{3}H-PAROXETINE AND EFFECTS OF MDMA. U. Scheffel and P.R. Hartig*. Div. Nuclear Medicine and Dept. Environ. Health Sciences, The Johns Hopkins Medical Inst., Baltimore, MD. 21205.

3H-Paroxetine (^{3}H-P) is a potent and selective serotonin uptake site inhibitor in vitro. The purpose of this study was to characterize the in vivo binding of ^{3}H-P in mice with a view towards the development of a ligand for imaging 5-HT uptake sites in the living human brain.

^{3}H-P showed high uptake in the mouse brain, regional localization in agreement with the known distribution pattern of 5-HT uptake sites, and good regional selectivity. 3H-P in vivo binding to the hypothalamus was inhibited by 5-HT uptake blockers in a dose-dependent manner. There was an excellent correlation (r=0.99) with respect to the potency of these drugs in inhibiting ^{3}H-P binding in vivo vs. inhibition of 5-HT uptake in synaptosomes.

The effect of MDMA on in vivo 3H-P binding to 5-HT uptake sites in mice was studied: At one day after the last MDMA dose (8x 20 mg/kg), an up-regulation of 5-HT uptake sites was observed. There was no late (7 -14 days) neurotoxic effect on 5-HT uptake sites, in agreement with previous results in mice.

The data indicate that ^{3}H-paroxetine is a potent and selective in vivo label of 5-HT uptake sites which can be used to monitor 5-HT uptake site regulation in vivo.

222.11

EFFECTS OF MDMA ON CENTRAL SEROTONERGIC NEURONS IN NON-HUMAN PRIMATES: PERMANENT OR TRANSIENT? G. A. Ricaurte, L. S. Forno, S. G. Wiener, L. E. DeLanney, I. Irwin, J. W. Langston. Dept. of Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; Pathol. Dept., Vet. Adm. Med. Ctr., Palo Alto, CA 94304; Psychiat. Dept., Stanford Univ., Palo Alto, CA; Inst. Med. Res., San Jose, CA 95128.

The purpose of this study was to determine if the effects of 3,4-methylenedioxymethamphetamine (MDMA) on central serotonergic neurons in non-human primates are reversible. Previous studies indicate that administration of 5 mg/kg of MDMA s.c. twice daily (0900 and 1700 hours) for 4 days to squirrel monkeys results in an 75-86% depletion of regional forebrain serotonin, and in the appearance of abnormal inclusion bodies in nerve cells in the dorsal raphe nucleus (Ricaurte et al., JAMA, in press). These observations were made in monkeys killed two weeks after MDMA administration. We now report that monkeys killed ten, rather than two, weeks after an identical regimen of MDMA have only a 30-47% depletion of regional forebrain serotonin, and no inclusions in the dorsal raphe nucleus. Moreover, these monkeys also show recovery of 5HIAA in CSF over the 10 week period. There was no evidence of cell loss in the midbrain raphe of ten-week survival animals. These findings suggest that serotonergic deficits produced by the aforementioned regimen of MDMA in the monkey are reversible, and that inclusion bodies in the dorsal raphe nucleus do not herald cell death, but more likely reflect reaction of the cell body to axonal injury.

222.12

ELECTRON MICROSCOPIC STUDY OF THE DORSAL RAPHE NUCLEUS IN THE MDMA-TREATED SQUIRREL MONKEY. L. S. Forno, G. A. Ricaurte, L. E. DeLanney, I. Irwin, J. W. Langston. Pathology Dept., Vet. Adm. Med. Ctr., Palo Alto, CA 94304; Dept. of Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; Inst. for Med. Res., San Jose, CA 95128.

We have recently shown that when 3,4-methylenedioxymethamphetamine (MDMA) is administered systemically to squirrel monkeys, it produces not only damage to serotonin-containing nerve terminals, but also marked changes in nerve cell bodies in the dorsal raphe nucleus (Ricaurte et al., JAMA, in press). Specifically, MDMA induces formation of PAS-positive inclusions in nerve cells of the dorsal, but not median, raphe nucleus. These inclusions were present in three of three six year old monkeys that had received MDMA two weeks previously. Electron microscopic examination of the dorsal raphe nucleus of a one year old monkey administered MDMA two weeks earlier shows loss of cisternae of rough endoplasmic reticulum, constricted, thread-like mitochondria, and an increase in dense bodies with early stages of lipofuscin or ceroid formation. In another young monkey, examined ten weeks after MDMA, no inclusion bodies could be found by light microscopy, and by electron microscopy, only small amounts of lipofuscin were present. These preliminary studies demonstrate an effect of MDMA on the phagolysosomal system, and suggest that nerve cell changes after MDMA may be reversible.

222.13

EVIDENCE FOR A MONOAMINERGIC LINK IN THE NEUROTOXICITY OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA). V.L. Taylor* and C.J. Schmidt* (SPON: P.J. Robinson). Merrell Dow Res. Inst., 2110 E. Galbraith Road, Cincinnati, OH 45215

The psychedelic amphetamine analog, MDMA, is a selective serotonergic neurotoxin when administered to rats in high doses. A single dose of MDMA (20 mg/kg) resulted in a decrease in cortical tryptophan hydroxylase (TPH) activity and 5-HT concentrations for one month post-drug. Pretreatment with the centrally active inhibitor of aromatic l-amino acid decarboxylase, α-monofluoromethyl-dopa (MFMD, 100 mg/kg daily x 3), antagonized the MDMA-induced loss of both cortical TPH activity and transmitter concentrations observed at 1 week. The neurotoxicity of MDMA was also antagonized when rats were pretreated with the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine. To determine if dopamine was involved, unilateral 6-OH-dopamine lesions of the substantia nigra were performed on rats 1 week prior to MDMA administration; serotonergic markers in contralateral and ipsalateral structures then were compared. Although the lesions reduced ipsalateral dopamine to less than 10% of control, striatal 5-HT and cortical TPH activity were reduced to a similar extent on both sides 3 hr after MDMA. Identical results were observed in animals allowed to survive 1 week after MDMA. The results suggest a catecholaminergic link in the serotonergic neurotoxicity of MDMA; however, the anatomical basis for the role of catecholamines is unresolved.

222.14

5-HIAA IN CEREBROSPINAL FLUID REFLECTS DAMAGE OF SEROTONERGIC NEURONS INDUCED BY MDMA IN CNS OF NON-HUMAN PRIMATES. S.G. Wiener, G. A. Ricaurte, L.E. DeLanney, I. Irwin and J.W. Langston. Department of Psychiatry, Stanford University School of Medicine, Stanford, CA 94305; Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205; Institute for Medical Research, San Jose, CA 95128.

This study assessed the feasibility of using 5-hydroxyindoleacetic acid (5HIAA) in cerebrospinal fluid (CSF) as a marker for serotonergic neurotoxicity induced by (±)3,4-methylenedioxymethamphetamine (MDMA) in non-human primates. Monkeys were administered doses of MDMA known to produce toxic effects on central serotonergic neurons. Two weeks later, the animals were lightly anesthetized with ether and samples of CSF were collected by means of cervical puncture. Later that same day, monkeys were killed for determination of brain and spinal cord serotonin concentrations. Monoamines and their major metabolites were measured using HPLC-EC methods. Monkeys with 80-90% depletions of regional forebrain serotonin and a 42% depletion of cervical cord serotonin had a 60% reduction in CSF 5HIAA, with unchanged homovanillic acid (HVA) or 3-methoxy-4-hydroxyphenethyleneglycol (MHPG). These findings suggest that CSF 5HIAA can be used to index MDMA-induced serotonergic neurotoxicity in non-human primates, and indicate that it may be possible to probe for MDMA-induced neuronal damage in humans by means of CSF 5HIAA analysis.

222.15

EFFECT OF OPTICAL ISOMERS OF 3,4-METHYLENEDIOXY-METHAMPHETAMINE (MDMA) ON STEREOTYPED BEHAVIORS AND NEUROTRANSMITTER RELEASE INTO RAT BRAIN DIALYSATE IN VIVO

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MDMA elicits behavioral effects characteristic of both the amphetamine and the hallucinogenic phenylalkylamines. High doses of MDMA were found to cause a dramatic depletion of 5-HT and its metabolite, 5-HIAA and these effects are similar to the 5-HT releaser, p-chloro-amphetamine (PCA). However, little information is available regarding the differences in optical isomers of MDMA on behavioral effects and neurotransmitter release in vivo. When examined within 1 hr S(+)-, R(-)-MDMA and S(+)-MDA produced stereotyped behavioral responses such as sniffing, head-weaving, backpedalling and turning. These responses were similar to those induced by PCA. S(+)-MDMA was more potent than the R(-) enantiomer in eliciting head-weaving, backpedalling and turning behaviors. It is known that MDMA and PCA interact with both the 5-HT- and dopamine-systems by causing an increased efflux or release of these transmitter in vitro. PCA increased both transmitter release and decreased their acidic metabolites in brain dialysates. The differences in transmitter release between S(+)- and R(-)-MDMA will be presented.

222.16

NOVEL METHAMPHETAMINE ANALOG CAUSES LONG-LASTING DEPLETION OF DOPAMINE IN THE RODENT BRAIN.

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N,N-dimethylamphetamine (N,N-DMA) is an analog of methamphetamine (METH) which has recently surfaced on the illicit drug market in various parts of the United States. This new analog ("designer drug") is synthesized via reduction of N-methylephedrine, a non-restricted analog of ephedrine. The aim of the present study was to evaluate the neurotoxic potential of N,N-DMA, and compare it to that of METH. Mice repeatedly administered various doses (10, 20 and 40 mg/kg) of N,N-DMA and analyzed for brain monoamine content one week later showed a dose-related depletion of dopamine, with the highest dose producing approxiamately a 30% depletion. N,N-DMA did not alter the concentration of serotonin in various regions of the mouse brain. As mice tend to be relatively resistant to the serotonin-depleting effects of amphetamines, the effects of N,N-DMA were also examined in rats. Again, N,N-DMA produced a depletion of dopamine but not serotonin. Compared to METH, N,N-DMA was approximately one fifth as potent at depleting caudate dopamine in both mice and rats. These results suggest that both the dopamine- and serotonin-depleting effects of N,N-DMA are less pronounced than those of METH, and indicate that methylating the nitrogen group of METH attenuates its neurotoxic activity.

222.17

EFFECT OF THE INHIBITION OF DEAMINATION OF 5-HYDROXY-TRYPTAMINE WITHIN SEROTONERGIC NERVE ENDINGS ON THE UPTAKE OF 5-HYDROXYTRYPTAMINE.

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Km and Vmax of the uptake of 14C 5-hydroxytryptamine (5-HT) into 5-HT synaptosomes were determined in presence and absence of pargyline (100 uM), a monoamine oxidase (MAO) inhibitor. Synaptosomes of rat brain were prepared according to the discontinuous sucrose density gradient method. It was confirmed that the preparation is free of extrasynaptosomal MAO. 14C 5-HT uptake was expressed as the sum of total 14C 5-HIAA formed plus 14C found in synaptosomes minus 14C 5-HIAA in the synaptosomes. In absence of pargyline, Km of 14C 5-HT uptake was o.2 uM, Vmax 29 p mol/mg protein/2 min. In presence of pargyline, Km of 14C 5-HT uptake was 0.059 uM, Vmax 5.9 p mol/mg protein/2 min. From these results, we concluded that the inhibition of 5-HT deamination in 5-HT synaptosomes significantly decreases the maximum capacity of 5-HT synaptosomes for 14C 5-HT uptake without significant change in their affinity for 14C 5-HT. We propose the reduced uptake may result from an increase in the cytoplasmic concentrations of 14C 5-HT in 5-HT synaptosomes following MAO inhibition.

222.18

A DOSE-RESPONSE STUDY OF p-CHLOROAMPHETAMINE (PCA) EFFECTS ON AUDITORY AND TACTILE STARTLE HABITUATION IN RATS AND MICE.

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Davis et al. (1986) suggested that 5-HT may differentially alter startle if it acts primarily in the forebrain or spinal cord, i.e., 5-HT may depress startle via actions in the forebrain but increase startle via actions in the spinal cord. In our laboratory, 5 mg/kg PCA has produced an ca 25% increase in auditory startle in rats during expression of the "5-HT syndrome," but no change in startle following 5-HT depletion 24 hrs later. In this study, auditory and tactile startle was evaluated in groups of 13 male and 13 female CD rats (0, 5, 10, or 20 mg/kg PCA ip), and 8 CD-1 mice (0, 10, or 20 mg/kg PCA ip) before and 15-min and 48-hrs following dosing. Startle testing occurred in SDI chambers and each 50-trial session was made up of alternating 5-trial blocks of auditory (120 dBA noise) and tactile (20 psi air puff) stimuli presented at 8-sec intervals. One-hr activity levels were monitored in these animals prior to and 3-4 days following PCA administration. Across all treatment groups rats, but not mice, showed greater tactile startle than auditory startle amplitudes. Within-session habituation on both auditory and tactile trials was more evident in rats than mice. All PCA-treated rats, but not mice, displayed the characteristic 5-HT syndrome 15-min after dosing. All doses of PCA produced slight increases in auditory and tactile startle 15-min post dosing in male rats and mice, but not in female rats. At 48 hrs, no change in startle elicited via either modality was evident in rats, but a slight (ca 10-15%) dose-related decrease was seen in mice. These data do not suggest that PCA-induced release and depletion of 5-HT differentially alters auditory or tactile startle in this paradigm.

PAIN PATHWAYS: LONG-TERM CHANGES

223.1

ALTERATIONS WITHIN LAMINA I OF THE RAT DORSAL HORN FOLLOWING NEONATAL CAPSAICIN TREATMENT.

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We have been examining the secondary changes that the injection of capsaicin and the subsequent early loss of central C fiber terminations may have on the organization of spinothalamic tract cells. Four adult rats that had previously been injected subcutaneously on postnatal day 2 with capsaicin (50mg/kg), two adult animals injected on postnatal day 2 with vehicle and two uninjected adult animals were deeply anesthetized and perfused with 10% phosphate buffered formalin. Spinal segments C2-C4 and L3-L4 were embedded in plastic, serially sectioned at 2μm and stained with cresyl violet. The number of neuronal somata and the number of neurons per unit area of lamina I were determined from camera lucida drawings and photographs of the dorsal horn of every 15th section. While there was a trend for lamina I of capsaicin treated animals to have fewer neurons, no significant differences were detected at any spinal level in the total number or density of neurons within lamina I between any groups. These data indicate that either the present method was not sufficiently sensitive to detect the death of spinothalamic tract neurons within lamina I or that the previously reported inability to label these neurons following capsaicin treatment is due to the reorganization of their central connections.

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223.2

SPINOTHALAMIC TRACT (STT) CELLS SIGNAL THE PAIN AND HYPERALGESIA FOLLOWING INTRADERMAL CAPSAICIN.

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Intradermal (ID) injection of capsaicin (CAP) produces burning pain, hyperalgesia to heat at the injection site, and mechanical hyperalgesia in a large area surrounding the injection site. In the present study, we examined the effect of ID CAP on responses of identified STT cells. Recordings from single lumbosacral STT cells were made in anesthetized monkeys. Cells were classified according to their responsiveness to graded mechanical stimulation as high threshold (HT) or wide dynamic range (WDR). Responses to a 10 μl injection of vehicle or 100 μg CAP were recorded for 5 min after injection. Four of 5 HT and 9 of 10 WDR cells responded vigorously to ID CAP. At the injection site, the median heat threshold of WDRs was lowered from >50° to 34°C. Mechanical responsiveness of 2 HT and 7 WDR cells to von Frey filaments and to stroking the skin lightly with a cotton swab was enhanced within a radius of >4 cm around the injection site. Vehicle effects were weak or absent. These results correlate with those from psychophysical studies in humans and demonstrate an important role for STT cells in mediating pain and hyperalgesia.

(This work was supported by NIH grants NS09743, NS11255, NS21266 and NS14624.)

223.3

ALTERATIONS IN TRIGEMINAL NUCLEI FOLLOWING APPLICATION OF TOXIC RICIN TO ADULT FELINE DENTAL PULPS. R.C. Canfield*, M.A. Henry, and L.E. Westrum (SPON: D. Farrell). Depts. of Neurol. Surgery, Biol. Structure, Restorative Dent., Univ. of Washington, Seattle, WA 98195.

Previous studies (J. Neurocytol. 16:601, 1987) showed extensive axonal and terminal degeneration of primary afferents in brain stem trigeminal nuclei (TNC) at 6-10 days after intrapulpal application of toxic ricin. We are using light (LM) and electron microscopic (EM) methods to investigate the more chronic effects of ricin application to pulps on TNC. At 20 days survival silver stains show persisting degeneration bilaterally in the same regions as in those from shorter survivals. Although, argyrophilic neurons were not seen in the silver stains, semi-thin sections of EM preparation show "dark neurons" in regions known to receive dental afferents. EM shows dramatically increased glycogen near blood vessels and within glia and altered terminals and dendrites suggesting a response to injury. Electron dense somata and dendrites are seen as are dendrites with abnormal vacuolization. Terminal alterations occur in EM preparations as earlier, but markedly dense ones appear to be reduced. Growth-cone-like profiles, not seen previously, are also seen. The findings show increased degenerative changes in postsynaptic structures and possible new axonal growth. (Supported by NIH Grants DE00219, DE04942 and NS09678. LEW is an affiliate of the CDMRC.)

223.4

MYELINATED AFFERENTS IN AN ACUTELY INJURED NERVE DEVELOP A RESPONSE TO VIBRATORY STIMULI. C. M. Koschorke*, R. A. Meyer, and J. N. Campbell. Dept. Neurosurgery and Applied Physics Lab., The Johns Hopkins University, Baltimore, MD 21205

Recently, we demonstrated that regenerating nerve sprouts in mature neuromas exhibit a neural response to vibratory stimuli over the frequency range of 5 to 100 Hz. In the present study, we sought to determine when this vibratory sensitivity first occurs. In pentobarbital anesthetized monkeys, the sural nerve was acutely crushed, ligated, and transected immediately distal to the ligature. Six small strands were dissected from the parent nerve proximal to the transection site, and action potential activity from myelinated afferents was recorded from these strands for the next 13 hours. A displacement-controlled mechanical stimulator was used to apply sinusoidal stimuli to the transection site. A neural discharge to vibratory stimuli first appeared 6.5 hours after the nerve was transected in 7 A-fibers (5%). By 13 hours after the transection, 22 A-fibers (17%) had developed a response to vibratory stimuli. These results indicate that mechanical sensitivity develops within hours of a nerve injury. This is consistent with the hypothesis that the mechanical-to-electrical transducer, which is responsible for the mechanical sensitivity of normal cutaneous mechanoreceptive afferents, is transported down the nerve fibers and becomes functional in the regenerating membrane.

223.5

Dose related actions of capsaicin on nociceptive afferent fibres of the neonatal rat tail in vitro. **A.Dray, J.Bettaney*, P.Forster* and M.N.Perkins* (SPON:R.M.Lindsay). Sandoz Institute for Medical Research, Gower Place, London, England.**

Capsaicin produces a variety of effects on polymodal nociceptors including activation, desensitization and conducation block. We have studied the relationship between these effects and determined whether they are dependent on extracellular calcium.

In an in vitro preparation of the spinal cord and attached tail isolated from 1-2 day old rats, ventral root (L3-L5) depolarization was used to measure the activation of peripheral fibres by noxious stimuli. During superfusion of the tail with normal calcium (1.26mM) the depolarization produced by a submaximal dose of capsaicin (ED50 = 0.5 uM) was reproducible. Prolonged administration of capsaicin at 0.2 - 2.0 uM produced complete desensitization which was reversible after many hours but the effects of other noxious (heat, bradykinin) and innocuous stimuli were unchanged. Desensitization following 20-50 uM doses was accompanied by impaired responsiveness to other noxious stimuli. Complete removal of calcium did not reduce capsaicin evoked depolarization or desensitization. However in 20mM calcium depolarization by capsaicin was reduced and capsaicin evoked impairment of afferent fibere responsiveness to other stimuli was increased.

These data suggest that capsaicin evoked desensitization and the impairment of C-fibre transmission are separable. In addition external calcium does not play an important role in the activation or desensitization of nociceptors.

223.6

SENSITIVITY OF CULTURED NEONATAL RAT DRG NEURONS TO LOW DOSES OF CAPSAICIN: A PATCH-CLAMP STUDY. M. Alreja,*[1] D. Lo,[2] and R.H. LaMotte[1]. Department of Anesthesiology[1] and Section of Molecular Neurobiology[2], Yale Univ. Sch. of Med., New Haven, CT 06510.

The sensitivity of membrane currents of cultured rat DRG neurons to low doses of capsaicin was tested. Neurons were enzymatically dissociated from 1-7d old rat DRGs and 3-30 days later whole-cell voltage clamp recording was carried out using standard patch-clamp techniques. Early inward and delayed outward currents were produced by step depolarizations from a prepulse of -90 mV. In between trials the cells were maintained at a potential of -70 mV. Steady state inactivation was determined using depolarizing pre-pulse protocols. Capsaicin (54.5 mM stock solution in ethanol) was diluted to its final concentration (100pM-10μM) in the bath solution and applied for 15 sec to 10 min. Capsaicin but not vehicle, decreased and then completely abolished the early inward and delayed outward currents in >80% of the cells. This effect was seen both in small (>20 μm diam.) and large (>28 μm) cells. Some cells were responsive even to the smallest doses. The decline in the currents was more gradual with lower doses. Recovery of the response was not seen with the micromolar doses for up to 90 minutes after capsaicin. An increase in cell size was sometimes observed following capsaicin.

Supported by NIH grant NS14624

223.7

ELECTROPHYSIOLOGICAL PROPERTIES OF ADULT RABBIT TRIGEMINAL NEURONS IN DISSOCIATED CELL CULTURE.
T.K. Baumann and R.H. LaMotte, Dept. of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510

While dorsal root ganglion (DRG) neurons have been studied extensively with intracellular methods, trigeminal neurons have so far received much less attention. Also, the majority of DRG neurons studied in cell culture were derived from newborn animals. We used whole cell patch clamp recording as the first step in the electrophysiological characterization of cultured adult rabbit trigeminal neurons. Neurons with relatively small cell bodies (soma diameters ranging from 24 to 36 μm) were selected. In response to prolonged (80 ms) depolarizing current pulses most fired only one action potential. Fewer fired repetitively, with the frequency of discharge increasing with the strength of the stimulus, reaching 200 Hz when 1 nA of current was injected. Two kinds of neurons were found, based on responses to hyperpolarizing current pulses. One type showed a pronounced time-dependent rectification, whereas the other did not. The latter had action potentials of relatively long duration (>4.5 ms). Thus, adult trigeminal neurons in cell culture display characteristics of in vivo trigeminal neurons studied recently by others.

Supported by NIH NS 14624 and the Johns Hopkins University Center for Alternatives to Animal Testing.

223.8

ELECTROPHYSIOLOGICAL EVIDENCE SHOWING GROWTH OF VENTRAL ROOT AFFERENTS AFTER INJURY TO SCIATIC NERVE IN NEONATAL RATS. K.J. Kim, U.T. Oh*, S.C. Nam* and J.M. Chung. Marine Biomed. Inst. and Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77550.

We previously reported that many ventral root (VR) afferent fibers are the third branches of dorsal root (DR) ganglion cells. In this study, we examined the possibility of growth of these processes in the VR after an injury to the peripheral branches.

In 10 anesthetized neonatal rats (1-7 days after the birth), the sciatic nerve on one side was cut at the level of mid-thigh. Ninety to 120 days after surgery, compound action potentials were recorded from the L5 VR while electrically stimulating the DR of the same segment. The size of the C-fiber elicited compound action potentials was compared between the operated and unoperated control sides for 17 pairs. As compared to the control side, the size of compound action potentials recorded in the operated side was increased at least 50% in 9 cases. In 6 pairs, the size was similar, while smaller in the injured side for 2 other pairs. The size of response recorded at the distal site was larger than that at the proximal site in the operated side. This difference, however, was not seen in the control side.

These results suggest that some VR afferents in the rat grow centrally when sciatic nerve is injured in the neonatal stage. (Supported by NIH NS21266 and RCDA NS00995)

223.9

EFFECT OF SCIATIC NERVE TRANSECTION ON THE NUMBER OF UNMYELINATED FIBERS OF THE L5 VENTRAL ROOT IN ADULT RAT. J.W. Leem*, S.C. Nam*, K.J. Kim, K. Chung and J.M. Chung. Marine Biomed. Inst. and Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77550

The present study examined the possibility of unmyelinated axonal sprouting into the ventral root (VR) after peripheral nerve injury in the adult rat.

The right sciatic nerve of 5 adult rats was tied and cut at the level of mid-thigh under sodium pentobarbital anesthesia. After 5 months of survival time, the animals were perfused and fixed. Bilateral L5 VRs were removed and divided into three segments along the length of the root. Each segment was processed for electron photomicrography. The number of both myelinated and unmyelinated fibers was counted and compared between the operated and unoperated sides.

In both sides, the number of unmyelinated fibers in the VR was largest at the distal segment and decreased as the spinal cord was approached. There was no significant difference in the number of myelinated fibers between the control and experimental sides at any segment. As compared to the control side, the number of unmyelinated fibers on the experimental side was about 3 times larger in all 3 segments.

These data suggest that there is unmyelinated axonal sprouting in the VR after sciatic nerve injury in adult rats. (Supported by NIH NS21266, NS11255 and RCDA NS00995)

223.10

THE DEAFFERENTATION SYNDROME IN THE RAT: EFFECTS OF BILATERAL PARAFASCICULARIS LESIONING. Mark A. Lyerly, Janice Ovelmen-Levitt, and Blaine S. Nashold, Jr., Div. of Neurosurgery, Duke Univ. Med. Ctr., Durham, NC 27710

A deafferentation syndrome (DS), produced in rats by C5-T2 dorsal root ganglionectomies (DRGs), is expressed as scratching of partially afferented &/or biting (autotomy) of anesthetic limb areas. This objective evidence is used as an experimental model to study chronic dysesthesias &/or pain from deafferentation. This study included behavioral observations of the DS and the effects of bilateral parafascicularis nuclei lesions (PFL) on its expression. A total of 28 male Sprague-Dawley rats were used: 5 underwent PFL without deafferentation (PO); 14, DRGs (DO); and 9, PFLs 5 days prior to a DRG. The PO animals exhibited no autotomy. The DO rats demonstrated early onset, more severe and later onset, less severe biting groups ($p<0.01$ Mann-Whitney U), with 6/14 DO rats biting by the 3rd post-op day (13/14 by day 90), and 9/14 having scores of >5. Among rats having PFLs then DRGs, only 1/9 bit by the 3rd post-deafferentation day (5/9 by day 90), and only 4/9 had scores >5; both a lower % of animals biting and a lower total autotomy score than the DO rats. These results indicate that parafascicularis nuclei may play a role in the development/control of chronic pain; their lesioning apparently decreases both the degree of autotomy and percentage of animals expressing it.

223.11

THE DEAFFERENTATION SYNDROME IN THE RAT: EFFECTS OF FRONTO-PARIETAL CORTICAL LESIONS. J. Ovelmen-Levitt, E. Rossitch, Jr.,* J. Young,* and B. S. Nashold, Jr. (SPON:I. Diamond) Div. Neuros., Duke U. Med. Ctr., Durham, N.C. 27710

Forelimb sensory-motor cortical lesions in the rat have been found to suppress the deafferentation syndrome which follows C5-T2 ganglionectomy-avulsions whether the cortical lesions were made prior to or several days after the dorsal root lesions. The syndrome consists of biting and scratching of the affected limb. The self mutilation was observed daily for 45 days post-ganglionectomy and the degree of this was assigned a score of 0-19. Ten rats received dorsal root lesions only (G/A(0); 5 animals had cortical lesions only (SI(0); 6 rats had craniotomy only before or after dorsal root lesions (CR(1), CR(2); while 13 animals had cortical lesions either preceding (SI(1)) or following (SI(2) the ganglionectomy-avulsions. Only 2 of the 6 SI(1) rats (33%) bit by 45 days post-op, while 100% (10/10) of the G/A(0) rats had bitten by that time. The G/A(0) animals achieved a mean score of 15.2, while the SI(1) rats had a mean score of 2 at 45 days. The SI(2) achieved a mean score of 4.5. Although 85% of SI(2) bit by 45 days, 37% had begun biting before the cortical lesions were made. SI(1) and SI(2) groups of rats are significantly different populations from G/A(0) Mann-Whitney U:$p < .05$. SI(0) animals did not bite. CR(1) and CR(2) were not different from G/A(0). These results indicate that the fronto-parietal neocortex may be involved in the development and/or expression of chronic central pain.

223.12

ALTERATIONS OF THE THALAMIC SOMATO-SENSORY MULTIUNIT RESPONSE BY AN EXPERIMENTAL PERIPHERAL NERVE INJURY IN THE RAT. A. Louveau *, L. Jeaugey * and A.L. Benabid. LMCEC, INSERM U. 318 , Grenoble Univ., 38700 La Tronche, FRANCE

Studies about mechanical lesions of peripheral nerves are of particular interest because of their high frequency in traumatology. We developped an experimental model in the rat to investigate the suffering of the different groups of neural fibers, during the gradual compression of the sciatic nerve. 40 adult OFA rats (190-210 g) were investigated under chloral hydrate anaesthesia (400 mg/kg i.p.), paralysed with Pancuronium Bromide (0.3 mg/kg i.v.), tracheostomized and artificially ventilated. Electrical stimulation (0.5-50 V) was delivered through bipolar hook electrodes to the dissected isolated sciatic nerve. Multiunits responsive to sciatic stimulation were recorded in the VPL nucleus of the Thalamus. Changes in cell activity were studied using post stimulus time histograms (PSTH). Calibrated compression of the sciatic nerve was performed by a weighted piston . The stimulation induced somatosensory VPL response was made of a short latency (15 ms) low threshold (1 V) input carried by Ab fibers (peak 1) and followed, after a 60 ms inhibitory period, by a discharge lasting from 100 to 500 ms (peak 2). This late discharge was thought to be carried by Aδ and C fibers for it was elicited at higher thresholds (up to 50 V), it was selectively suppressed by systemic morphine (10 mg/kg i.v.) and was restored by systemic naloxone(1 mg/kg i.v.).This evoqued pattern in VPL was altered by a gradual compression of the sciatic nerve downstream to the stimulation site. Peak 1 was more sensitive to compression since it was selectively suppressed by a weak compression (50 g). Peak 2 was erased by a 3 to 4 times stronger pressure (150-200 g). Under constant weight, the early inputs suffered faster than the late inputs and recovered later after the release of compression, depending on its duration. These thalamic recordings confirm that the large myelinated fibers are more sensitive to traumatism than the thin unmyelinated fibers and show that the thalamic cells activity reflects these peripheral nerve lesions.

223.13

Do Calcium Spikes Account for the Bursts of Action Potentials which occur in Sensory Thalamus of Central Pain Patients? F Lenz, J Dostrovsky, H Kwan, T Hirayama*, R Tasker*, J Gorecki*. Univ. of Toronto, Toronto, Canada.

Previous work has demonstrated abnormal activity in the sensory thalamus of patients with central pain secondary to spinal cord injury (Lenz et al. Pain 31:225). Neurons which had lost their normal sensory input exhibited increased activity and tended to fire in bursts of action potentials. These bursts have now been characterized by application of techniques for the analysis of neuronal firing patterns (25 cells).

Bursts consisted of 3 to 7 action potential (AP) occurring at frequencies of greater than 200 Hz. Within a burst, each interspike interval (ISI) was longer than the preceding one. The duration of the first ISI in a burst was inversely related to the number of APs in the burst. When a single neuron had APs consisting of an initial positive and subsequent negative component, the negative component was larger for the first AP in the burst than for subsequent APs in the burst. This pattern is characteristic of bursts associated with the occurrence of underlying calcium spikes in intracellular studies of thalamic nuclei and suggests that an underlying calcium spike may explain the bursting activity of thalamic neurons in pain patients. If the perception of pain is related to this abnormal activity then the mechanism of bursting suggested by the present results may have significant therapeutic implications.

224.1

Sensitization of high threshold mechanoreceptors in the oral cavity of the goat. Friedman*, R. Cooper, B.Y., Ahlquist*, M.L., and Loughner*, B.A. (Spon: C.J. Vierck, Jr.) Departments of Neuroscience, Oral and Maxillofacial Surgery, and Pain Center, University of Florida, Gainesville, Florida, 32610.

Properties of high threshold mechanoreceptors (HTM) of the goat oral cavity were examined.

HTMs in fresh tissue and had relatively high thresholds (.68 nt/mm2), punctate receptive fields and slow conduction velocities (.5-25 m/sec). With repeated stimulation, afferents either fatigue or remain stable in response to slowly accelerating mechanical forces (n=24). Tonic activity could be occasionally observed from units previously quiescent (n=12). This activity fell into two categories: continuous and bursting.

HTMs in carrageenan inflamed tissue had generally lower thresholds (.55 nts/mm2) and much improved dynamic response properties (n=35). Tonic activity of both kinds (see above) could be obtained from either quiescent or driveable afferents (n=23). The success rate of sampling HTMs was greatly improved in inflamed tissue (250%). Similar improvements were apparent for observations of tonic activity. These differences are likely to be due to release or synthesis of endogenous substances.

Studies of afferent chemosensitivity, in fresh tissue, suggested that afferent response properties could be greatly improved by exposure to serotonin (5 ug/ul, i.a.). Both increases in dynamic response properties and transitions to tonically active forms could be observed.

224.2

RESPONSES OF T_2-T_7 DORSAL HORN CELLS TO DISTENSION OF INFLAMED AND NORMAL DISTAL ESOPHAGUS IN CAT. **DW Garrison, MJ Chandler and RD Foreman. Depts. Physical Ther. and Physiol., Univ. Okla. HSC, Okla. City, OK 73190.**

Esophageal disease can be associated with an inflamed esophagus. We hypothesized that T_2-T_7 dorsal horn cells would have a lower threshold of activation to distension of inflamed distal esophagus as compared to normal esophagus. In 21 anesthetized vagotomized cats the internal distal esophagus was exposed to a gauze soaked in turpentine for either 1.5 or 3 hr. Histological examination confirmed that inflammation had occurred. The left gray matter of the T_2-T_7 spinal cord was searched with microelectrodes to record activity of dorsal horn cells. Seventeen cells were excited with distension of the distal inflamed esophagus by water injected into the balloon of a Foley catheter. A distension volume of 2.4 $\pm$ 0.3 ml was required to reach threshold for increasing cell activity. In experiments where the distal esophagus was not inflamed, 18 cells required a threshold distension volume of 8.8 $\pm$ 0.8 ml to increase cell activity. The threshold for activating T_2-T_7 dorsal horn cells was significantly less for distension of inflamed esophagus as compared to normal esophagus ($P<0.001$). These data may explain why mechanical events such as swallowing or esophageal spasm are innocuous in normal individuals but may elicit pain in patients with esophageal disease. (Supported by HL22732, NS08150, Presby. Hlth. Found.)

224.3

ADJUVANT-INDUCED INFLAMMATION IS ASSOCIATED WITH INCREASED DYNORPHIN IMMUNOREACTIVITY IN BOTH LOCAL CIRCUIT AND PROJECTION NEURONS IN LUMBAR SPINAL LAMINA I OF THE RAT. R.L. Nahin, J.L.K. Hylden and R. Dubner, Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD., 20892.

Increases in spinal dorsal horn dynorphin (DYN) levels are associated with peripheral inflammation induced by local injections of Complete Freund's Adjuvant (CFA) (Ruda et al., PNAS, 85(1988) 622-626; Iadarola et al., BR, In Press). The present study combined the retrograde transport of Fluorogold with the immunocytochemical identification of DYN A_{1-8} in superficial dorsal horn neurons to examine whether the CFA-induced DYN increase is found in local circuit neurons only or also in projection neurons.

Lamina I projection neurons were retrogradely labeled following injections of Fluorogold (0.1-0.2 μl) into the ponto-mesencephalic parabrachial area. Ten to 14 days after injections, rats were administered colchicine (70 μg) onto the lumbar spinal cord. Twenty-four hours later, the rats were perfused with 4% paraformaldehyde. Some rats received injections (75 μg : s.c.) of CFA into one hindpaw 1 to 5 days prior to colchicine administration. The lumbar spinal cord (L3-6) was cut and processed for standard fluoresence-immunocytochemistry using an antibody against DYN A_{1-8}.

Ipsilateral to CFA injections, the total number of DYN-like immunoreactive (DYN-LI) neurons increased 300% ($p<.01$) in lamina I as compared to the contralateral side. Although this increase consisted of both single-labeled and retrogradely-labeled DYN-LI neurons, the proportional increase was greater (275% vs 1100%; $p<.01$) for retrogradely labeled neurons. Mirroring this, the percentage of retrogradely labeled lamina I DYN-LI neurons rose from 5% of the total lamina I DYN-LI pool in naive animals or contralateral to CFA injections, to 20% ipsilateral to CFA injections. We conclude that CFA-induced inflammation has a differential effect on the increase in the number of local circuit and projection DYN-LI neurons in lumbar spinal lamina I.

224.4

TEMPORAL RELATIONSHIP BETWEEN INCREASES IN C-FOS mRNA AND PREPRODYNORPHIN mRNA IN RAT SPINAL CORD DURING PERIPHERAL INFLAMMATION. G. Draisci*, M.J. Iadarola and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

We have shown previously that peripheral inflammation induces a rapid (within 8 hrs) increase in preprodynorphin mRNA in rat dorsal horn neurons. Recently, Hunt et al (Nature, 328:634,1987) using similar types of stimuli reported an even more rapid increase (within 30 min) in the protein(s) coded by the c-fos proto-oncogene. The somatotopic and laminar location of the dynorphin positive and c-fos positive neurons were very similar suggesting that the two products may be in the same cell and that c-fos may be involved in the transcriptional control of the dynorphin gene. We have examined the time course of increase in the dynorphin mRNA and c-fos mRNA in rat lumbar spinal cord during inflammation induced by intraplantar injection of carrageenan (6mg in 150 ul). Total RNA was isolated from lumbar spinal cord at 0.5,1,2,4,8,and 24 hrs after injection. RNA blots were prepared and hybridized with ^{32}P-labeled probes for rat c-fos (kindly provided by Dr. T. Curran) and rat preprodynorphin (kindly provided by Drs. J. Douglass and O. Civelli). Peak increases in c-fos mRNA were observed between 0.5 and 2 hrs, declined to 1/2 maximal by 4 hrs and were essentially at control levels by 8 hrs. In contrast, the initial increase in dynorphin mRNA was not apparent until 4 hrs and substantial increases (100% over control) were seen by 8 hrs. While progressively greater elevations were observed in dynorphin mRNA at later times, c-fos was not increased (e.g. at 4 days) despite the maintained inflammation and hyperalgesia seen in this model. Thus, the increase in c-fos mRNA is an early event in the present inflammatory model, preceding the increase of dynorphin mRNA. Assuming a similar cellular location, this temporal relationship suggests that the c-fos protein may interact in transcriptional regulation of the dynorphin gene.

224.5

POTASSIUM- AND CAPSACICIN-EVOKED RELEASE OF SUBSTANCE P FROM DORSAL AND VENTRAL RAT SPINAL CORD SLICES. R.E. Solomon[1], L.D. Aimone[2], T.L. Yaksh[2] and G.F. Gebhart[1]. [1]Department of Pharmacology, University of Iowa College of Medicine, Iowa City, IA 52242 and [2]Laboratory of Neurosurgical Research, Mayo Clinic, Rochester, MN 55905.

The release of substance P (sP) from superfusates of rat spinal cord *in vitro* was studied. Portions of the lumbar enlargement were dissected into dorsal and ventral halves, sliced into 0.5 x 0.5 mm pieces and perfused with an artificial, HEPES-buffered CSF. Four-min perfusates were collected and, utilizing previously published procedures (J. Physiol. 391: 141), assayed for sP by radioimmunoassay. Elevation of the potassium (K^+) concentration to 10 - 70 mM, or addition of capsaicin (0.01 - 100.0 μM) to the perfusate evoked concentration-dependent release of sP from dorsal spinal cord. Release of sP evoked by 50 mM K^+ was 3-fold greater from dorsal than from ventral spinal cord and was proportional to the dorsal/ventral difference in sP content. In contrast, 10.0 μM capsaicin increased sP release from dorsal spinal cord almost 9-fold over basal levels but did not evoke sP release from ventral spinal cord. Depletion of calcium from the perfusate and addition of EGTA (3.0 mM) abolished sP release evoked by 50 mM K^+ or by 10.0 μM capsaicin from dorsal spinal cord. Preliminary pharmacological experiments indicated that both morphine and clonidine (10^{-4} M) significantly attenuated sP release evoked by 0.1 μM capsaicin from dorsal spinal cord. The results are consistent with the putative role of sP as a nociceptive transmitter of primary afferents. Supported by T32 GM 07069, DA 05329, NS 16541 and DA 02879.

224.6

SUBSTANCE P-LIKE IMMUNOREACTIVITY IN THE RAT DORSAL HORN FOLLOWING HINDPAW INJECTION OF ALGESIC AND ANALGESIC AGENTS. L.N. Holland* and B.D. Goldstein (SPON: M.L. Kirby). Department of Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

Hindpaw injection of formalin has been shown to increase substance P (SP) levels in the rat dorsal horn (DH). Morphine has also been shown to increase SP levels. Thus, agents of opposing roles in nociception produce similar changes in SP. In order to better characterize these changes, we tested the action of a hindpaw injection of either eugenol, an anesthetic, or bradykinin, an algesic agent, on SP levels in the rat DH.

Urethane anesthetized rats (1200 mg/kg) were injected with either eugenol, bradykinin or formalin in a concentration of 1% into the right hindpaw. After sixty minutes, rats were perfused, the spinal cord removed (L4-L6), and stained immunohistochemically for SP. SP levels were quantified using microdensitometry.

Formalin and eugenol produced higher levels of SP compared to control while bradykinin produced significantly lower levels of SP.

In conjunction with data which show that naloxone blocks formalin induced changes of SP in the rat DH at sixty minutes, these data suggest that formalin injection may not be a good model for nociception.

224.7

NALOXONE BLOCKS THE INCREASE OF SUBSTANCE P IN THE DORSAL HORN DURING A NOCICEPTIVE STIMULUS. K.E. McCarson, B.D. Goldstein. Dept of Pharmacol and Toxicol, Medical College of Georgia, Augusta, GA 30912-2300.

Substance P (SP) found in primary afferent terminals has been proposed as a mediator of nociception. Formalin (FM) injected into the hindpaw of a rat as a nociceptive stimulus has been shown to increase the amount of immunoreactive SP in the dorsal horn. This increase may be due to a blockade of release of SP by enkephalinergic interneurons. Therefore, anesthetized rats were treated with naloxone (NAL) 10 mg/kg or saline (SAL) s.c. either 30 min before or 2 min after the delivery of either a 0.4 ml 5% FM or SAL injection into the plantar aspect of the right hindpaw. After various time intervals, the animal was perfused and the lumbar enlargement removed. SP was quantitated using immunohistochemical staining. SAL caused an increase in SP lasting 20 min while FM produced a biphasic effect with early (0-10 min) and late (20-60 min) increases in SP. NAL pretreatment partially blocked the initial increase in SP due to SAL or FM, but not if NAL was injected 2 min following SAL or FM. The late increase of SP resulting from FM was blocked by NAL only if it was administered prior to the FM. The SP time course resulting from FM is consistent with behavioral and electrophysiological studies. Blockade by NAL suggests an influential role of endogenous opioids in the control of SP levels in the dorsal horn.

224.8

EFFECTS OF DIFFUSE NOXIOUS STIMULI AND INFLAMMATORY AGENTS ON TRIGEMINAL MEDULLARY DORSAL HORN NOCICEPTIVE NEURONES IN THE RAT. J.W. Hu*, X. Chen* and B.J. Sessle. Fac. Dentistry, Univ. Toronto, Toronto, Ont. M5G 1G6 Canada.

Single neurones were recorded extracellularly in the medullary dorsal horn (N. caudalis) of anaesthetized, paralyzed rats, and classified as nociceptive-specific (NS, n=35) or wide dynamic range (WDR, n=37) on the basis of their responses to natural and electrical cutaneous stimuli. Diffuse noxious stimuli applied for 30 s outside the orofacial mechanoreceptive field (e.g. to tail, paw) produced a strong inhibition (onset<5 s; duration 40-60 s) of evoked A and C-fibre activity in both WDR and NS neurones. The injection of 2-5 µl of the inflammatory agent mustard oil (5%) into the deep masseter muscle in contrast produced a facilitation (20-75% increase above control activity; latency 1-3 min; duration 10-30 min) of especially C-fibre activity in 50% of the 16 neurones tested; WDR neurones in particular were affected. This facilitatory effect was usually associated with an expansion of the cutaneous mechanoreceptive field of the neurones. Electrical stimulation (1 min, 2 Hz, 2 ms, 5 mA) of XII nerve muscle afferents also produced facilitation of C-fibre activity in the nociceptive neurones. These findings emphasize the marked plasticity and sensitivity of caudalis nociceptive neurones to noxious and inflammatory conditions in sites distant from their mechanoreceptive field. Supported by NIH grant DE04786.

224.9

SYMPATHETIC ACTIVATION OF HYPERACTIVE WDR NEURONS FOLLOWING RECEPTIVE FIELD TRAUMA. W.J. Roberts & R.C. Kramis.* R.S. Dow Neurol. Sci. Inst., Good Samaritan Hosp. and Medical Center, Portland, OR 97209.

Mechanoreceptor input to hyperactive wide-dynamic-range (WDR) neurons is thought to cause both the spontaneous pain and the touch evoked pain (allodynia) characteristic of sympathetically maintained pain (Roberts, Pain, 24: 297-311, 1986). We tested this hypothesis by recording extracellularly from spinal neurons in barbiturate anesthetized cats. The responses of WDR neurons to sympathetically evoked afferent activity were recorded before and after trauma (noxious pinch or heating) to the receptive fields.

Brief (1 min) noxious stimulation elevated background WDR activity for tens of minutes. Sympathetic stimulation resulted in even higher rates of activity during the post-trauma period. These findings disclose a persistent trauma-induced hyperactivity in some WDR neurons following brief noxious input and further demonstrate that sympathetic efferent actions can increase the already elevated WDR output, as is hypothesized to occur during chronic sympathetically maintained pain.

224.10

DISCHARGES IN SMALL AND LARGE MYELINATED PERIPHERAL FIBERS EVOKED BY FORMALDEHYDE INJECTED IN THEIR CUTANEOUS RECEPTIVE FIELDS. N.E. Saadé, M. Tabbara*, S.F. Atweh and S.J. Jabbur. Fac. of Med., American University of Beirut, Beirut, Lebanon.

We have earlier shown that subcutaneous (sc) injection of formaldehyde (F) solution in the receptive fields of dorsal horn neurons elicited an immediate and continuous discharge in wide-dynamic-range (WDR) neurons and no effect in the low-threshold mechanoreceptive (LTM) neurons (Exp. Neurol., 93, 275, 1986). These differences could have been determined by the responses to F of peripheral nerve terminals. In spinal cats, 31 fiber filaments were isolated from sural or superficial peroneal nerves and their receptive fields (RFs), sensory modalities and conduction velocities (CVs) were determined. The response discharges of each fiber to sc injection of 0.1-0.15 ml of 2.5% F in RF were studied. F effects were categorized as (1) none, (2) short-term (<10 min) excitatory generally followed by complete loss of sensitivity of the RF and (3) long-term (15-80 min) excitatory.

We observed that F injection results in sustained discharges from the majority (11/15) of small myelinated fibers (with CV<30 m/sec), and short-term or no discharges from the majority (15/16) of large myelinated fibers (with CV 30-80 m/sec), which could account for the respective behaviours of WDR and LTM spinal neurons. (Supported from Lebanese N.R. Council and D.T. Sabbagh Fund).

224.11

PROLONGED EFFECTS ON CA1 POPULATION SPIKE IN RAT HIPPOCAMPUS PRODUCED BY NOXIOUS STIMULI. S. Khanna* and J. G. Sinclair. Div. of Pharmacol. & Toxicol., Fac. of Pharmaceutical Sciences, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

There are indications that the hippocampus is involved in nociceptive mechanisms (Prado, & Roberts, Brain Res. 340: 219, 1985; Sinclair, & Lo, Neurosci. Lett. 68: 47,1986; Soulairac, *et al.*, Int. J. Neuropharmacol. 6: 71, 1967). We have investigated the effect of peripheral noxious heat on monosynaptic transmission through region CA1 of the dorsal hippocampus (dH). Experiments were done in rats (250-350 g) lightly anaesthetized with urethane 1.0 g/kg, i.p. Deep anaesthesia during surgery was produced by supplementation with halothane. A submaximal CA1 population spike (PS), evoked by field CA3 stimulation, was recorded. In addition, the contralateral dH electroencephalogram (EEG) was recorded. Noxious heat (50-60°C, 15 sec) applied to the distal 3.0 cm of the tail produced a temperature-dependent depression of the CA1 PS amplitude. While 50°C did not consistently reduce the amplitude of the PS, 55 and 60°C resulted in a mean decrease to 39±14 % of control (n=7) and 17±6 % of control (n=5), respectively. At 55°C the mean recovery time was 8 min following exposure whereas at 60°C the depression lasted 18 min. Qualitatively, a similar response was observed when the distal 2.0 cm of the left hind foot was exposed to noxious heat at 55°C. On repeated exposures of a receptive field to noxious heat, the CA1 depression was markedly attenuated. However, a subsequent application of noxious heat to a different topographical area resulted in a depression of the PS. A noxious stimulus usually changed the EEG activity from an irregular pattern to a 4-6 Hz rhythmic slow wave activity (RSA) which lasted for a short period of time. Interestingly, in the presence of continuous spontaneous RSA, noxious stimuli failed to depress the PS amplitude. These results implicate the hippocampus in nociceptive processing mechanisms. (Supported by the Medical Research Council of Canada and B.C. Health Care Research Foundation).

225.1

MUSCARINE DEPRESSES AN APV-INSENSITIVE FORM OF LTP IN CA3 HIPPOCAMPAL NEURONS. *S.H. Williams & D. Johnston*, Neuroscience Program, Baylor College of Medicine, Houston, Tx 77030.

Long-term potentiation (LTP) of the hippocampal mossy-fiber CA3 pyramidal cell synapse is insensitive to NMDA receptor antagonists such as APV. A previous investigation showed that β-adrenoreceptor agents could modulate LTP at this synapse, agonists produce a potentiation and antagonists block (Hopkins & Johnston, *J.Neurophysiol*, 59 667-687). We report here that cholinergic agonists can also modulate LTP: in contrast to norepinephrine, however, muscarine produces a *depression* of LTP. Transverse hippocampal slices were prepared using standard techniques and incubated in a picrotoxin containing media to block inhibition. Single CA3 neurons were studied under current and voltage clamp. Tetanic stimulation of the mossy fiber input to CA3 neurons (3 episodes of 1s duration, 100Hz), elicited LTP in 13/15 control cells (> 20% increase in epsp or epsc 15 min after tetanus). When 1μM muscarine was present during tetanization only 3/10 cells showed LTP. The current-voltage relationship for the synaptic current showed that muscarine blocks the increase in synaptic conductance (G, normally associated with LTP. These data are summarised below.

	%change normalized to pre-tetanus control (mean ± sem)		
	EPSP	EPSC	G,
Control	+50 ± 9%	+43 ± 11%	+41 ± 2%
+ 1μM muscarine	+ 7 ± 9%	+ 6 ± 6%	+ 5 ± 8%

The bidirectional modulation of LTP by β-adrenergic and muscarinic agonists may be mediated by modulation of calcium channel activity in the postsynaptic membrane. (NIH grants HL31164 & NS11535, and AFSOR 85-0178).

225.2

DECREASE IN SYNAPTIC VESICLE CALCIUM DEPOSITS DURING LTP IN THE HIPPOCAMPUS. C.K. Meshul and W.F. Hopkins. V.A. Med Center and Dept. Neurol., Oregon Health Sciences Univ., Portland, OR 97201.

Long-term potentiation (LTP) displays a stringent calcium dependence, and may be due to a long-term increase in transmitter release. We assessed the possibility that LTP is accompanied by changes in the distribution of calcium in the presynaptic terminal. Calcium was localized ultrastructurally within terminals located in stratum radiatum of the CA1 subfield according to the method of Borgers (1981). Rat hippocampal slices were prepared and maintained in vitro using standard techniques. The pEPSP in CA1 was monitored following a stimulus train (100 Hz, 1 sec), and a significant increase in the amplitude of the pEPSP beyond 15 min was called LTP. Control slices were given the same number of stimuli at a frequency that did not result in LTP. The slices were fixed between 15 and 60 min following stimulation and processed for calcium localization. Seventy percent of the presynaptic vesicles from control slices contained calcium deposits, whereas 42% did so from slices that displayed LTP ($p < .05$). The decrease in the percentage of synaptic vesicles containing calcium deposits might suggest that during LTP, vesicular calcium is released into the nerve terminal cytoplasm. Such an increase in free calcium could subserve the long-term increase in transmitter release that may underlie LTP.

225.3

EFFECT OF POTASSIUM CONCENTRATION ON CALCIUM INDUCED POTENTIATION IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS. **L. Grover & T. Teyler. Neurobiol. Dept., N.E. Ohio Univ. Coll. Med., Rootstown, OH, 44272.**

Ten min exposure to high extracellular calcium produced potentiation of population spike and EPSP in area CA1 of hippocampus (Turner et al. Neurosci. 7: 1411-6, 1982). We examined effects of high calcium and potassium on EPSPs recorded intracellularly from CA1 pyramidal cells of in vitro rat hippocampus. Baseline responses were obtained during a 10 min period when slices were superfused (0.5 ml/min) with normal medium (3.4 mM potassium, 2.0 mM calcium). Slices were then exposed for 10 min to: (1) high calcium (4.0 mM), (2) high potassium (6.25 mM), or (3) high calcium and potassium. High calcium produced transient potentiation (reversed within 10 min following return to normal medium) in 4 of 5 cells. Mean change in EPSP amplitude ± SEM (all 5 cells) = 12.8 ± 7.5 %. Effects of high potassium (n=4) also reversed quickly upon return to normal medium. Nine of 10 cells exposed to high calcium and potassium showed potentiation; in all but one cell, potentiation persisted for the duration of the recording: up to 60 min following return to normal medium. Change in EPSP for time periods following return to normal medium were: 1-10 min, 39.0 ± 10.7 % (n=10); 11-20 min, 27.9 ± 6.8 % (n=10); >21 min , 21.8 ± 11.3 (n=6). **Supported by ONR 86K0664, NIH DA03755, EPA CR813394.**

225.4

HOMO- AND HETEROSYNAPTIC EXPRESSION OF LTP IN HIPPOCAMPAL CA3 NEURONS: SENSITIVITY TO INTRACELLULAR EGTA INJECTIONS

J.E. Bradler* and G. Barrionuevo (SPON: D.M. Barnes) Depts of Behavioral Neuroscience and Psychiatry, and Center for Neuroscience, University of Pittsburgh, Pittsburgh PA 15260

Last year we described the homo- and heterosynaptic expression of LTP in the CA3 region in vitro (Soc. Neurosci. abstr. 13(3): 976, 1987). Recently, we have investigated the sensitivity of homo- and heterosynaptic LTP expression in voltage-clamped CA3 neurons to intracellular EGTA injections (.5 M ; 1.25 nA 450 ms pulses at .5 Hz for 4-5 min). The disappearance of I_{ahp} measured at -62mV in hybrid clamp demonstrated EGTA effectiveness. Following the disappearance of I_{ahp}, 5 trains of 6 pulses at 100 Hz were delivered to one of three stimulation sites: CA1 s. radiatum, the s. granulosum of the dentate gyrus, or the fimbria. An extracellular electrode confirmed the induction of LTP in the CA3 cell population. In 4/4 cells, the mossy fibers (MF) expressed homosynaptic LTP following tetanization of the MF. In 2/2 cells, LTP was heterosynaptically expressed in the fimbria (Fi) and CA1-evoked EPSCs following a MF tetanus. In an additional 2 cells, homosynaptic LTP was expressed in the Fi-evoked EPSC following a Fi tetanus. In one cell, homosynaptic LTP was expressed in the CA1-evoked EPSC. We have not observed the extracellular expression of LTP in the absence of intracellular LTP expression in an EGTA-injected cell. Supported by an RCDA (NS01196) to G.B.

225.5

CALCIUM INFLUX THROUGH NMDA-RECEPTOR CHANNELS MODELED IN A HIPPOCAMPAL DENTATE GRANULE CELL. W.R. Holmes and W.B Levy, Math. Research Branch, NIDDK, NIH, Bethesda, MD 20892, and Dept. of Neurosurgery, U. Virginia School of Medicine, Charlottesville, VA 22908.

Calcium influx through NMDA-receptor channels is thought to play a major role in the development of LTP. Calcium influx through channels linked to voltage-dependent NMDA receptors was modeled at a synapse on a dendritic spine on a dentate granule cell. The cell received synaptic inputs on spines at anywhere from 1-117 different dendritic locations. The frequency of synaptic activity was varied from 25-800 Hz while holding the number of active afferents and the number of activations of each afferent constant.

The Ca^{++} influx was dependent on both the frequency of the input and on the number of co-active inputs. With few active inputs Ca^{++} influx was small, and there was little or no increase of Ca^{++} influx with increasing input frequency. However, with larger numbers of co-active inputs (e.g. 39 or 57), increasing input frequency resulted in a steep non-linear transition between low Ca^{++} influx and high Ca^{++} influx. This transition occurred at lower frequencies as the number of active inputs was increased. After this transition, further increases in input frequency or in the number of co-active inputs produced little additional increase in Ca^{++} influx, and in some cases influx decreased.

Parameters related to conductances mediated by non-NMDA receptors could be doubled or halved with only minor effects on total Ca^{++} influx. This was not true for the NMDA mediated conductance. Changes in NMDA receptor affinity, the number of receptors, and to a lesser extent the mean channel open time had a large effect on total Ca^{++} influx. Changes in the amount of neurotransmitter released, the transmitter uptake rate, and changes in extracellular Mg^{++} also had large effects on Ca^{++} influx.

Although the model helps to illuminate the function of the NMDA receptor regarding Ca^{++} influx, the best temporal input patterns for LTP found by Levy and Steward (1983) were not the same ones found for highest Ca^{++} influx in the model. Another conductance or some other factor needs to be added to explain these LTP timing studies. (This work was supported by NS07845 (WRH) and NS15488 and NIMH RSDA K02-MH00622 to WBL.)

225.6

LONG-TERM POTENTIATION IS ACCOMPANIED BY AN INCREASE IN EXTRACELLULAR RELEASE OF ARACHIDONIC ACID. T.V.P. Bliss*, M.P. Clements*, M.L. Errington*, M.A. Lynch* and J.H. Williams*. (SPON: European Neuroscience Association). Div'n. of Neurophysiology and Neuropharmacology, National Institute for Medical Research, Mill Hill, London NW7, U.K.

These experiments are part of an investigation into the possibility that arachidonic acid and/or its lipoxygenase metabolites are the retrograde messenger(s) postulated to carry information from the postsynaptic to the presynaptic side of the synapse to trigger the sustained increase in release of glutamate associated with LTP. We have measured, using HPLC separation and UV/fluorometric detection, the concentrations of arachidonic acid and glutamate in (i) push-pull perfusates from the dentate gyrus following the induction of LTP by tetanic stimulation of the perforant path, and (ii) superfusates from hippocampal slices in which LTP was induced by 15 min exposure to 5mM calcium. In both cases there was a sustained increase in the concentration of glutamate and arachidonic acid in the two hours following the induction of LTP. The phospholipase A_2 and lipoxygenase inhibitor, nordihydroguaiaretic acid, which blocks the induction of both tetanus-induced and calcium-induced LTP (Bliss et al., Pflug. Archiv., 411, Suppl. 1, 229, 1988), also suppressed the increase in release of glutamate and arachidonic acid. Although we do not know the site of release of arachidonic acid, the LTP-associated increase in extracellular release is consistent with the idea that it exerts a messenger and/or trophic role in LTP.

225.7

NALOXONE BLOCKS MOSSY FIBER, BUT NOT COMMISSURAL OR HETEROSYNAPTIC LONG-TERM POTENTIATION IN THE HIPPOCAMPAL CA3 REGION IN VIVO. B.E. Derrick and J.L. Martinez, Jr., Dept. of Psych., University of California, Berkeley, CA 94720.

Hippocampal CA3 responses were observed in male Sprague-Dawley rats under pentobarbital anesthesia via stimulation of the mossy fibers and the contralateral CA3 field. Naloxone (NAL, 5 mM, pH 6.8 in Ringer's, 1.0 ul pressure ejected) did not consistently alter field responses given to either pathway during the 10 min following drug application. Two 1-sec, 100 Hz trains, which did not produce afterdischarges, were given to each pathway to induce LTP. NAL blocked the usual increase in the primary mossy fiber population spike (NAL, x = 1.4%, Ringer's, x = 55%). Increases in the commissural spike were unaffected by NAL (x = 72%, Ringer's, x = 55%). NAL also abolished the short-term potentiation in both pathways usually seen post tetanus (PTP). LTP was, however, observed in the later negative components of the mossy fiber response following NAL. Heterosynaptic LTP also was observed in the later components of the commissural response following tetanization of the mossy fibers, and less frequently seen in mossy fiber responses as a result of commissural tetanus. In both cases it was not affected by NAL. These data suggest that (1)heterosynaptic LTP is postsynaptic to the mossy fiber-CA3 synapse, and may be a result of potentiation of a common final recurrent pathway; and (2) our previous studies (Derrick, et al., Neurosci. Abstr, 13, 1987) suggest that opioid peptides do not alter inhibition in the CA3 region, thus opioid peptides may be directly involved in the initiation of mossy fiber LTP, rather than indirectly modulating it via a disinhibitory effect.

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225.8

Long-term Potentiation (LTP) and c-fos: is there a link?

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The nuclear "third messenger" c-fos has recently been shown to be massively induced in adult neurons by kindling stimulation (Dragunow and Robertson Nature 329, 414), suggesting that it might be involved in plastic changes associated with seizure activity. In another model of CNS plasticity, LTP, c-fos is not consistently induced in urethane-anaesthetized rats (Douglas,Dragunow and Robertson, in press). In the present series of experiments we investigated the production of c-fos following LTP of the perforant-path-dentate gyrus synapse in unanaesthetized rats. Low-frequency stimulation, or high frequency stimulation in the presence of CPP, urethane or MK801 did not induce c-fos in dentate granule or entorhinal cortical cells. However, in some rats (4/6) stimulated at high frequency there was a slight (an average of three times the contralateral side) induction of c-fos protein in the dentate gyrus, but not the entorhinal cortex. Thus, high frequency stimulation can induce c-fos in some animals but the relationship of c-fos with LTP is unclear.

225.9

PATHWAY-SPECIFICITY OF NOREPINEPHRINE-INDUCED LONG-LASTING POTENTIATION (NELLP) IN DENTATE GYRUS OF RAT HIPPOCAMPAL SLICE. D. Dahl, G. Decker, and J.M. Sarvey. Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

This laboratory and others have reported NELLP of dentate gyrus granule cells that is manifested as a persistent (> 30 min) increase of the excitatory postsynaptic potential (EPSP) and the population spike evoked by perforant pathway stimulation.

We have differentially stimulated the medial and lateral components of the perforant pathway to the dentate gyrus and confirmed isolation by the distinctive response profiles associated with activation of each afferent. NELLP of the medial perforant pathway-evoked EPSP and population spike was reliably obtained by superfusion of a combination of 1.0 or 10.0 μM NE + 50.0 μM phentolamine, whereas no potentiation was apparent at these concentrations in response to activation of the lateral perforant pathway. An NE concentration of 1.0 μM without phentolamine was not effective in eliciting NELLP, and phentolamine alone had no effect on responses to activation of either pathway. Isoproterenol, however, at a concentration of 1.0 μM, induced the same pathway-specific NELLP of medial perforant pathway-evoked responses as the concurrent administration of NE and phentolamine.

These results indicate that NE acts differentially on entorhinal cortical projections to the dentate gyrus, and that NELLP of the medial perforant pathway can be obtained with NE concentrations as low as 1.0 μM with concurrent administration of phentolamine.

225.10

LONG-TERM POTENTIATION IN THE DENTATE GYRUS IS ENHANCED BY STIMULATION OF THE MEDIAN RAPHE NUCLEUS. J.M. Klancnik[1,2], A.G.Phillips,[1], Depts. of Psychology[1], and Physiology[2], University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The dentate gyrus (DG) receives significant serotonergic projections from the median raphe nucleus (MR), and contains a dense population of serotonergic binding sites. Therefore it is significant that population spikes evoked in the DG perforant path (PP) stimulation can be enhanced during a 90 msec period following a single pulse of stimulation to the MRN. It is well established that population responses in the DG undergo long-term potentiation (LTP) after tetanic stimulation to the PP. However, there is a degree of uncertainty surrounding serotonergic modulation of LTP in the DG. This issue was addressed in the present study which investigated the effects of electrical stimulation of the MR on LTP of the PP-DG population response. Urethane anesthetized adult male Wistar rats were implanted with stimulating electrodes in the MR and PP, and a recording microelectrode in the hilus of the DG. Population responses in the DG were evoked by PP stimulation, at an intensity that produced a half-maximal amplitude population spike. A 100 Hz., 1 sec stimulus train was applied either to the PP alone, or concurrently to the PP and MR. Subsequently, PP-DG responses were evoked and recorded at the rate of 1/min. Concurrent stimulation of the MR and PP produced a significantly greater increase in population spike amplitude and population EPSP slope than was seen after tetanic stimulation of the PP alone, both at one and sixty min. after tetanus. These results suggest a modulatory influence of serotonergic afferents on the processes underlying neuronal plasticity in the DG.

225.11

A CONTEXT DEPENDENCY FOR LONG-TERM POTENTIATION? K-A. Moore and R. J. Racine (SPON: C. Hawryshyn). Dept. of Psychology, McMaster Univ., Hamilton, Ontario, CANADA, L8S 4K1.

It is well known that memory is context-dependent (e.g. state-dependent learning). We have attempted to determine whether long-term potentiation (LTP), a memory model, might also show a context-dependency. If it does, then at least some of this dependency may be determined by interactions that occur at the cellular level. A "cooperativity" effect has been demonstrated for LTP in which co-activation of separate inputs serves to strengthen the LTP produced in one or both inputs. Although the tetanizing trains are applied contiguously, the test pulses have always been restricted to one or the other pathway. We have attempted to reproduce the "context" that is present during tetanization by co-activating the pathways during the test phase as well. We chose the medial septal input to provide a context for perforant path input, because the septal input enhances LTP in the perforant path/granule cell circuit without being altered itself (Robinson, G.B. and Racine, R.J., Brain Res. 1982, 249, 162-166). A compound stimulus, consisting of a brief septal train followed, 70 msec later, by a pulse to the perforant path, was used to trigger the baseline response (with context). Similar trains were then applied 70 msec prior to perforant path trains, after which the test stimuli were reapplied. Results thus far indicate that responses evoked "in context" show a greater LTP effect than those evoked by perforant path pulses alone.

225.12

TWO FORMS OF LONG-TERM POTENTIATION IN THE CA3 REGION OF THE RAT HIPPOCAMPUS IN VIVO. M.K. Washington, D.N. Lieberman, B.E. Derrick, & J. L. Martinez, Jr. (SPON: C. BARRIT) Psych Dept., Univ. of Calif., Berkeley, CA 94720

Hippocampal CA3 responses were observed in male Sprague-Dawley rats under pentobarbital (50 mg/kg) or urethane (1.0 g/kg) anesthesia. Mossy fiber (MF) responses were elicited using bipolar nichrome electrodes placed in the stratum lucidum of the CA3 region. Commissural (COM) responses were produced by stimulating the homotopic contralateral CA3 pyramidal layer. Two 1 sec trains of 100 Hz were delivered to either the MF or the COM afferents. Long-term potentation (LTP) of the COM response (N=8) consisted of an increase in both the population spike and the later components (5-15 msec) of the response. Both responses reached maximum at approximately 10 min post-tetanus. COM LTP was decremental, with a half-life of approximately 75 min for the population spike. In contrast, MF potentiation (N=8) consisted of an increase in only the population spike. This increase was gradual, reaching maximum at approximately 45 min and showed no decrement in response over the course of the experiment (>2 hrs). Heterosynaptic LTP was observed in the later components of the commissural response following tetanization of the mossy fibers, but was less frequently seen in mossy fiber responses following commissural tetanus. COM and MF LTP are differentially affected by pharmacological agents (see Derrick, et al., this volume). LTP of the MF response is blocked by urethane but not pentobarbital anesthesia. Urethane does not affect LTP of the COM response, nor does it affect post-tetanic potentiation of COM or MF responses. We currently hypothesize that the non-decremental LTP of the mossy fiber response is dependent on both opioid receptor activation and phosphoinositol turnover.

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225.13

IS BEHAVIORAL LONG TERM POTENTIATION DUE TO LEARNING OR CHANGES IN MOTOR ACTIVITY? A PRELIMINARY REPORT. E.L. Hargreaves* and D.P. Cain (SPON: R. Steenhuis). Dept. Psychology, U. of Western Ontario, London, Ontario, CANADA N6A 5C2.

In light of the finding that changes in ongoing behavior can affect the hippocampal evoked potential (EP), reports of LTP-like changes in EPs after the acquisition of a learned behavior could be due to changing motor patterns instead of learning. To explore this possibility ten rats were chronically implanted with stimulating and recording electrodes in the perforant-path/Dentate system of the hippocampus and then run through an eight arm radial maze and a one way active avoidance task. Daily recording sessions occurred three hours prior to placement in the learning environment. During the recording session EPs were collected under strict observation of two different homogenous behavioral activities, wheel running and immobility.

Preliminary results indicate consistent differences in EPs collected under the two behavioral states as measured by EPSP slope and pop-spike amplitude. Measures of learning show strong improvements in the behavioral performance of both tasks over time. However, no changes in EPs were observed as a function of acquisition of the tasks, and the EPs in general remained fairly consistent with baseline measures. Further research is now being conducted to confirm that the neural system under study can support a plastic change through the induction of traditional LTP. Supported by Grants from NSERC to D.P.C.

225.14

EVIDENCE FOR AN LTP REFRACTORY PERIOD J. Greenberg*, J. Larson*, & G. Lynch. (SPON: J. Turnbull) Center for the Neurobiology of Learning & Memory, University of California, Irvine, CA 92717.

Use of stimulation patterns consisting of brief, high frequency bursts has provided evidence for several postsynaptic events controlling induction of long-term potentiation (LTP) in hippocampus. In particular, burst stimulation given to one set of inputs to a CA1 pyramidal cell induces LTP only if preceded by priming stimulation of the same inputs or a separate set of inputs to the same cell (Larson & Lynch, Science, 232:985, 1986); when several inputs to a primed cell are stimulated in sequential but overlapping order, the degree of LTP induced at the activated synapses depends on their order in the temporal sequence (Larson & Lynch, this meeting). The present experiments provide evidence that burst stimulation in a primed cell is followed by a brief period during which further burst stimulation of a separate input is less effective for LTP induction.

Two stimulating electrodes were positioned to activate separate sets of Schaffer/commissural axons converging on CA1 neurons in the rat hippocampal slice. Dendritic population EPSP's were measured for a 10 min period before and 20 to 30 min following patterned stimulation. This consisted of a priming pulse to both inputs followed by a burst (4 pulses , 100 Hz) to S1 at a delay of 180 msec and to S2 at a delay of 220 msec. The resultant potentiation (% increase in EPSP slope) was 49.1% (±5.9) for S1 and 29.5% (±5.3) for S2. S1 potentiated to a greater degree than S2 in 12 of 17 cases; S2 potentiated more than S1 in only 3 cases. Control experiments in which either S1 or S2 (at the corresponding delays of 180 or 220 msec) were stimulated alone resulted in LTP of 52.2% (±5.7). In the combined pattern then, prior stimulation of S1 reduced the efficiency of the S2 burst by about 40%.

Inspection of the extracellular responses recorded in the apical dendritic field revealed that the responses to the S2 bursts were considerably reduced in magnitude, compared to either the S1 responses or responses to bursts given at the same priming delay without S1 stimulation. We have previously reported that the magnitude of burst responses is highly correlated with the degree of LTP induced by those bursts; therefore, the reduced potentiation at S2 synapses may result from shunting of postsynaptic responses to S2 bursts by currents activated by S1.

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225.15

DEGREE OF LTP IN CONVERGING INPUTS IS DETERMINED BY THEIR POSITIONS IN A TEMPORAL STIMULATION SEQUENCE. J. Larson* and G. Lynch (SPON: M.-L. Kean). Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA 92717.

Recent evidence suggests links between the hippocampal theta rhythm and the induction of long-term potentiation (LTP). Short stimulation bursts (mimicking complex-spike discharges) produce maximal LTP when repeated at 5Hz-the theta frequency. This appears to be due to a suppression of subsequent IPSPs by the first burst, allowing enhanced postsynaptic depolarization in response to later bursts and consequent activation of NMDA receptors (Larson & Lynch, *Science* 232:985, 1986; *Brain Res.* 441:111, 1988). The present study examines the effects of asynchronous input activation--such as might occur during a single theta wave -- on LTP induction.

Three stimulation electrodes (S1, S2, S3) were used to activate independent inputs to apical dendrites of field CA1 neurons in rat hippocampal slices. Field EPSPs evoked by single test stimuli to each electrode were monitored for 10 min before and 20-30 min after patterned stimulation as follows: A "priming" pulse was given to each input to suppress IPSPs; then a burst (4 pulses, 100Hz) was given to each electrode at a different delay: 180 msec (S1), 200 msec (S2), and 220 msec (S3). Thus, pulses 3 and 4 of S1 overlapped pulses 1 and 2 of S2; pulses 3 and 4 of S2 overlapped pulses 1 and 2 of S3. The pattern was repeated 10 times at 5 sec intervals. Stimulus intensity was set such that activation of any input separately would not induce LTP. After stimulation of the three inputs together, the degree of LTP induced (% increase in field EPSP) was greatest for S1 (25 ± 3%), intermediate for S2 (17 ± 2%) and least for S3 (8 ± 2%), measured 20 min after patterned stimulation.

Intracellular recordings were made from pyramidal neurons in order to quantify postsynaptic responses during patterned stimulation. The degree of postsynaptic depolarization was greatest during the period of S2 stimulation. Since S1 synapses showed greater LTP than S2 synapses, we conclude that the degree of LTP induced does not depend simply on the degree of depolarization during input activation. Rather the depolarization during and after input activation appears to determine the magnitude of LTP. This is probably a consequence of the unusual kinetics of NMDA receptor-linked ion channels. The relatively weak LTP effects at S3 synapses may also reflect the activation of shunting currents by the earlier inputs.

(Supported by ONR N00014-86-K-0333)

225.16

STABLE DEPRESSION OF POTENTIATED SYNAPTIC RESPONSES IN THE HIPPOCAMPUS INDUCED BY LOW FREQUENCY STIMULATION. U. Staubli and G. Lynch. Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA 92717.

Reversal of long-term potentiation (LTP) figures prominently in discussions of 1) the possible causes of forgetting and 2) the weakening of synapses as part of learning algorithms in neuronal network models. Results from experiments done in acute animal preparations suggest that depressive mechanisms exist in the hippocampus (Barrionuevo *et al.*, 1980). However, there is no evidence a) that the depression is synaptic in nature as opposed to more global changes of neuronal properties and b) that the depression lasts long enough to be a factor in memory.

To analyze these points, we have carried out a study using chronically implanted rats with two stimulating electrodes and one recording electrode in the Schaffer collateral/commissural system. This allowed us a) to assess the duration of the LTP reversal, b) to verify if the depression is specific to responses evoked by the experimental electrode, and c) to induce LTP again in cases where the previous potentiation remained reversed for at least 24 hrs. We found that repetitive low frequency stimulation (LFS) at 1 Hz (100 sec) induced a specific and lasting (>24 hrs) reversal of LTP in 21 of the 33 animals tested while control responses remained unchanged. No effect was observed when LFS was administered to the unpotentiated pathway. In six animals the potentiation was only temporarily depressed (<15 min) by LFS, and no effect was observed in the remaining animals. In 18 of the animals that showed a lasting depression LTP was re-established and remained stable for at least 24 hrs.

Current studies seek to identify (1) optimal parameters (stimulation pattern and time point of administration) for the reversal effect and (2) physiological characteristics of potentiated vs. unpotentiated responses during low frequency stimulation. The results may have important implications for hypotheses about (a) the substrates of LTP and (b) the mechanisms underlying forgetting.

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225.17

LONG-TERM POTENTIATION IN AMYGDALA BRAIN SLICES. P. F. Chapman* and T. H. Brown. Division of Neuroscience, Beckman Research Institute, City of Hope, Duarte, CA 91010

The amygdala is a structure with demonstrated importance to affect and learning (Davis, 1986, *Behav. Neuro.*, 100, 814; Mishkin, 1982, *Nature*, 273, 297). Investigations of this structure have generally focussed on the effects of lesions, drugs, or electrically-induced epiliptiform activity on behavior. Due to the complexity of its intrinsic and extrinsic connections, few examinations have been made of the relationship between cellular neurophysiology, and function.

We have used an *in vitro* slice preparation to make intracellular current- and voltage-clamp analyses of long-term potentiation (LTP) in the amygdala of rats. Either coronal or horizontal slices (450 µm) through the amygdala were prepared by isolating a block of tissue from each hemisphere. Glass recording electrodes (50-90 MΩ) were filled with 4M KAc and placed in the lateral or basolateral amygdala. EPSPs and EPSCs were recorded using a single-electrode current- and voltage-clamp device.

Stimulation of the external capsule with 20 µsec constant current pulses produced an EPSP with a latency of 2-3 msec and peak amplitude of 10-15 mV. The corresponding EPSC varied in amplitude between 0.3 and 1.5 nA. Baseline measures were made for each cell by selecting the stimulus intensity that produced EPSPs greater than 5 mV, but at least 10 mV less than the spike threshold for that cell. Test pulses were delivered at a rate of five per minute. After 10 minutes, three trains of pulses were delivered at 300 Hz, each lasting 100 msec, with a five second inter-train interval. Following tetanic stimulation, EPSPs and EPSCs increased between 20 and 150 percent for at least 20 minutes.

The occurrence of LTP in the amygdala slice should facilitate detailed biophysical and pharmacological analysis of the mechanisms that control its induction and expression. Such knowledge may enable tests of the behavioral role of LTP in this structure. *(Supported by the AFOSR).*

225.18

Comparative Extracellular Current Flow at Dendrites and Soma of Dentate Gyrus Granule Cells During Long-Term Potentiation. C. Pavlides*, Y.J. Greenstein & J. Winson. The Rockefeller University, 1230 York Ave., NY, NY 10021.

In the study of long-term potentiation (LTP) in the dentate gyrus, the magnitude of the initial slope of the evoked synaptic potential (ESP) in the region of the granule cell layer, is commonly used as a measure of the increase of synaptic efficacy at the site of the perforant path (PP) input. To investigate the viability of this assumption, in rats anesthetized with either Chloropent (N=5) or Urethane (N=5), LTP was induced by tetanic stimulation of the PP and ESPs were measured simultaneously at both the soma and the dendritic site of the PP input.

Decoupling of somal and dendritic ESPs occurred in both groups of animals, the nature of the decoupling depending on the anesthetic used. Thus in animals anesthetized with Urethane the somal (14.5%) increase of the ESP was significantly lower than the dendritic (38.9%) ($P < .001$). In contrast, in animals anesthetized with Chloropent, the somal (23.3%) increase of the ESP was significantly higher than the dendritic (14.9%) ($P < .05$). We conclude that in assessing changes of synaptic efficacy, following the induction of LTP, measurements should be taken at the site of synaptic input.

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225.19

ASYMMETRY OF ASSOCIATIVE LONG-TERM POTENTIATION (LTP) IN THE HIPPOCAMPUS ACROSS THE CELL BODY OF CA1 PYRAMIDAL CELLS. J. Jester, P.K. Stanton, and T.J. Sejnowski. Dept. of Biophysics, Johns Hopkins University, Baltimore, MD 21218.

Long-term potentiation (LTP), a lasting increase in synaptic strength, occurs in hippocampus following high-frequency stimulation. Further, associative LTP has been shown in pathways receiving a weak stimulus concurrent with a strong stimulus to a converging input. We have investigated the association of inputs using patterned stimuli on the same or opposite sides of the cell bodies of CA1 hippocampal neurons.

Extra- and intracellular recordings were made from 400 μm thick slices of rat hippocampus in an interface chamber at 34°C. The stimulus sites were stratum oriens (SO), which synapses on the basal dendrites, and the Schaffer/commissural (SCH) pathway in stratum radiatum (SR) which synapses on the apical dendrites of CA1 pyramidal cells. The "strong" stimulus pattern (which alone induced LTP) consisted of trains of 10 bursts of 5 stimuli given at 100 Hz, with an interburst interval of 200 msec. The "weak" stimulus pattern (which alone had no effect) was a series of single shocks at 5 Hz which either coincided with or came between bursts to the strong site.

Pairing of the SO site as the strong stimulus and SCH as the weak resulted in no change of the epsp slope (+5.4 ± 4.8%, n=8) 15-60 minutes post-tetanus. However, when strong and weak sites were reversed, the SO site showed significant increases in both the epsp (+62.7 ± 19.9 %, n = 7) and the amplitude of the population (pop) spike (+58.5 ± 15%, n = 7). When the stimulus sites were in SR on opposite sides of the recording site, pairing strong and weak led to an increase of +37.0 ± 11.1 % (n = 10) of the epsp and +65.4 ± 16.0% (n=14) of the pop spike, whereas interleaving weak site shocks and strong site bursts resulted in long-term depression of the pop spike with no change in the epsp. Thus, associative LTP occurs across CA1 cell somata preferrentially when the weak input is in the basal dendrites.
(Supported by Office of Naval Research Grant #N00014-88-K-0198)

225.20

CA3 COMMISSURAL, BUT NOT MOSSY FIBER, SYNAPSES IN HIPPOCAMPUS EXHIBIT ASSOCIATIVE LONG-TERM POTENTIATION (LTP) USING RYTHMICALLY COACTIVE INPUTS. S. Chattarji, P.K. Stanton, and T.J. Sejnowski. Dept. Biophysics, Johns Hopkins Univ., Baltimore, MD 21218.

Long-term potentiation (LTP) is characterized by a persistent increase in synaptic efficacy following brief, high-frequency stimulation of afferent pathways in the hippocampus. There is evidence that weak excitatory inputs that do not exhibit LTP when stimulated alone do elicit LTP when coactivated with stronger inputs. Recent studies suggest that associative LTP can be induced in field CA1 of the hippocampus depending on the phase of rythmically active inputs. The present study evaluates whether associative LTP can be induced using such rythmically coactive inputs in field CA3, where mossy fiber synapses exhibit LTP not involving N-methyl-D-aspartate (NMDA) receptor activation.

Extracellular recordings were made in 400 μm thick rat hippocampal slices in an interface recording chamber at 33°C. Responses of CA3 pyramidal cell somata and apical dendrites were recorded following stimulation of the Commissural/Schaffer (COM) and mossy fiber (MF) afferents. Strong (potentiating) stimuli consisted of trains of 10 bursts of 5 pulses each at a frequency of 100 Hz, with an interburst interval of 200 msec. The weak stimuli, a train of single shocks at 5 Hz, was given either superimposed on the middle of each burst (in phase), or symmetrically between the bursts (out of phase).

A strong tetanus alone induced LTP at both COM and MF synapses, while weak inputs alone produced no change. When the COM side received a weak input in phase with a strong MF tetanus, associative LTP (>30 min) was induced at COM synapses. In contrast, a weak MF stimulus failed to elicit associative LTP when applied in phase with a strong COM input. Furthermore, when the COM side received a weak input out of phase with a strong MF train, a long-term depression (LTD) of COM evoked population spikes was observed. Thus, MF synapses, which lack NMDA receptors, do not exhibit associative LTP. (Supported by Naval Research Grant #N00014-88-K-0198)

LEARNING AND MEMORY: PHYSIOLOGY III

226.1

NMDA-RECEPTOR-MEDIATED ACTIVITY AND LONG-TERM POTENTIATION (LTP) IN RAT NEOCORTEX. L.J. Bindman and K.P.S.J. Murphy*. Dept. of Physiology, University College London, London, WC1E 6BT, U.K.

We have examined the involvement of N-methyl-D-aspartate (NMDA)-receptor-mediated activity in the production and maintenance of LTP in rat neocortex. Both intracellular and field potential recordings were made from slices of neocortex in vitro, bathed in c.s.f. containing 1.1 mM Mg^{++}. D-2-amino-5-phosphono-valerate (D-AP5, 10 to 20μM) was bath applied to block selectively NMDA-receptor-mediated activity. Test shocks were applied to the subcortical white matter at 0.1 Hz.

We found that (1) e.p.s.p.s in layer V neurons did not potentiate over tens of min. although subsequent application of D-AP5 showed there was a significant component due to activation of NMDA-receptors (Aram, J. et al., J. Physiol. (Lond), 394: 117P, 1987). (2) The presence of D-AP5 prevented the induction of LTP in neurons of layers V and VI following conditioning trains, thus extending the observations on cells in layers III and IV (Artola, A. and Singer, W., Nature 330: 649, 1987); (3) Application of D-AP5 after establishment of LTP in layers III, V and VI reversibly reduced its magnitude, showing that NMDA-receptor-mediated activity was also involved in the maintenance of LTP in neocortex.

226.2

BATH APPLICATION OF PROGESTERONE INHIBITS THE INDUCTION OF LONG-TERM POTENTIATION IN AREA CA1 OF THE HIPPOCAMPUS. C.L. Dolorfo* and S. Veraggo. (SPON: L. Denner) Dept. of Biol. Sciences, San Jose State Univ., San Jose, CA 95192.

Long-term potentiation (LTP) is a phenomenon that has been proposed to be involved in both learning and memory and in epilepsy. It has been suggested that certain cognitive abilities, as well as seizure susceptibility in epileptic women, vary during the menstrual cycle. Such effects may be related to fluctuations in progesterone and estrogen levels. In a previous study, we observed that the duration of LTP in hippocampal slices from rats in metestrus (a time when the mean progesterone to estrogen ratio (P/E) is highest) was shorter than that generated in slices from rats in diestrus (when the mean P/E is lowest). Also, the duration of LTP produced in slices from progesterone treated ovariectomized (OVX) rats was shorter than that produced in slices from estradiol treated and untreated OVX rats. These results suggested that progesterone might have an inhibitory effect on the maintenance of LTP. The purpose of the present study was to determine if progesterone has a short-latency effect on LTP. Hippocampal slices from OVX Sprague-Dawley rats were perfused with artificial CSF (ACSF) containing progesterone (40ng/ml). A control population spike was produced in region CA1 (0.2Hz, 0.1ms) and maintained for 20 min before production of LTP (15Hz, 10s--3 trains, 10s intervals) was attempted. LTP could not be generated in progesterone treated slices (n=8; $p<0.001$), but LTP was produced in the control ACSF (n =10; $p<0.001$). These data suggest that progesterone may have a direct, short-latency effect that prevents the induction of LTP. These observations suggest a possible mechanism by which fluctuations in gonadal steroids may modulate cognitive processes and seizure frequency. Supported by the Epilepsy Foundation of America and by MBRS Support grant no. 2S06RR008192-08.

226.3

DANTROLENE BLOCKADE OF LONG-TERM POTENTIATION (LTP) IN THE RAT HIPPOCAMPAL CA1 REGION. A. Obenaus, I. Mody and K.G. Baimbridge. Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5

Previous reports from our laboratory and others have suggested an important role for calcium (Ca^{2+}) in the induction of LTP with a particular emphasis being placed on Ca^{2+} entry through NMDA activated channels. Using Wistar rat hippocampal slices maintained in vitro, we have examined the possible contribution of intracellular Ca^{2+} release in LTP. Dantrolene (DANT) blocks Ca^{2+} release from the sarcoplasmic reticulum of skeletal muscles, and our results indicate that DANT (20μM) also blocks LTP. The blockade is highly dependent upon the time of exposure to the drug; most likely due to slow diffusion of DANT into the hippocampal slices. Total blockade of LTP requires exposures of 2 hours or more. This blockade is unlikely to be via a direct effect on the NMDA receptor (see Mody et al. this vol.). We suggest that intracellular release of Ca^{2+} may contribute to the Ca^{2+}-dependent component of LTP in the rat hippocampus. (Supported by a Canadian MRC Program Grant to KGB.)

226.4

APPLICATIONS OF A FREEZING SYSTEM DESIGNED TO CAPTURE CNS DYNAMIC MORPHOLOGY. C.S. Wallace, F.-L. Chang and W.T. Greenough. Neur. & Beh. Biol. Prog., Depts. Psych. & Cell & Struct. Biol., Univ. IL, Champaign, IL 61820.

Previously we have reported the design of an inverted slam freezer (Wallace et al, Soc. Neurosci. Abs., 1987) which brings a mirror freezing surface in contact with a stationary specimen, in contrast to conventional freezers which require that the specimen be mounted in an inverted position prior to freezing. Beginning with this instrument, we have developed a system for studying the morphology of CNS synapses as a function of recent activation history. To keep the tissue in a properly controlled environment prior to freezing, we have designed a detachable chamber that mounts on the inverted slammer. By incorporating stimulating and recording electrodes directly into the freezer's specimen holder we have eliminated the need to transport the tissue prior to freezing. Timing of the stimulation and the freezing can be coordinated, with at least .04 msec precision, by optical sensors. A tangential dentate slice (Chang & Greenough, adjacent) permits rapid freezing of neural circuits lying immediately beneath a natural (pial) surface. It is hoped that this development can provide insights into the morphological correlates of central synaptic function. Supported by ONR, EFA

226.5

LONG TERM POTENTIATION IN UPRIGHT TANGENTIAL MOUSE DENTATE SLICES. F.-L. Chang & W.T. Greenough, Col. Med., Neur. Beh. Bio. Prog., Dept. of Psych., & Cell Struct. Bio., Univ. of Illinois, Champaign, IL 61820

One of the most significant drawbacks of transverse hippocampal slices is the loss of fiber projections that run perpendicular to the cut. In 1968, Yamamoto and Kawai (Jap. J. Physiol., 18, 620) described a slice preparation cut from the ventral surface of guinea pig dentate; however, the viable slice thickness allowed only the ventral portion of the hilus to be included, and this was potentially damaged due to proximity to the cut. We took advantage of the small size of the mouse dentate; within a tangential slice, most of the hilus is intact, as are the local interneurons and their projections.

Mouse hippocampus was dissected out and followed by 2 coronal cuts 150 um apart to isolate the mid-segment. A third cut produced a 450 um slice from the ventral surface. After an incubation of 1.5 hours, spontaneous unit activities could be recorded throughout the dentate. LTP was produced successfully via stimulation of the perforant path in over 70% of the slices. Among the successful slices, LTP of population spikes ranged from 20 to 59% (mean: 35%), and lasted for at least 1.5 hours.

This dentate slice, with its 2-D fiber projections and uncut ventral surface, combined with Wallace et al's inverted slam freezer (adjacent) provides an unique opportunity to study CNS plasticity. Funded by EFA & ONR.

226.6

BLOCKING CHOLINERGIC FUNCTION WITH ATROPINE FAILS TO ALTER THE ESTABLISHMENT OF LTP IN HIPPOCAMPAL SLICES *IN VITRO*. Y. McQueen* and W. D. Knowles (SPON: C. L. Cherniсky). Cleveland Clinic Foundation, Cleveland, OH 44195.

Cholinergic synaptic function exists in hippocampal slices, and may play a role in memory function. We tested the role of cholinergic function in the establishment of long term potentiation (LTP) in CA1 of the hippocampal slice by applying atropine during tetanization. There was no difference in the potentiation seen with atropine.

Guinea pig hippocampal slices (300-450 μm) were maintained at 35°C in physiological saline (3 mM K^+, 2 mM Ca^{++}, 2 mM Mg^{++}) in an interface chamber. Test stimuli (0.2 Hz) were delivered to the Schaffer collaterals on both the subicular and CA3 side of the recording electrode in CA1 stratum pyramidale. Stimuli amplitudes were adjusted to produce a half-maximal population spike. Two minutes prior to tetanization, atropine methyl nitrate (100 μM in physiological saline) or physiological saline was applied to the surface of the slice as a microdrop. LTP was produced by a 2 sec train of 100 Hz, 100 μs stimuli to one of the stimulating electrodes, randomly chosen. LTP was measured 15 minutes after tetanization. We saw no difference in the amplitude of LTP with atropine or vehicle. We conclude that muscarinic cholinergic function does not influence the establishment of LTP in CA1 pyramidal cells.

Supported by the Ohio Department of Aging.

226.7

COMPARISON OF DENDRITIC AND SOMA-INDUCED DEPOLARIZATION AS CONJUNCTIVE STIMULI FOR PAIRING WITH SYNAPTIC ACTIVATION, GIVING LONG-TERM POTENTIATION IN RAT HIPPOCAMPAL SLICES. Ø. Hvalby*, G.-Y. Hu*, J.-C. Lacaille and P. Andersen* (SPON: A.Njå) Inst. of Neurophysiology, University of Oslo 0162 - Oslo 1, Norway.

Temporal coupling of postsynaptic depolarization with low-frequency synaptic activation induce long-lasting potentiation of synaptic transmission across the radiatum fibre/CA1 hippocampal synapses.

Glutamate was iontophoretically delivered (200-800 ms) at a "hot spot" in the apical dendritic tree, giving a mean depolarization of 9.9 ± 6.0 mV, n=28). A single afferent volley to the same dendritic area was delivered simultaneously, and the conjunction was repeated 10-120 times at 0.2 Hz. In 20 of 28 cells this paradigm caused an improved synaptic transmission in the paired pathway, lasting more than 15 minutes. A separate non-paired pathway to the same cells was unchanged.

In contrast, when the same paradigm was applied for current-induced depolarization of comparable magnitude (12.2 ± 5.9 mV, n=18), no changes in the synaptic transmission was observed. Stronger depolarization, causing the cells to discharge above 50 Hz were effective.

The results suggest that dendritic depolarization is essential for an effective conjunctive effect. Stronger currents may be needed at the soma in order to spread sufficiently far into the dendritic tree.

226.8

THETA STIMULATION OF THE LATERAL OLFACTORY TRACT SERVES AS A CUE IN A SUCCESSIVE-CUE OLFACTORY DISCRIMINATION TASK. T.A. Otto, U. Staubli and G. Lynch. Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA 92717.

Electrical stimulation of the olfactory bulbs or of the lateral olfactory tract (LOT) has been reported to serve effectively as a discriminative stimulus in olfactory learning tasks (Mouly *et al.*, 1985; Roman *et al.*, 1987). The present report provides additional evidence of the efficacy of such stimulation to serve as a learning cue, and further describes a paradigm in which this stimulation activates piriform cortex at the same time as natural odors, permitting a description of the physiological events in a cortical network occurring during learning.

Stimulating electrodes were implanted bilaterally in the LOT and recording electrodes bilaterally in the piriform cortex at levels maximizing the dendritic monosynaptic population EPSP. Following recovery, water-deprived rats were trained to discriminate natural odors in a successive cue, "go, no-go" task. One sec nosepokes into an odor port resulted in the delivery of one of two odors--one 'positive', the other 'negative'. Sessions consisted of discriminating two session-unique odors to a criterion of 18 correct responses in 20 consecutive trials; normal rats generally require no more than 5 or 6 such sessions to reach criterion within the first 20-25 trials.

The first session of training with LOT stimulation required a discrimination between clean air as the 'negative' cue, and clean air plus 2 sec of bursts of 4 pulses at 100Hz delivered at theta frequency (7Hz) to one LOT as the 'positive' cue. The first burst was delivered 200 ms after the onset of clean air ejection; we have recently discovered that the firing of single units in piriform cortex to natural odors in this task is delayed by approx. 200 ms following odor presentation (McCollum *et al.*, 1988). The second session of LOT stimulation training required the subject to discriminate stimulation of one LOT from stimulation of the contralateral LOT. In both cases, subjects display nearly perfect transfer of learning from natural odors to electrical stimulation.

The procedures described herein permit an analysis of the monosynaptic responses accompanying behaviorally relevant and precisely controlled input to cortex, thereby permitting future analyses of the cellular events accompanying learning in a defined cortical network. Preliminary data suggest that synaptic changes in piriform similar to LTP may occur following stimulation training.

Supported by grants NIH 1FS NS08136 to TO, and ONR N00014-86-K-0333 to GL.

226.9

EFFECTS OF SCOPOLAMINE AND GLUCOSE ON LONG-TERM POTENTIATION IN DENTATE GYRUS. R.C. Michaelis and P.E. Gold, Department of Psychology, University of Virginia, Charlottesville, VA 22903-2477.

Glucose administration enhances memory in humans. Glucose also antagonizes scopolamine induced amnesia and hyperactivity, and augments physostigmine induced tremors in mice. These interactions between glucose and scopolamine, together with the evidence of cholinergic involvement in memory storage, led us to evaluate the effects of scopolamine and glucose on long term potentiation. Under physiological control in anesthetized rats, bipolar stimulating electrodes were implanted in the perforant path and monopolar recording electrodes were implanted in the hilus of the dentate gyrus. Test pulse amplitude (0.1 msec duration) was adjusted so that the population spike (PS) amplitude was 33-50% of the extracellular EPSP amplitude. High frequency stimulation (10 trains, 20 msec each, 400 Hz, 10 second ITI) resulted in a 356 ± 57% (mean ± sem) increase in the PS amplitude in control animals. Scopolamine (2.0 and 3.0 mg/kg) produced a dose-dependent reduction in the amount of potentiation (218 ± 41% and 165 ± 25%, respectively), without any marked changes in the baseline PS amplitude. Glucose (1000 and 1500 mg/kg) reversed the scopolamine (3 mg/kg) induced deficit in a dose-dependent manner (256 ± 32% and 313 ± 53%, respectively). These glucose doses by themselves had no effect on the response to test pulses or on potentiation. Scopolamine impairs the establishment of long-term potentiation, and this impairment can be attenuated with glucose. These pharmacological characteristics are similar to those observed in behavioral studies of memory. The findings add further support to the view that circulating glucose levels may regulate cholinergic function. [Supported by ONR N00014-85-K0472 and MH 31141].

226.10

AN ALGEBRAICALLY ADDITIVE RELATIONSHIP EXISTS BETWEEN BEHAVIORALLY AND ELECTRICALLY ELICITED INCREASES IN DENTATE GYRUS RESPONSIVENESS TO PERFORANT PATH STIMULATION. K.R. Isaacs, F.-L. Chang, and W.T. Greenough, Neur. & Beh. Biol. Prog. & Depts. Psych. & Cell & Struct. Biol., Univ. of Illinois, Champaign, IL 61820.

If increased dentate gyrus responses to synaptic activation resulting from experience (Green & Greenough, 1986) and conventional LTP have a common mechanism, they should show an additive relationship. Rats were housed at P30 in either EC, together in a large cage filled with toys, or IC, in individual cages, for 30-35 days. Littermates from each condition were operated on the same day. Rats were anesthesized with nembutal and electrodes were placed in the hilus of the dentate gyrus for recording and in the angular bundle for stimulation. An input/output curve (IO) was collected after positioning the electrodes to produce the maximal response. LTP inducing stimulation was then administered using 100Hz, 1 sec duration at a maximal response amplitude determined from the initial IO. LTP trains were separated by 5 min and were administered until a stimulus produced no further increases in response amplitude ($\bar{x}$=5 trains). After 30 minutes a second IO was collected and compared with the pre-LTP IO. In all five pairs of rats, the IC EPSP potentiated more than the littermate EC. Differences in EPSP potentiation ranged from 22.6% to 64.3%, with a mean of 48.08%. (Supported by ONR, EFA & NIMH)

226.11

EFFECTS OF GENETICS AND CHRONIC NICOTINE TREATMENT ON ACTIVATION OF NMDA RECEPTORS. J. M. Wehner, R. K. Freund, M. K. Anderson*, K. Kelly*, and A. C. Collins. Instit. for Behav. Genetics, Univ. of Colorado, Boulder, CO 80309

C57BL mice perform better on a spatial learning task than do DBA mice. This task and the phenomenon of long-term potentiation (LTP), may involve activation of NMDA receptors in the hippocampus. We tested granule cell population spike (PS) responses from hippocampal slices for sensitivity to the NMDA antagonist DL-2-aminophosphonopentanoic acid (AP5) in Mg^{2+}-free medium. Responses from C57 slices were slightly more sensitive than those from DBA mice to the inhibitory effects of AP5 (2-16 uM). These strains did not differ in the LTP response to high frequency stimulation (HFS; 400 Hz; 250 ms) in area CA1. Because nicotine may alter learning, the effects of chronic nicotine infusion (4 mg/kg/hr; 8 days) on CA1 PS responses were measured. Increased sensitivity to stimulation (increased I-O slopes) for control and post-HFS responses was observed in nicotine-treated C57 mice; no changes were observed for DBA mice. Slices from nicotine-infused C57 mice were also less responsive to HFS (smaller I-O shift). These results suggest a basal difference in NMDA receptor activation between these mouse strains, and chronic nicotine treatment elicits a larger strain difference on measures of CA1 cell responsiveness. (Supported by AFORS 85-0369 and R. J. Reynolds Tobacco Co.)

226.12

LONG-TERM SYNAPTIC DEPRESSION PRODUCED BY ANTIDROMIC CONDITIONING STIMULI IN CA1. S.Pockett, N.Brookes,* L. Bindman, Department of Physiology, University of Auckland, Auckland, New Zealand.

Pockett & Lippold (Exp.Neurol.91 481, 1986) found, using extracellular recording techniques at room temperature, that when the pyramidal neurons of the CA1 region or hippocampal slices were stimulated antidromically, by a train of pulses in a pattern that would produce long-term potentiation if delivered through a synaptic pathway, a long-term depression (LTD) of the synaptically derived population spike resulted. Here we report that LTD can also be demonstrated when recording intracellularly from slices at 34°C.

E.p.s.p.s were recorded in response to stimulation of the Schaeffer collateral/commissural pathway every 20 sec. Their amplitude remained constant during a 1.5 to 2 hour recording period. Stimuli applied antidromically to the alveus caused antidromic action potentials and sometimes inverted i.p.s.p.s in the CA1 neurons. If the antidromic stimuli were applied in a conditioning pattern of 6 bursts of 50 impulses at 100 Hz, at 10 sec. intervals, a depression of e.p.s.p. amplitude resulted which lasted as long as the recording period (1-2 hours).

It was possible to apply 25mM $Mg2+$ to the preparation and see total obliteration of e.p.s.p.s and i.p.s.p.s, then wash it off and see complete recovery. If the conditioning antidromic stimuli were applied in the presence of $Mg2+$, e.p.s.p.s remained depressed after the washout period. This shows that LTD is not a special case of heterosynaptic depression nor long lasting feed-back inhibition.

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226.13

OLFACTION AND IMMUNITY FROM CONDITIONED FOOD AVERSION IN MICE. N.E. Kinney, J.W. Wright and J.W. Harding.(Depts. of Psychology and Vet. Comp. Anat., Pharm. & Physiol., Washington State Univ.,Pullman), Dept. of Psychology, SE Missouri State Univ., Cape Girardeau, MO 63701.

Nerve sectioned mice demonstrate a degree of immunity to food aversion formation while anosmic (taste only mediated aversion), but lose this immunity once recovered (Kinney et al, Soc. Neurosci. Abstr. 11:527, 1985). Food deprived male albino mice were given access to novel food (almond) followed by 50 min of rotary motion (60 rpm). These mice then underwent a bilateral olfactory nerve section procedure (Harding et al., Brain Research, 132:11-18, 1977) and after 7 days recovery were given second access to almond. Immunity to conditioned food aversion was provided by nerve section induced anosmia introduced between first and second access. But it was not provided by injection of the centrally-acting antimuscarinic scopolamine hydrobromide (0.1 mg/kg) given prior to rotation. Nor was immunity provided by scenting the almond with a familiar odor (musk) present in home cage from birth to weaning. These results suggest that olfaction plays a central role in the formation of motion induced food aversions since anosmia introduced after first access is not followed by reduced second access consumption as found in controls.

Supported by SE Missouri State University GRFC.

226.14

BODY ROTATION-INDUCED CONDITIONED TASTE AVERSIONS IN RATS: COMPARISON OF SOME CONTINUOUS AND INTERMITTENT SCHEDULES OF ROTATION. K.-P. Ossenkopp, K. Desborough* and F. Boon*. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, CANADA N6A 5C2.

Several studies have shown that horizontal body rotation can be used as an effective unconditioned stimulus (UCS) to produce conditioned taste aversions (CTA) in rats. What is not clear from these studies, is whether some schedules of intermittent rotation are as effective as continuous rotation in producing the CTA. This issue was examined in two experiments. In Experiment 1 rats were given a saccharin solution to drink and then sham rotated, rotated for 1 hr (70 rpm), rotated for 1/2 hr, rotated for 1/2 hr after a 1/2 hr delay, or intermittently rotated for 1 hr on a schedule of 10 sec on - 10 sec off. Rats were conditioned every other day for a total of 4 conditioning days. Three days after the last conditioning trial they were given a two-bottle choice test. The strongest CTA was seen after 1 hr continuous or intermittent rotation. In Experiment 2 intermittent rotation schedules (1 hr duration) of 10 sec on-off, 60 sec on-off, or 900 sec on-off, were compared with sham rotation. The strongest CTA was obtained with the 60 sec on-off schedule. Thus, the amount of rotation, the length of the UCS period, and the number of on-off transitions in the UCS period, are important in determining strength of the subsequent CTA. (Supported by NSERC).

226.15

MEMORY ENHANCEMENT INDUCED DURING POST-LEARNING PARADOXICAL SLEEP. B. Hars, E. Hennevin, V. Bloch (SPON: J. Serviere). Depart. de Psychophysiologie, CNRS, Gif sur Yvette and Univ Paris XI, Orsay, France.

The hypothesis that newly acquired information is processed during paradoxical sleep (PS) following learning, suggests that the memory trace is in an active state at this time. In a previous work (Hars et al. Behav. Brain Res., 1985, 18, 241-250) rats were submitted to an active avoidance conditioning. After the conditioning session, the conditioned stimulus was given as a cue during PS phases: retention performances were enhanced. When cueing treatment was given during wakefulness or slow wave sleep (SWS) it had no facilitative effect. In the present work rats were trained to run in a maze for food reward. After each daily trial, mild electrical stimulation of the mesencephalic reticular formation was delivered during PS phases: there was a marked improvement in learning curves. The same stimulation was ineffective when delivered, at the same delays after trials, either during periods of SWS or wakefulness. These results give strong support to the hypothesis that the new memory trace is reactivated during post-learning PS.

226.16

VASOPRESSIN (AVP) IS RELEASED FROM THE DORSAL HIPPOCAMPUS (DH) OF FREELY BEHAVING MALE RATS: ABSENCE OF A RELATIONSHIP WITH SOCIAL MEMORY. A.D. Ramirez* and E.J. Roy. Depts. of Physiology and Psychology, Univ. of Illinois, Urbana, IL 61801.

Five adult male rats were implanted with push-pull cannulae aimed at the DH. Four rats were perfused (PPP) at a flow rate of 12-13 ul/min for 3.4-5h. A control period of 2h was followed by a 5-min exposure to a juvenile male rat (20-35 days) then re-exposed after 30 min to a second 5-min exposure to the same juvenile (Noninterference). Three rats (two from the previous experiment) were perfused (2.2-4h) in the absence of social interaction (Controls). In the noninterference experiments the behavior of the adult male rats was similar to that described by Thor and Holloway (1982) with a mean investigation value of 119.8 ± 25.5 sec for the first exposure and 79.6 ± 15.5 sec for the second 5-min exposure. Mean values of AVP released ranged from 0.035 to 0.35 pg/10min. No change was observed during these exposures (Pre-exposure x=0.18 ± 0.08, 12 intervals; Exposure period x=0.18 ± 0.03, 9 intervals). In the three control PPP the overall mean release rate of AVP was 0.17 ± 0.06 pg/10min (Range = 0.06-0.43).

In summary these results demonstrate that the DH releases AVP from freely behaving rats. In addition, the function of this peptide from the DH appears not to be correlated to social memory when using this paradigm.

226.17

THE NAPLES HIGH AND LOW-EXCITABLE RAT STRAINS: A REVIEW OF PUBLISHED AND UNPUBLISHED DATA. A.G. Sadile, Inst. Human Physiol. & Biomed. Phys., Univ. Naples,, Naples, Italy.

Increased locomotor activity in a novel environment is thought to depend mainly on the integrity of hippocampal formation. For, the Naples High- (NHE) and Low-Excitable (NLE) rat strains, selected on the basis of divergent activity in a modified Làt's box, have been proposed as genetic models to study locomotor hyper/hypo-activity in spatial novelty situations, under a similar background of emotionality, arterial blood pressure and learning ability. Aim of our report is to review evidence from a series of correlative analyses of multidisciplinary nature, which further validates this hypothesis. Behaviorally, both NHE/NLE-rats show an impaired working memory in an unreinforced tunnel maze and an impaired rate of acquisition of lever pressing for water compared to random-bred controls. Electrophysiologically, they are both sensitive to vasopressin and enkephalin induced slow potentials, with the NHE being hyper-sensitive. Both NHE/NLE-rats have fewer mossy fibers than controls. Immunoreactive vasopressin and corticosterone high-affinity binding show different strain dependent correlative profiles. The "metabolic" DNA, a DNA fraction with fast turnover, is not inhibited by spatial novelty in NHE-rats only. Both strains can be used as tool to study spatial information processing in the mammalian brain.

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EPILEPSY: HIPPOCAMPUS AND NEOCORTEX I

227.1

EXTRACELLULAR K+ AFFECTS HIPPOCAMPAL IPSPs BY ALTERING THE ACTIVITY OF OUTWARD POTASSIUM/CHLORIDE CO-TRANSPORT. Scott M. Thompson*,† and Beat H. Gähwiler (Spon: Dr. S. DiMauro). Brain Research Institute, University of Zürich, Zürich, Switzerland;† and Dept. of Neurology, Columbia University, NY, NY 10032*.

Activity-dependent elevations of $[K^+]_o$ affect the amplitude of GABAergic IPSPs due to alterations in their reversal potential. Single-electrode voltage-clamp techniques were used to record currents from CA3 cells in organotypic slice cultures of hippocampus in order to investigate the effect of $[K^+]_o$ on E_{Cl^-}.

Reducing $[K^+]_o$ from 5.8 to 1 mM shifted E_{IPSC} from -67.6 to -81.9 mV, and E_{GABA} from -64.7 to -81.1 mV, when V_M was held constant and depolarized with respect to E_{IPSC}. This shift was unaffected by block of K^+ channels with intracellular Cs^+. The dependence of E_{GABA} on $[Cl^-]_o$ was close to that predicted by the Nernst equation in both $[K^+]_o$ = 5.8 and 1 mM saline. Reducing the extracellular Na^+ concentration 50% had no effect on E_{GABA}. Furosemide caused a 69% decrease in IPSP driving force when V_M was clamped depolarized to E_{IPSC}. Reducing $[K^+]_o$ from 5.8 to 1 mM in the presence of furosemide produced a small shift in E_{IPSC} from -61.0 to -71.2 mV, however, washout of furosemide from $[K^+]_o$ = 1 mM saline shifted E_{IPSC} further to -89.8 mV.

We conclude that reducing $[K^+]_o$ lowers the intracellular Cl^- concentration by increasing the efficacy of outward potassium/chloride co-transport. Inhibition of outward Cl^- transport by elevated $[K^+]_o$ may be important in the genesis and spread of epileptiform discharge.

227.2

DEPRESSANT EFFECTS OF LIDOCAINE ON REPETITIVE FIRING OF CA1 PYRAMIDAL CELLS IN THE HIPPOCAMPAL SLICE. R. Capek and B. Esplin. Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, H3G 1Y6, Canada.

The effects of lidocaine on the CA1 pyramidal cells were studied in the superfused hippocampal slice preparation of the rat by a conventional intracellular recording technique. The cells were activated by injecting a single 500 ms depolarizing current pulse or a train of twenty 3 ms pulses at 5 to 100 Hz through the recording micropipette.

Lidocaine (20 to 100 µM) diminished the repetitive firing evoked by a single depolarizing pulse. The firing evoked by low current intensities was little affected but that evoked by high currents was substantially reduced.

The maximal rate of rise ($\dot{V}_{max}$) of action potentials evoked by trains of short depolarizing pulses at higher frequencies gradually declined. In the presence of lidocaine, $\dot{V}_{max}$ of the first action potential in the train was reduced only slightly or not at all but the decline of $\dot{V}_{max}$ was distinctly steeper.

A use-dependent block of sodium conductance can explain these findings and is the likely mechanism of anticonvulsant action of lidocaine. Since the reported effects of valproate were qualitatively identical (Capek and Esplin, Soc. Neurosci. Abstr. 12: 46, 1986), the present data support the notion that valproate acts the same way.

(Supported by the MRC of Canada).

227.3

HIPPOCAMPAL CA3 PHYSIOLOGY FOLLOWING ISCHEMIA-INDUCED LOSS OF CA1. J. Franck, B. Fadeel* and P. Schwartzkroin (SPON: A.B. Harris). Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

Hippocampal subfield CA1 often degenerates in individuals with intractable temporal lobe epilepsy. While the mechanism of this cell death is not clear, evidence suggests that remaining cell groups in hippocampus are part of the epileptic "focus."

We have induced a similar loss of CA1 by transient forebrain ischemia and begun to examine the physiologic consequences on remaining CA3 and dentate neurons. Adult male Wistar rats were made ischemic for 15 minutes by clamping the internal carotid arteries following permanent vertebral artery cautery. Several weeks later CA3 single cell and population responses were studied in *in vitro* hippocampal slices in a standard interface chamber perfused with reduced Mg^{++} medium (1.2 mM).

Following verified atrophy of CA1, pyramidal cells in CA3 discharge in burst patterns in response to synaptic activation (stimulation of the dentate gyrus). Furthermore, synchronous population afterdischarge activity and spontaneous multiple population spike episodes are recorded extracellularly. Studies are currently ongoing to determine the mechanisms and chronicity of this aberrant activity.

Long-term alterations in intrinsic properties of remaining hippocampal cells following selective CA1 atrophy and/or in synaptic restructuring between remaining cells may provide a substrate for focal discharge from "sclerotic" hippocampus.

Supported by NIH-NINCDS grants NS20482 and NS25155.

227.4

MECHANISMS UNDERLYING SPREAD OF POTASSIUM-INDUCED HIPPOCAMPAL TONIC-CLONIC SEIZURE DISCHARGES. P. Rabenu*, Y. Yaari and M.S. Jensen*. (SPON: D.H. Silberberg). Dept. of Physiol. Hebrew Univ., Jerusalem, Israel and Inst. of Physiol., Aarhus Univ., Aarhus, Denmark.

Exposing rat hippocampal slices to elevated [K] (7 or 8 mM) induces two types of epileptic foci: (i) "spiking" focus in area CA3, which regularly produces brief bursts of population spikes, and (ii) "seizure" focus in area CA1, which recurringly generates tonic electrical seizures. The bursts discharges from CA3 propagate into CA1, where they synaptically evoke burst activity. The bursts triggered in CA1 prior to a seizure episode (interictal) are small, while those evoked immediately after the tonic seizure are gigantic. The latter bursts constitute the clonic phase of seizure.

Seizure discharges commenced in subarea CA1c and the discharge zone slowly (ca. 1 mm/s) expanded towards CA1a. Within this zone, population spikes locally generated during the tonic phase, or synaptically triggered during the clonic phase, spread much faster (ca. 0.3 m/s) in the same direction. The data suggested that the slow spread of the seizure discharge zone is mediated by the spatial dispersion of [K]o, while the fast spread of population spikes within this zone is due to ephaptic interactions among CA1 neurons.

Supported by the Israel National Academy of Science and ETP.

227.5

ROLE OF EXCITATORY AMINO ACID RECEPTORS IN POTASSIUM-INDUCED HIPPOCAMPAL SEIZURE DISCHARGES. M.S. Jensen*, P. Rabenu* and Y. Yaari. (SPON: A.K. Asbury). Inst. of Physiology, Aarhus University, Aarhus, Denmark and Dept. of Physiology, Hebrew University, Jerusalem, Israel.

The role of non-NMDA (i.e. quisqualate/kainate) and NMDA receptors in hippocampal interictal and seizure discharges was examined using specific non-NMDA (quinoxaline derivative FG-9041) and NMDA (+-APV) blockers.

Interictal bursts and tonic-clonic seizure episodes were induced in the CA1 fields of rat hippocampal slices by elevating [K] in the perfusing media to 7-8 mM. Interictal and clonic burst generation was totally suppressed by FG-9041, but apparently unaffected by APV. Neither drug alone nor in combination interfered with the generation of tonic seizure episodes.

In between seizures, EPSPs evoked in area CA1 by stratum radiatum stimulation, were markedly reduced by FG-9041 but only mildly attenuated by APV. During the tonic phase of seizure, however, a prominent late component of the EPSP was blocked by APV. This APV-sensitive component could be isolated by perfusing FG-9041.

Thus, while seizure episodes are not initiated by glutamatergic excitation, synaptic activation of NMDA receptors is enhanced during the tonic phase of seizure.

Supported by Danish BTC, ETP and Israel National Academy of Science.

227.6

EPILEPTIFORM ACTIVITY INDUCED IN RAT HIPPOCAMPAL SLICES BY HIGH BICARBONATE-CONTAINING MEDIA. J. Church* and H. McLennan* (SPON: J.A. Pearson). Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

The HCO_3'/H_2CO_3 buffer pair is largely responsible for maintaining c.s.f. pH within normal limits. We have examined the effect of altering $[HCO_3']_o$, and thus pH_o, on rat CA1 pyramid function recorded intracellularly.

Perfusion with medium containing 72mM HCO_3' (pH 7.9 after equilibration with $95\%O_2:5\%CO_2$) resulted, within 5 min, in an increase in input resistance and a reduction in threshold for spike generation. Neurons previously quiescent at resting potential often became spontaneously active. Accommodation of spike discharge to depolarizing current injection was enhanced, as was the afterhyperpolarization (AHP) following the spike train, and many neurons developed the ability to generate intrinsic burst discharges. Within 30-120 min spontaneous epileptiform bursts, which could also be elicited synaptically, were seen. These were abolished by addition of 20 μM ketamine. Ketamine did not, however, affect the intrinsic ability of the neurons to generate burst potentials.

It is unclear whether the observed activity results from a direct effect of changing $[HCO_3']_o$ or from the resultant change in pH_o. It is unlikely, however, to be a consequence of the reduced $[Ca^{2+}]$ seen in high-HCO_3' media as Ca^{2+}-dependent processes, including synaptic activity and $G_{K(Ca)}$-mediated AHPs, are well maintained.

227.7

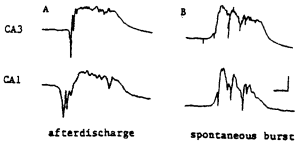
LITHIUM AGGRAVATES MALAOXON-INDUCED BRAIN CELL INJURY. A. Naukkarinen*, L. Paljärvi*, M.R. Hirvonen*, H. Komulainen* and K.M. Savolainen. Depts. of Pathology and Industrial Hygiene, University of Kuopio, SF-70210; and Dept. of Hygiene and Toxicology, National Public Health Institute, SF-70700 Kuopio, Finland.

Acute brain cell injury induced by a single dose of malaoxon (26.2 or 39.2 mg/kg i.p.) was studied in the Han/Wistar rat by light and electron microscopy. Astrocytic edema was found in cortical layers 2-3, and hippocampus. The neuronal injury was characterized either by cytoplasmic vacuolation due to enlarged RER cisternae or by cell shrinkage with or without microvacuolation. Cell injury is likely to be epileptic in nature, since the type and distribution of cell injury were similar to those reported in some other models of epilepsy (e.g. Söderfeldt et al., Acta Neuropathol. (Berl), 54: 219, 1981), and definite injury was found only in convulsing animals. Pretreatment with lithium chloride (10 mEq/kg) aggravated both convulsions and cell injury. Parallel experiments suggest that the effects of malaoxon could be mediated by changes in phosphoinositide signalling.

227.8

CA3 TO CA1 DELAY REVERSED DURING INTERICTAL TO ICTAL TRANSITION IN 0 Mg^{++} INDUCED ICTAFORM EVENTS IN RAT HIPPOCAMPAL SLICE. L.S. Jones, D.D. Mott* and D.V. Lewis. Dept. of Peds. and Pharm. Duke Univ. Med. Ctr., Durham, NC

It is known that spontaneous interictal epileptiform bursts probably start in CA2-CA3 and spread to CA1 (Wong and Traub, 1983). With ictal activity, however, CA1 may be the primary generator (Traynelis and Dingledine, 1988; Kojima, personal com.). We studied the ictal events produced by removing extracellular Mg^{++} from the perfusate bathing hippocampal slices from adult, male rats. These events are periodic, comprising a preictal build-up of bursting, followed by an ictal event, and a subsequent postictal quiescent phase. Recording from CA1 and CA3, we find preictal bursts occurred first in CA3, but as they became larger and more complex, the CA3-CA1 latency decreased then reversed until CA1 lead by up to 10 msec during the ictal discharges. Also, in stimulus train induced bursting CA1 lead CA3 during electrical afterdischarges following a stimulus train, but CA3 lead CA1 during spontaneous bursts (see figure).



Supported by NS 22170.

227.9

Cl^--DEPENDENT AFTERPOTENTIALS OF EPILEPTIC DISCHARGES IN THE MOTOR CORTEX OF THE RAT. S.Uhlig*, O.W. Witte and E. Valle* (SPON.: EBBS), Neurologische Universitätsklinik, Moorenstr. 5, 4000 Düsseldorf, F.R.Germany

In the present experiments the role of Cl^- currents in generation of afterpotentials of penicillin-induced PDS was further analyzed using intracellular recordings in the motor cortex of anesthetized rats in vivo.

Afterdepolarizations with an average duration (100% to 10% decay time) of 700 ms (fast afterpotentials) could be transformed into afterhyperpolarizations and v.v. by intracellular current injection. Reversal usually occurred between -60 and -70 mV. Intracellular Cl^- injection reversably shifted the inversion potential towards more positive values. It did not affect the slow afterpotentials. The equilibrium potential of the fast afterpotentials also shifted into the positive direction with high intensity stimulation of the brain. The positive shift was already observed following a single stimulus. It increased with repetitive stimulation.

The experiments indicate that Cl^--dependent membrane currents are involved in the generation of the fast afterde- and afterhyperpolarizations and that strong synchronization can lead to significant intracellular Cl^- accumulations.

227.10

HYPOXIA-INDUCED ELECTROGRAPHIC SEIZURES IN RAT HIPPOCAMPAL SLICES. K.Kawasaki*, S.F.Traynelis and R.Dingledine (SPON: D.L.McIlwain). Dept. of Pharmacol., Univ. of North Carolina, Chapel Hill, NC 27514.

It has been previously reported that a small proportion of rat hippocampal slices bathed in 8.5 mM $[K^+]_o$ generate spontaneous electrographic seizures. We have found that, in slices not undergoing spontaneous seizures in 8.5 mM K^+, brief periods of hypoxia produced seizures in the CA1 region. Rat hippocampal slices bathed in high K^+ were held in an interface chamber. Hypoxia was induced by changing the overflow gas from 95%O2/5%CO2 to 95%N2/5%CO2 for 5 to 60 sec. In 39% of 176 slices generating interictal bursts in CA3, hypoxia produced seizures in CA1 similar in appearance to spontaneous seizures. The CA3 region appeared considerably more resistant to hypoxia, since only 2% of slices had seizures in CA3. CA3 interictal burst frequency and intensity were not markedly changed before CA1 seizure but were increased during and reduced after seizure. The intensity of hypoxia-induced seizures was decreased by the NMDA receptor antagonist D-APV (30 μM), but not by L-APV. Lowering the temperature from 36-37° to 33-34°C, or cutting the Schaffer collateral input, abolished hypoxia-induced CA1 seizures. Intracellular recordings revealed gradual depolarization of CA1 pyramidal cells preceding seizures. Long-lasting hyperpolarization was also seen after seizures. Hypoxia might decrease uptake of K^+; the resulting acceleration of $[K^+]_o$ accumulation following each incoming interictal could then trigger CA1 seizures.

(Supported by NS17771)

227.11

INVERSE RELATION BETWEEN CA1 EXCITABILITY AND OSMOLALITY IN HIPPOCAMPAL SLICE. B.A. Ballyk and R.D. Andrew. Anatomy Dept., Queen's University, Kingston, Ont. K7L 3N6.

Plasma hyperosmolality draws water from neurons, leading progressively to CNS depression and coma. Similar symptoms result with progressive hyposmolality, but may include increased seizure susceptibility. Using rat hippocampal slices, we examined if CA1 pyramidal cell excitability changed when superfusate osmolality ranged between ± 60 mOsm (normally 290 mOsm). The amplitude of the orthodromic and antidromic population spikes (PS) were inversely related to osmolality: both decreased by ~1% with every mOsm increase (using NaCl or mannitol) and increased by ~1% with each mOsm decrease (using H_2O). The amplitude of the evoked EPSP also decreased with hyperosmolality and increased with hyposmolality, as recorded extra- and intracellularly. Other intracellular parameters (resting potential, input resistance, spike threshold) remained unchanged. The amplitude of the field interictal burst in 0 Mg^{2+} saline was also inversely affected by osmolality. Freely permeable DMSO had no effect at +60 mOsm. These data show that osmolality alters population events but not single neuron properties, probably by changing cell volume and thus ephaptic interactions during synchronous firing. As an example, hyposmotic swelling increases extracellular resistance which increases the field PS and EPSP, possibly making hippocampus more prone to epileptiform activity.

227.12

NEURONAL EXCITABILITY AND OSMOLALITY IN THE RAT NEOCORTICAL SLICE. A.S. Rosen* and R.D. Andrew. Anatomy Dept., Queen's University, Kingston, Ontario, K7L 3N6.

Our hippocampal studies (Ballyk and Andrew, this meeting) prompted an examination of osmolality effects upon neocortical excitability. Plasma osmolality is normally maintained around 287 mOsm and regulated within ±1%. However, clinical disorders can lead to hyperosmolar states (eg diabetes mellitus) or hyposmolar states (eg water intoxication). Clinical manifestations of either state are neurologic, progressing from lethargy to coma. Notably, seizure susceptibility increases in hyposmolar states. We therefore examined neuronal excitability in layers II and III of rat neocortical slices when superfusate osmolality was raised with mannitol or lowered with H_2O. Within a ±60 mOsm range, intracellular recordings (n = 12) displayed little change in resting potential, input resistance, VI relation or spike threshold. However, evoked EPSP amplitude declined 31-66% with increases of +30 to +60 mOsm and increased 25-43% in changes of -30 to -50 mOsm. These effects reversed in normal saline. Orthodromic field potentials were reduced with hyperosmolality and increased with hyposmolality. Thus, the osmolar state affects synchronous population discharge but not single neocortical cell properties. Freely permeant DMSO had no effect at + 60 mOsm. We suggest that cellular swelling augments field interactions and increases evoked EPSPs which may help explain increased seizure susceptibility in hyposmolar states.

227.13

MANGANESE AND EPILEPSY: ROLES FOR BOTH SEIZURES AND GENES IN LOWERED MANGANESE CONCENTRATIONS IN GENETICALLY EPILEPSY PRONE RATS. G.F. Carl,[1,2] J.W. Critchfield,[1] J.L. Thompson[1,2], B.B. Gallagher[3] and C.L. Keen[3]. Dept. Neurol Med. Coll. GA and Med. Res., VA Med. Ctr., Augusta, GA 30910 and Dept. Nutr., U. Cal, Davis, CA 95616.

It has been shown by several investigators that epileptics exhibit lower whole blood concentrations of manganese (Mn) than do controls. One group has suggested that the seizure activity itself is responsible for the depletion. Another group has suggested a possible genetic effect causing lowered blood Mn concentrations. In order to differentiate between these effects we used 14 9 wk old genetically epilepsy prone rats (GEPR-9) and 10, 9 wk old genetically epilepsy resistant rats (GERR) derived from GEPR progenitors. The GEPR-9 animals were divided into two groups of 7 each, and one group was exposed to a 100 decibel sound (ringing bell, 15 sec) twice a day for three weeks producing a 73% seizure rate, while the other group was handled identically but protected from noise. The animals in which seizures were induced consumed more food but gained less weight than either the unexposed GEPR group or the GERR group. Concentrations of Mn were lowered in brain blood and heart of GEPR animals compared to GERR animals while Mn concentrations were lower in blood and liver of seized GEPR animals, compared to unexposed GEPR animals. Therefore, it seems that both seizure activity and genetic difference contribute to the lower Mn concentrations found in genetically epilepsy prone rats.

227.14

IONIC CHANGES AND INCREASED MEMBRANE EXCITABILITY FOLLOWING REPLACEMENT OF EXTRACELLULAR Cl^- IN THE RAT HIPPOCAMPUS. J. Louvel*, P. Perreault, C. Drapeau, R. Pumain*, and M. Avoli. (SPON: G. Kostopoulos). MNI, Montreal, Canada and INSERM (U97), Paris, France.

Ion sensitive and intracellular recordigs were performed in the CA1 subfield of the rat hippocampal slice to study the effects evoked by low Cl^- artificial cerebrospinal fluid (ACSF). Measurements performed directly in the ACSF where 95% of Cl^- had been replaced with Methylsulphate or Isethionate revealed marked decreases in the concentration of Na^+ (17 -28%), Ca^{++} (40-50%), K^+ (21-29%) and Mg^{++} (3-15%). Similar changes were observed for $[Ca^{++}]$ and $[K^+]$ when their activity was measured in the extracellular space of the slice during perfusion with low Cl^- ACSF. During such procedure hippocampal pyramidal cells depolarized by 2-7 mV and their input resistance increased by 12-82% (n=8). A 2-6 mV decrease in action potential (AP) threshold and a decrease in AP amplitude were also observed. The repetitive firing evoked by an intracellular depolarizing pulse increased in low Cl^- ACSF, but the subsequent, slow afterhyperpolarization augmented in both duration and amplitude even when the injected current was adjusted to trigger the same number of APs as in control. Our data indicate that cations concentrations decrease in low Cl^- ACSF. These changes might contribute to the increased excitability induced by the low Cl^- ACSF.

227.15

EPILEPTIFORM DISCHARGES INDUCED BY LOW Cl^- ARTIFICIAL CEREBROSPINAL FLUID (ACSF) IN THE RAT HIPPOCAMPAL SLICE. R. Pumain*, C. Drapeau, P. Perreault, J. Louvel* and M. Avoli. (SPON: P. Gloor). MNI, McGill University, Montreal, Que., Canada and INSERM (U97), Paris.

$[K^+]_o$, extracellular and intracellular activities were recorded in the CA1 subfield of hippocampal slices maitained "in vitro" and perfused with ACSF in which 95% of Cl^- had been replaced with Isethionate or Methylsulphate. In this type of medium anti- or orthodromic stimuli evoked synchronous epileptiform bursts sometimes followed by a long-lasting depolarization (up to 15s). The latter was accompanied by an increase in $[K^+]_o$ (up to 11-12 mM) which was maximal at the border between s. pyramidale and s. oriens. NMDA receptors antagonists APV or CPP failed to block these discharges although more current was required to elicit them. Conversely in the presence of bicuculline or picrotoxin the late and long-lasting component of the epileptiform response was blocked and failed to appear even following high intensity stimuli. These data suggest that in the presence of low Cl^- the late component of the epileptiform discharge generated by hippocampal pyramidal cells involves the efflux of Cl^- ions through channels coupled to GABA receptors. Supported by MRC of Canada, FRSQ, INSERM and NATO.

227.16

PHARMACOLOGICAL FEATURES OF THE Mg^{++}-FREE EPILEPTIFORM BURSTS IN THE HUMAN NEOCORTEX MAINTAINED "IN VITRO". C. Drapeau, M. Avoli, A. Olivier and J.G. Villemure, MNI, McGill University, Montreal, Que., Canada.

Human tissue was obtained during surgery performed on 16 patients with epilepsy resistant to conventional therapy. Extracellular recordings were performed in slices maintained "in vitro". Spontaneous, synchronous epileptiform bursts were observed within 45-90 min of perfusion with Mg^{++} -free medium. Epileptiform bursts were observed in 60% of the slices studied and occurred at a frequency of .0 9 ± .04Hz, each lasting 4.5±1.9s. The amplitude of the bursts varied according to the position of the electrode in the neocortical layers showing maximum amplitudes in the superficial layers. Bicuculline (50uM) decreased the burst frequency by 37±8% and increased the duration of the burst by 43±28%. The competitive antagonist of NMDA receptors, (1-5 um) CPP reversibly blocked the bursts in a concentration-dependent manner by inducing a gradual increase in the interburst interval. Similar findings were also obtained with the non competitive NMDA antagonist MK-801, although the effects induced by this drug were not reversible. These data further demonstrate that Mg^{++} -free induced bursts in the human tissue are mediated through the activation of NMDA receptors and suggest the presence of active GABAergic mechanisms in this model. Supported by MRC of Canada, FCAR, FRSQ.

227.17

KINDLED SEIZURE SUSCEPTIBILITY OF ADULT RABBITS EXPOSED POSTNATALLY TO HYPOXIA. J.A. DeLeo*, C.D. Applegate, A.V. Lorenzo, D.H. Hsi*, J.L. Burchfiel, C.T. Lombroso. Developmental Neurophysiology, Children's Hospital, Harvard Medical School, Boston, MA 02115.

It is suggested that perinatal hypoxia increases the susceptibility to seizures. The effect of postnatal hypoxia on subsequent seizure susceptibility was assessed by amygdaloid kindling in adult rabbits. Rabbits, like humans, are classified as perinatal brain developers. Rabbits from 1 to 44 days were exposed to 100% N_2 for an average of 7 min or until the heart rate was reduced by 70%. Upon removal into room air, they recovered within 1 to 3 min. Non-hypoxic littermates served as controls. At 2 months of age, animals were implanted with bilateral amygdalae electrodes. Control animals required an average of 19 trials to elicit a stage 6 generalized convulsion. By contrast, the experimental animals exhibited significantly advanced motor behavior (average of stage 5) on the first trial. In the experimental group, the behavioral responses were observed at the onset of afterdischarges, indicating a decreased number of stimulations to produce stages of seizures. The duration of afterdischarges on the first trial was longer in experimental animals. These results suggest that exposure to severe, acute hypoxia during postnatal development increases the susceptibility to kindling stimulation. (The Kaplan Foundation, NIH HD 15304, Vallely Family Fund)

227.18

ZERO MG++ INDUCED SEIZURE DISCHARGE IN THE PERIRHINAL-PYRIFORM SLICE PREPARATION. J.R. Plant* and D.C. McIntyre. Dept. of Psychology, Carleton University, Ottawa, Canada, K1S 5B6

Recent in vivo studies have reported a NMDA receptor-dependent epileptogenic effect of magnesium-free (Omg++) perfusate on hippocampal and neocortical slice preparations. Due to the important role NMDA plays in limbic seizures, the present study investigated the effects of Omg++ on spontaneous and evoked activity in coronal slice preparations of the perirhinal-pyriform cortex (CTX) of the rat. Exposure to Omg++ resulted in the gradual ($\bar{x}$ = 12 min) development of ictal discharges which originated in the perirhinal CTX and propagated to the pyriform CTX. Although discharges in the perirhinal CTX usually preceded those in the pyriform CTX ($\bar{x}$ = 21 msec), prolonged exposure to Omg++ resulted in the development of independent discharges in the amygdala-pyriform CTX region. In Omg++, isolated minislices of the perirhinal CTX exhibited characteristic discharge patterns at onset times shorter than those seen in intact coronal sections.

These results suggest an important role for both NMDA receptors and the perirhinal CTX in the development of ictal activity in the amygdala-pyriform CTX region. Difference between kindled and control tissue will be described.

227.19

PERSISTENT SYNAPTIC FAILURE AND NEURONAL DEPOLARIZATION DURING EXPERIMENTAL EPILEPSY. G.B. Watson, R.K. Rader, T.H. Lanthorn. CNS Diseases Research, G.D. Searle, Chesterfield, MO 63198.

Status epilepticus can result in permanent brain damage. We have recently begun to investigate the effects of epileptiform activity in the rat hippocampal slice in low levels of glucose which approximate those occurring during experimental status epilepticus. Low glucose in conjunction with a convulsant results in persistent synaptic failure (PSF), just as low glucose plus hypoxia (experimental ischemia) does (Rader, et al., this meeting). Such models may prove useful in the evaluation of compounds which can prevent brain damage due to status epilepticus and related syndromes. In the zero Mg^{++} model of epileptiform activity, high frequency electrical stimulation (50Hz/5sec) of the Schaffer collateral/commissural fibers can elicit a spreading depression-like event as recorded in CA1. In 10mM glucose, synaptic responses are initially depressed, but recover fairly rapidly following this depolarization. At lower glucose concentrations (2mM), synaptic responses do not recover following high frequency stimulation or following spontaneous depolarizations which occur in 2mM glucose/zero Mg^{++}. Application of the NMDA antagonist CPP (100μM) immediately after the depolarization event has proven effective in reducing PSF, with at least partial recovery seen in all slices tested.

EPILEPSY: SECOND MESSENGERS AND mRNA

228.1

A CALMODULIN ANTAGONIST RETARDS THE DEVELOPMENT OF KINDLING AND REDUCES THE PRODUCTION OF C-FOS PROTEIN FOLLOWING A KINDLING STIMULUS. D.G. Herrera*, M.R. Peterson* and H.A. Robertson. Dept. Pharmacology, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

We have recently shown that kindling stimuli in the rat are accompanied by activation of the proto-oncogene c-fos in the dentate gyrus. This proto-oncogene may be a master-switch, regulating protein production. However, there is only circumstantial evidence suggesting that c-fos is involved in kindling. In cultured cells, the activation of c-fos production is dependent on calcium influx and activation of calmodulin. We therefore studied the effects of the specific calmodulin inhibitor W7 on both kindling and the production of c-fos protein. Here we report that the calmodulin antagonist W7 retards the development of kindled seizures and reduces the production of c-fos protein.

Rats were given saline or W7 (N6-aminohexyl-5-chloronaphtalene-sulphonamide) (15 mg/kg, i.p.) followed by a kindling stimulation through a bipolar electrode implanted in the amygdala. This dose of W7 usually eliminated c-fos protein accumulation in the dentate gyrus. W7 also significantly retarded the development of kindled seizures. These results add to the evidence which suggests that activation of the proto-oncogene c-fos is involved in long-term changes in brain.

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228.2

LASTING INCREASE IN EXCITATORY AMINO ACID RECEPTOR-MEDIATED POLYPHOSPHOINOSITIDE HYDROLYSIS IN SLICES OF THE AMYGDALA/PYRIFORM CORTEX OF AMYGDALOID AND HIPPOCAMPAL KINDLED RATS. K. Akiyama, N. Yamada* and S. Otsuki*. Dept. of Neuropsychiatry, Okayama Univ. Med. Sch., Okayama 700, JAPAN.

In search of neurobiological alterations underlying lasting seizure susceptibility of kindling, we previously demonstrated that ibotenate (IBO)-stimulated polyphosphoinositide (PPI) hydrolysis increased significantly in the amygdala/pyriform cortex (AM/PC) 7 days after the last seizure in the AM kindled rats (Akiyama et al., Exp Neurol 98:499-508, 1987). The present study examined 1) whether the increase in IBO-stimulated PPI hydrolysis in the AM/PC is more lasting and 2) whether hippocampal (HIPP) kindling elicits similar changes. Electrodes were implanted into either the left AM or the left dorsal HIPP to prepare kindled rats. In the AM-kindled rats, IBO-stimulated accumulation of [^{3}H]inositol 1-phosphate ([^{3}H]IP_1) significantly increased in the AM/PC 1, 2 and 4 weeks after the last seizure. 4 weeks after the last seizure, there was a similar magnitude of significant increase in the contralateral (right) AM/PC and the ipsilateral (left) AM/PC. In the HIPP-kindled rats, IBO-stimulated accumulation of [^{3}H]IP_1 increased significantly in both the AM/PC and HIPP 24 hours, 5 days and 15 days after the last seizure. The remarkable and lasting increase in IBO-stimulated PPI hydrolysis coupled to excitatory amino acid receptors in the AM/PC in the AM and HIPP kindling may be associated with development of kindling and long-term maintenance of kindled events.

228.3

SEIZURE-INDUCED ALTERATIONS IN LIPID METABOLISM IN HIPPOCAMPUS AND CEREBRAL CORTEX. D. L. Birkle, P. Kurian*, N. G. Bazan. LSU Eye Center, New Orleans, LA 70112

Alterations in endogenous glycerolipids and free fatty acids (FFA) during electroconvulsive shock (ECS) in hippocampus (HPC) and cerebral cortex (CTX) of Wistar rats and C57BL mice were quantified by analysis of fatty acid methyl esters (FAME). Arachidonic acid (20:4, n-6) turnover in lipid pools were measured using in vivo labeling by intraventricular injection of ^{14}C-20:4. PolyPI of CTX were more highly enriched in 20:4 than HPC polyPI. 30 sec after ECS, FFA in CTX and HPC increased 50%, with major changes in stearic acid (18:0) and 20:4. Diacylglcyerols (DG) accumulated in HPC, with 18:0, 18:1 and 20:4 affected most. There was loss of 20:4 from PIP and PIP_2 in CTX; changes in HPC polyPIs were minimal. Triglycerides (TG) in CTX decreased, particularly polyunsaturated species. There were no alterations in endogenous levels of other glycerolipids, but there was increased ^{14}C-20:4 turnover in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of HPC and CTX. These data indicate major differences between CTX and HPC in lipid responses to stimuli and imply lesser correlation between alterations in polyPI, DG and FFA; thus, other glycerolipids (TG, PC and PE) may contribute to DG accumulation. Also, phospholipase A_2-mediated release of 20:4 from polyPI may account for some polyPI loss and accumulation of free 20:4. These ECS-induced changes in cerebral lipids are not observable in whole cerebrum. (NS23002 and BRSG SO-RR5376).

228.4

PERTURBATION OF HIPPOCAMPAL MEMBRANE-ASSOCIATED ACTIVITY OF PROTEIN KINASE C AND AN INHIBITOR AFTER A GENERALIZED SEIZURE. J.T. Slevin, G.N. Barnes*, and T.C. Vanaman*, Veterans Administration & Univ. of KY Lexington, KY 40536

Brain Protein kinase C (PKC), implicated in long term potentiation and neurosecretion, may play a role in epilepsy. Here we report partial purification of an inhibitory activity of PKC which, in concert with PKC activity associated with hippocampal membranes, changes following administration of electroshock seizures to rats. To separate PKC from the inhibitory activity, particulate and soluble fractions of hippocampi were subjected to DE-52 chromatography. A decrease in membrane-associated PKC activity was seen by four minutes after electroshock with complete return of activity by 10 minutes post-stimulus. No changes were observed in cytosolic activity. PKC inhibitory activity associated with hippocampal membranes demonstrated a parallel time dependent increase with recovery by 10 min while cytosolic activity showed a concomitant loss. This inhibitory activity appears to be a phosphoprotein phosphatase, as judged by sensitivity of the activity to phosphatase inhibitors and ability to release ^{32}[Pi] from histone IIIS. Data suggest that time-dependent changes in the phosphorylation state of PKC substrates in hippocampal neurons may be partially responsible for the post-ictal depression observed in animals, including humans, after a generalized seizure. Supported by the V.A. and NIH grant #5-RO1-NS21868.

228.5

LITHIUM ENHANCES MALAOXON-INDUCED CONVULSIONS BUT ATTENUATES ASSOCIATED INCREASES OF CEREBRAL INOSITOL-1-PHOSPHATE LEVELS IN WISTAR RATS. K.M.Savolainen[1], M.R.Hirvonen[2]*, H.Komulainen[1], L.Paljärvi[3]*, and A.Naukkarinen[3]* (SPON: E.J.Walaszek). [1]Natl. Public Hlth Inst., Dept. Environm. Hyg. & Tox., SF-70700 Kuopio, [2]Dept. Industr. Hyg., and [3]Dept. of Pathology, University of Kuopio, SF-70210 Kuopio, FINLAND.

The potential of a single dose of malaoxon (26.2 or 39.2 mg/kg i.p.) to induce epileptic convulsions and to stimulate the hydrolysis of cerebral phosphoinositides (PI's), as assessed by brain regional inositol-1-phosphate (Ins1P) levels was studied in Han/Wistar rats 24 h after saline or LiCl (10 mEq/kg i.p.) pretreatment. Rats were observed for 1, 4 or 72 h, and then the brains were analyzed for regional Ins1P levels by GLC. Malaoxon induced convulsions at the higher dose only. It dose-dependently elevated Ins1P levels in nonconvulsing rats with a marked persistent additional increase of brain Ins1P during convulsions. LiCl significantly increased the potential of malaoxon to induce convulsions but it attenuated brain Ins1P elevations after malaoxon; the temporal patterns of Ins1P increases after malaoxon were the same with both pretreatments. These results suggest that, in this rat strain, lithium inhibits brain inositolpolyphosphate phosphatases decreasing the accumulation of Ins1P under malaoxon-induced stimulation of the brain (see Majerus et al., J. Biol. Chem., 263:3051, 1988).

228.6

MAINTENANCE BUT NOT INITIATION OF STIMULUS TRAIN INDUCED BURSTING (STIB) IS IMPAIRED BY PROTEIN SYNTHESIS INHIBITORS (PSIs). D.V. Lewis, L.S. Jones, and D. Lapadula. Dept of Pharm. and Peds., Duke Univ. Med. Ctr. Durham, NC 27710

We have been examining the effect of PSIs on the STIB model of epileptogenesis. 625 u hippocampal slices from adult, male rats were prepared in the normal manner. When a slice was stable, a PSI was bathed on for 30 min (cycloheximide 35 uM, emetine 15 uM, puromycin 25 uM, anisomycin 50 uM; protein synthesis inhibiton 80% or greater). Then, up to 10 stimulus trains were presented, one every five min, until there were afterdischarges following a stimulus train, and spontaneous bursts between trains. There were no statistically significant differences between the number of trains necessary to elicit afterdischarges in control and PSI-treated slices. Both emetine and puromycin slightly delayed the onset of spontaneous bursts, but the other PSIs were not different from controls. These data indicate that the development of bursts produced by STIB does not depend on protein synthesis. However, in slices that were continuously bathed in either cycloheximide or anisomycin for a further 5 hours following the induction of bursting, there was a more rapid and pronounced decay in the frequency of bursting, suggesting that the protein synthesis inhibitors are interfering with the maintenance of the bursting in CA3 and CA1 in some manner.

Supported by NS 22170.

228.7

DEFICIENT ATP HYDROLYSIS AS A MECHANISM OF EPILEPSY IN SEIZURE PRONE MICE. T. N. Seyfried and A. Wieraszko. Dept of Biology, Boston College, Chestnut Hill, MA 02167.

Inbred DBA/2 (D2) mice have an inherited susceptibility to audiogenic seizures (AGS), whereas inbred C57BL/6 (B6) mice are resistent to these seizures. AGS susceptibility is genetically associated with a deficiency of the ecto-Ca^{2+}-ATPase activity in the brain stem of B6 x D2 recombinant inbred strains and in F_1 hybrids from various strain crosses. Recent studies indicate that ATP, which has potent membrane depolarizing action, is co-released with different neurotransmitters into the neuronal synaptic cleft. The ecto-ATPase may function to terminate the action of ATP on the cell surface and to generate the energy needed for modulating the mechanisms of neural transmission. Moreover, the rapid hydrolysis of ATP will lead to the formation of adenosine (an endogenous anticonvulsant). We propose that the intense, seizure evoking auditory stimulation of D2 mice causes an immediate release of ATP which overwhelms the hydrolyzing capacity of the deficient ecto-Ca^{2+}-ATPase activity. This will cause a persistence of ATP action and will retard the accumulation of adenosine. A failure to quickly hydrolyze the released ATP could sustain membrane depolarization, disrupt neural modulation, and cause an avalanche of convulsive activity. The ecto-Ca^{2+}-ATPase is apparently critical only under intense auditory stimulation since D2 mice are phenotypically normal and do not seize spontaneously under normal physiological conditions. Supported by grants from the NIH (23355 and 2486) and NSF (BNS 8644955).

228.8

ANALYSIS OF NEUROTRANSMITTER mRNAS AFTER AMYGDALOID KINDLING OF RATS. H. Shinoda*, J.P. Schwartz and N.S.Nadi (SPON: P.H. Sheridan). CNB and MNB, NINCDS, NIH, Bethesda MD 20892

Changes in neurotransmitter content have been examined extensively in kindled animals. There are several reports of increases of somatostatin (SS), whereas results for GABAergic activity have been mixed. In this study, we have measured the levels of SS, proenkephalin (PE), and glutamic acid decarboxylase (GAD) mRNAs in various brain regions of kindled rats, as well as levels of SS and GAD. Rats were sacrificed 2 weeks after Stage V kindling had been established. Neither GAD mRNA content nor GAD enzyme activity changed in any brain region in the kindled rats. The SS content increased in cortex and striatum, as well as in hippocampus, of kindled rats relative to controls, whereas SS mRNA increased only in cortex and striatum. PE mRNA increased only in amygdala. Although no increase in PE mRNA was observed in hippocampus when analyzed by slot-blot hybridization, in situ hybridization demonstrated an increase in PE mRNA specific to hippocampal granule cells. None of these changes are seen in naive rats receiving only a single stimulus, demonstrating that the responses represent changes occurring at the final stage (Stage V) of kindling. Whether these increases in somatostatin and enkephalin play a causative role or are only secondary to the kindling process is under investigation.

228.9

KINDLED SEIZURES DECREASE BRAIN REGIONAL ACTIVITY OF A NAAG-HYDROLYZING ENZYME. J.L. Meyerhoff, M.B. Robinson, M.A. Bixler, S.S. Richards, R.A. Lyn* and J.T. Coyle. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307 and Dept. of Psychiatry, Johns Hopkins University, Baltimore, MD 21205.

N-Acetylated-α-linked acidic dipeptidase (NAALADase) is a Cl^- dependent, membrane-bound peptidase that hydrolyses the endogenous neuropeptide N-acetyl-L-aspartyl-L-glutamate (NAAG), a putative excitatory neurotransmitter. NAALADase cleaves NAAG into glutamate and N-acetyl-aspartate. We previously showed that kindled seizures increase NAAG levels in entorhinal cortex. The present study examined the effect of seizures on regional brain levels of NAALADase. Rats were subjected to daily amygdaloid-kindling electrical stimulation, or non-kindling cortical suprathreshold stimulation (STS), and killed 48 hours after the last of a series of 5 generalized convulsions. Brains were dissected, tissues were coded and then assayed in a blinded fashion. Compared to sham-operated controls, NAALADase activity was decreased in the amygdala, entorhinal cortex, pyriform cortex and hippocampus after kindled seizures. STS-induced convulsions also decreased NAALADase activity in the first 3 regions, but not the hippocampus. Activity was unaffected in the substantia nigra by either type of convulsion. Statistical analysis was by ANOVA ($p<0.01$), followed by a Newman-Keuls test ($p<0.05$).

228.10

ANALYSIS OF HIGH AND LOW AFFINITY OUABAIN BINDING SITES OF THE NA,K-ATPASE IN CEREBRAL CORTEX USING QUANTITATIVE AUTORADIOGRAPHY. M.C. Antonelli*, P.E. Filuk* and W.L. Stahl. VA Medical Center and University of Washington Sch. of Med., Seattle, WA 98108.

Isoforms of the Na,K-ATPase bind ouabain with high and low affinities and have been studied in cerebral cortex of rabbit and monkey brains. In rabbit brain, microsomal membranes were studied by a conventional filtration assay and brain slices were examined by quantitative autoradiography (QAR). Kd values of 11-12 mM for specific high affinity of [^{3}H]ouabain were found by both methods. High affinity [^{3}H]ouabain binding in tissue sections was stimulated by the ligands Mg^{2+}/Pi or Mg^{2+}/ATP/Na^+ and this binding was inhibited by K^+ (IC_{50} = 1 mM), thimerosal (IC_{50} = 0.16 mM) and the neurotoxin erythrosine B (IC_{50} = 0.01 mM). In the presence of erythrosine B, specific low affinity sites were found with a Kd = 1 μM. Specific low affinity sites accounted for approximately one-half of the total binding sites in rabbit cerebral cortex. Ouabain binding was examined in control and epileptic foci from monkeys lesioned with alumina. Specific high affinity ouabain binding was decreased 50% in the focus, which is consistent with the lower Na,K-ATPase activity previously found in the focus and supports the view that the total number of Na,K-ATPase molecules is decreased (Supported by the Veterans Administration and NIH grant NS 20482; M.C.A. was supported by a fellowship from CONICET, Argentina).

228.11

INCREASE OF KAINIC ACID BINDING SITES IN THE HIPPOCAMPUS OF EPILEPTIC INFANTS AND KINDLED RATS. A. Represa,* O. Robain,* E. Tremblay* and Y. Ben-Ari (Spon G. Barbin). INSERM U-29. 123 Bd Port-Royal. Paris 14. FRANCE.

In infants epileptics a highly significant increase of KA binding sites has been observed in the terminal field of the mossy fibers (stratum lucidum of CA3). The density was 42.6 ± 23 and 122 ± 24 f mol/mg tissue) in aged matched controls (n = 8) and epileptics (n = 5) respectively. This increase of labelling was apparently not associated to an aberrant mossy fiber sprouting indicating that it may be due to a change in the synthesis, or affinity of KA receptors.

In rats which had been kindled, aberrant kainic acid binding sites were found in the supragranular region and in the inferior region of CA3. These binding sites are associated with Timm deposits suggesting a sprouting of mossy fibers : a similar sprouting occurs also after seizures induced by kainate (A. Represa et al. Neurosci. 1987).

We conclude that in both human and rats, epilepsy is associated with an enhancement of kainate binding in the hippocampus. Since KA increased neuronal excitability the rise of KA binding observedin both, infant epileptics and kindled rats would compromisethe hippocampal function and facilitate seizure activity.

228.12

KINDLING STIMULATION INDUCES C-FOS M-RNA EXPRESSION IN RAT HIPPOCAMPUS. C. Shin, D.R. Cohen*, L.S. Butler*, and J.O. McNamara. Duke Univ. & VA Med. Ctr., Durham, NC 27705, and Roche Inst. of Mol. Biol., Nutley, NJ 07110

Periodic induction of focal electrical seizure (Afterdischarge [AD]) is an absolute prerequisite for kindling to occur. The molecular mechanism by which periodic ADs lasting tens of seconds result in lifelong kindling effect is unclear. One possibility is that ADs activate transcription of an "early gene" which in turn regulates expression of a host of target genes, the net effect being the kindled state. We determined whether a single kindling stimulation to a naive animal activated c-fos transcription, since PTZ seizures rapidly induced c-fos expression (Morgan et al,1987).

ADs were recorded in both hippocampi (HPCs) of adult male rats stimualted in the angular bundle with 2 sec train of 200 μA 60 Hz biphasic pulses. HPCs were rapidly removed 30 min later. Total RNA was isolated and electrophoresed (3 μg/HPC). Northern blot was hybridized with 32P-labeled cDNA probe to detect c-fos mRNA by autoradiography.

Unstimulated controls had minimal c-fos expression (OD=.009 ±.004; n=8). All 7 stimulated rats exhibited a dramatic induction of c-fos mRNA in HPC ipsilateral to stimulation (OD=.89 ± .03; n=7), and, in 5 of 7, in contralateral HPC (OD=.88±.04; n=5).

Thus a single kindling stimulation induced marked c-fos mRNA expression in HPC. This may lead to increased translation of c-fos protein (Dragunow & Robertson, 1987), and be a pivotal event in the presumed cascade of molecular mechanisms underlying the development of kindling.

RECEPTOR MODULATION AND REGULATION II

229.1

CHRONIC ADMINISTRATION OF BETA-ADRENERGIC AND SEROTONERGIC AGENTS ALTERS SEROTONIN TYPE 2-MEDIATED BEHAVIOR IN THE RAT. A.S. Eison, F.D. Yocca and G. Gianutsos. Section of Pharmacology and Toxicology, School of Pharmacy, Univ. of Connecticut, Storrs, CT 06268 and CNS Research, Bristol-Myers Co., Wallingford, CT 06492-7660.

Noradrenergic (NE) and serotonergic (5-HT) transmission may alter the functional sensitivity of serotonin type 2 ($5\text{-}HT_2$) receptors. We have examined the effects of chronic (14 day) administration of the beta antagonist propranolol and the beta agonist clenbuterol on the quipazine-induced head shake response and cortical beta and $5\text{-}HT_2$ receptor number. Chronic treatment with propranolol resulted in a 44% up-regulation of beta receptors and a potentiation of the head shake response. Chronic treatment with clenbuterol resulted in a marked reduction in head shakes with a concomitant 29% down-regulation of beta receptors. $5\text{-}HT_2$ receptor binding was not modified by propranolol or clenbuterol treatments. We have examined the head shake response following chronic administration of $5\text{-}HT_2$ agonists, antagonists, or imipramine, to naive rats and rats which sustained NE or 5-HT lesions (depletions > 90%). The ability of chronic $5\text{-}HT_2$ agents to produce $5\text{-}HT_2$-mediated behavioral subsensitivity was not altered by 5-HT or NE denervation. Imipramine-induced reductions in behavior were significantly attenuated by serotonin lesions. Saturation binding parameters for beta and $5\text{-}HT_2$ receptors will also be presented.

229.2

(±)-DOI, A HALLUCINOGENIC PHENYLALKYLAMINE, DOWN-REGULATES $5HT_2$ RECEPTORS IN RAT CORTEX A. Himeno,* D. J. McKenna, A. J. Nazarali,* and J. M. Saavedra (SPON:J. S. Gutkind) Section on Preclinical Neuropharmacology, LCS, NIMH, Bethesda, MD 20892

(±)-DOI, an iodinated analog of the hallucinogen DOM, is a $5HT_2$-selective serotonin agonist. We examined the effects of chronic administration of DOI (1 mg/kg) on binding of $5HT_2$ selective ligands in rat cortex. Ligands and concentrations used were: [^{125}I]-R-DOI (0.5 and 5 nM), [^{125}I]LSD (1 nM), and [^{3}H]ketanserin (1 nM). Membrane suspensions from treated and saline-injected control rats were pre-incubated for 10 or 60 min at 37 °C prior to use in the assay. In treated animals specific binding of all 3 ligands was significantly reduced in both the 10 and 60 min preparations. Saturation studies with [^{125}I]DOI and [^{3}H]ketanserin were conducted in controls and treated animals. In treated animals, the Bmax for [^{3}H]ketanserin was less than controls, but the Kd was similar to controls. The Kd for [^{125}I]DOI in treated animals was higher than controls, but the Bmax was similar. These results may help to clarify regulatory mechanisms governing $5HT_2$ receptors.

229.3

M-CHLOROPHENYLPIPERAZINE-INDUCED INCREASES IN PLASMA PROLACTIN AND CORTICOSTERONE AND DECREASES IN FOOD INTAKE ARE DIFFERENTIALLY ALTERED BY CLOMIPRAMINE TREATMENT IN RATS. K.M. Wozniak,* C.S. Aulakh,* J.L. Hill,* and D.L. Murphy* (SPON: C.R. Lake). LCS, NIMH and LCS, NIAAA, Bethesda, MD 20892.

The purpose of the present study was to use m-chlorophenylpiperazine (m-CPP, a 5HT agonist) as a challenge agent to explore functional adaptational changes in serotonergic mechanisms regulating prolactin and corticosterone secretion and food intake following long-term clomipramine (a tricyclic antidepressant) treatment in rats. Clomipramine (5 mg/kg/day) or saline was administered subcutaneously to male Wistar rats by means of osmotic minipumps. Both clomipramine and saline-treated animals were challenged with m-CPP (1.25 and 2.5 mg/kg) during short-term (3-5 day) and long-term (21-23 day) antidepressant treatment.

Administration of m-CPP to rats produced increases in plasma prolactin (peak effect at 15 min) and corticosterone (peak effect at 15-30 min) concentrations and decreases in food intake. Long-term but not short-term clomipramine treatment potentiated m-CPP's effect on prolactin levels whereas both short-term and long-term clomipramine treatment significantly attenuated m-CPP's effect on corticosterone levels. On the other hand, neither short-term nor long-term clomipramine treatment had any significant effect on m-CPP-induced decreases in food intake. These findings are consistent with other clinical and animal studies demonstrating a differential effect of antidepressant treatment on different 5HT-mediated neuroendocrine and behavioral functions.

229.4

DIFFERENTIAL EFFECTS OF ANTIDEPRESSANT TREATMENTS ON 5HT AGONIST-INDUCED INCREASES IN PLASMA PROLACTIN AND CORTICOSTERONE IN RATS. C.S. Aulakh,* K.M. Wozniak,* J.L. Hill,* and D.L. Murphy* (SPON: N.A. Garrick). LCS, NIMH and LCS, NIAAA, Bethesda, MD 20892.

The purpose of the present study was to use fenfluramine (a 5HT-releasing agent) and 8-hydroxy-2(di-n-propylamino) tetralin (8-OHDPAT, a selective $5HT_{1A}$ agonist) as challenge agents to explore functional adaptational changes in serotonergic neurotransmission involved in the secretion of prolactin and corticosterone following long-term treatment with imipramine, clomipramine (tricyclic antidepressants), clorgyline (a selective MAO type A inhibiting antidepressant), and lithium in male Wistar rats. Imipramine (5 mg/kg/day), clomipramine (5 mg/kg/day), clorgyline (1 mg/kg/day), or saline was subcutaneously administered continuously by means of osmotic minipumps. In case of lithium, the animals were given rat chow containing lithium carbonate.

Short-term treatment with lithium but not clorgyline or imipramine potentiated fenfluramine-induced increases in plasma prolactin but not corticosterone. Long-term treatment with clorgyline but not other antidepressants attenuated fenfluramine and 8-OHDPAT-induced increases in plasma prolactin but not corticosterone. Similarly, long-term treatment with clomipramine and to some extent imipramine also, accentuated 8-OHDPAT's effect on plasma prolactin but not on corticosterone. These findings are consistent with several other clinical and animal studies demonstrating dissimilar actions of different antidepressant treatments on two different 5HT-mediated neuroendocrine functions.

229.5

DOWN-REGULATION OF 5-HT_2 RECEPTORS IN RAT CORTEX AND ON PLATELETS BY ANTIDEPRESSANTS. C. Brazell*, S. McClue*, F. Brush* and S. Stahl. Merck Sharp & Dohme Research Labs., Terlings Park, Harlow, Essex, U.K.

The 5-HT receptor on platelet membranes may be analogous to the 5-HT_2 receptor in the CNS. If this analogy is functional then the down-regulation of central 5-HT_2 receptors by antidepressants may be paralleled by the platelet 5-HT receptor. We have tested this hypothesis by 21 day administration of desipramine and amitriptyline (15 mg/kg, i.p., daily) to rats. This is the first report of the use of [^{125}I]-ILSD to determine 5-HT_2 receptors on rat platelets.

Washed platelet and cortical membranes were incubated with [^{125}I]-ILSD (0.1-6 nM, 30 min, 37°C). Ketanserin (10 μM) defined non specific binding. Specific binding of [^{125}I]-ILSD to platelets identified a single receptor site. Competition binding studies confirmed that the ligand labelled a site characteristic of the 5-HT_2 receptor. Binding parameters (% reduction from controls) were:

Treatment (n = 4-7)	Platelet (K_D 1.2 ± 0.4 nM) B_{max} (fmol/mg protein)	Brain (K_D 1.1 ± 0.1 nM) B_{max} (fmol/mg protein)
Control	83.0 ± 24.7	96.6 ± 26.2
Desipramine	66.7 ± 23.7 (-9.3%)*	63.6 ± 14.9 (-22.4%)*
Amitriptyline	51.8 ± 9.7 (-37.7%)*	67.9 ± 26.0 (-31.1%)*

Values expressed as mean ± S.E.M. where *p < 0.05 Student's t-test. Results confirm previous reports that antidepressants can decrease the number of 5-HT_2 receptors in the rat cortex and suggest a similar modulation in the platelet. Thus the platelet 5-HT_2 receptor may be a dynamic model for the 5-HT_2 receptor of the CNS.

229.6

ENHANCED COUPLING OF G PROTEINS TO ADENYLATE CYCLASE SUBSEQUENT TO CHRONIC ANTIDEPRESSANT TREATMENT. Hiroki Ozawa and Mark M. Rasenick. Dept. of Physiology and Biophysics and the Committee on Neuroscience, Univ. of Illinois College of Medicine, Chicago, IL 60680.

Previous studies from this and other laboratories have demonstrated that chronic treatment with tricyclic antidepressants (TAD) or electroconvulsive shock augments adenylate cyclase in rat cerebral cortex synaptic membranes. It appeared in these previous studies that the coupling of G proteins to adenylate cyclase was involved in the effects of antidepressant treatment, but the nature of those effects was not established. Thus, rats were injected (IP) once daily for 21 days with 10 mg/kg of amitryptilline, imipramine or iprindol. Controls included 20 days of saline followed by a single drug injection (acute) or 21 days of saline. In animals from the chronic treatment group, activation of adenylate cyclase by GppNHp or NaF was 71.4-78.1% greater than observed in acute or saline injected animals. When membranes were assayed under conditions where the predominant effect of GppNHp on adenylate cyclase was inhibitory, basal adenylate cyclase activity was 83.5% greater in membranes from the chronic TAD treated animals, but the inhibition induced by GppNHp (as percent of basal activity) was not different in any of the groups (61.1-67.3%). The potency of GppNHp to stimulate or inhibit adenylate cyclase was not altered by antidepressant treatment. Further, the maximal binding of the photoaffinity GTP analog, azidoanilido GTP by synaptic membrane G proteins was not altered. These results are consistent with the hypothesis that chronic antidepressant treatment augments coupling between Gs and the catalytic unit of adenylate cyclase.

229.7

ALTERATIONS IN RECEPTOR BINDING IN THE RAT CNS FOLLOWING CHRONIC TREATMENT WITH TWO NEWER NON-TRICYCLIC ANTIDEPRESSANTS, CLOVAXAMINE AND FLUVOXAMINE. D.B. Vine*, M.F. Madar*, and J.K. Wamsley. (SPON: S.S. Stensaas) Depts of Psych and Pharm, U of UT Sch of Med, SLC, UT 84132.

Since the pharmacologic treatment of depression in humans has been shown, in general, to be efficacious only after the chronic administration of antipressant drugs, neurotransmitter receptor change has continued to be a prominent hypothesis for their mechanism of action. In the present study we have analysed several biochemical changes in the rat CNS following chronic treatment with two 5HT uptake inhibitors. Quantitative receptor autoradiography, using selective ligands for $5HT_2$, 5HT, α_2, β_1, and β_2 adrenergic receptors, has enabled us to determine which receptors and receptor subtypes have been altered following chronic treatment with these two antidepressants. Using [^{3}H]forskolin, adenylate cyclase receptor changes were not significantly different between control and treated animals. Previous reports of in vitro and in vivo studies of decreased α_2 adrenoreceptor mediated inhibition of cAMP production following exposure to imipramine, together with our findings suggest an uncoupling of these receptors without a change in receptor density. Quantitation of the α_2 adrenoreceptors, using [^{3}H]idazoxan, also supports this finding. [^{3}H]dihydroalprenolol and [^{3}H]5HT binding data correspond to the hypothesis of postsynaptic receptor down regulation following chronic treatment with antidepressants.

229.8

INCREASED SEROTONIN$_2$ (5-HT_2) BINDING IN THE BLOOD PLATELETS OF DEPRESSED PATIENTS. R.C. Arora and H.Y. Meltzer, Department of Psychiatry, Case Western Reserve University School of Medicine, 2040 Abington Road, Cleveland, OH 44106.

The role of serotonin (5-HT) in depression has been extensively studied by examining various serotonergic parameters in the blood platelets. We have now studied 5-HT_2 binding in the blood platelets of 29 drug-free, non-psychotic depressed patients and 13 normal controls.

5-HT_2 binding in platelet membranes was determined using ^{3}H-LSD as ligand following the modified method of Geaney et al. (1984). Analysis of covariance (ANCOVA) with group, sex and group x sex interaction as factors indicated a significant difference in B_{max} ($F=10.12$; $df=1,38$; $p=0.0029$) between normals and depressed patients; B_{max} was significantly higher in depressed patients compared to normal controls. There was a trend for group x sex interaction but no sex effect on B_{max}. Female and male depressed patients had 102% and 24% greater B_{max} than normal females and males, respectively. There was no significant difference in K_d between the two groups. There were no correlations between K_d and B_{max} of ^{3}H-LSD binding and admission total Hamilton Depression scale or SADS-C Depression scale score.

If the increased 5-HT_2 binding is confirmed for depressed patients and proves specific, it could serve as a biological marker for depression. Increased 5-HT_2 sites have also been found in suicide brains by us and others.

229.9

MODULATION BY SALTS OF BINDING AFFINITIES AND CAPACITIES AT μ-, δ- and κ-OPIOID BINDING SITES IN GUINEA-PIG BRAIN. L.E. Robson*, S.J. Paterson* and H.W. Kosterlitz. Unit for Research on Addictive Drugs, University of Aberdeen, Marischal College, Aberdeen, AB9 1AS, U.K.

The effects of salts on the saturable binding of [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin at the μ-site, of [^{3}H]-[D-Pen^2,D-Pen^5]enkephalin at the δ-site and of [^{3}H]-U-69,593 at the κ-site have been investigated in homogenates of guinea-pig brain (10 mg tissue/ml in 10 mM HEPES-KOH, pH 7.4 at 25°C). At the δ-site NaCl (50 mM) reduces binding affinity 1.7-fold but does not alter binding capacity. In contrast, at the μ-site NaCl (50 mM) reduces binding affinity 3.5-fold and also decreases the binding capacity by 25%. In further contrast, at the κ-site of whole brain NaCl (200 mM), which reduces binding affinity 5.5-fold, increases the binding capacity 2-fold. However at the κ-sites of guinea-pig cerebellum, the effect of NaCl (200 mM) is solely inhibitory; a 5-fold decrease in binding affinity is accompanied by a 40% decrease rather than increase in binding capacity. Thus the kinetic basis of the effects of salts on opioid binding may be different at each site and may be different in brain regions.

229.10

PHYSIOLOGICAL CORRELATE TO MU OPIOID RECEPTOR UP-REGULATION IN THE HIPPOCAMPUS. A.M. Moudy and M.B. Laskowski. Dept. Physiology, St. Louis U. Med. Sch., St. Louis, MO 63104.

Opioid receptor upregulation has been examined biochemically as well as behaviorally. The functional significance of an increased number of opioid receptors remains largely uncharacterized. To demonstrate a direct physiological correlate to such upregulation, a biochemical and electrophysiological approach was taken, utilizing the in vitro hippocampal slice preparation as a model system. Hippocampal membranes were prepared from rats treated for 7 (or 14) days with naltrexone (20 mg/ml) or saline. Homologous displacement of [^{3}H]DAGO binding determined that upregulation of mu receptors occurs in the hippocampus (71%) to a degree similar to that measured in whole forebrain (77%, Moudy et al., 1985). D-Ala^2, MePhe^4, Gly-ol^5 enkephalin (DAGO) is a peptide ligand highly specific to mu opioid receptors. Hippocampal slices were prepared from naltrexone treated rats. When superfused with DAGO, population spike output to the same EPSP input increased by 42% in CA1. Slices from animals treated for 14 days showed an additional increase in excitability. These results indicate that the effect of DAGO on pyramidal cell excitability was potentiated in hippocampi from animals exposed chronically to antagonist. Moreover, they demonstrate a significant physiological correlate of opioid receptor upregulation.

229.11

IN VITRO AGONIST-INDUCED DOWN-REGULATION OF HYPOTHALAMIC μ-OPIOID RECEPTORS. M. MacDonald* and M. Wilkinson. (SPON: R.E. Brown) Physiol. & Biophys., Dalhousie U., Halifax, Canada B3H 4H7

Studies of the down regulation of opioid receptors *in vitro* has been largely restricted to delta receptor agonists in neurotumor cell lines. Similar investigations of μ-receptor regulation have been contradictory. Here we examine the effect of DAGO, a specific μ-agonist, on μ-opioid receptors in hypothalamic tissue punches as a procedure for studying the possible down regulation of μ-receptors *in vitro*. Hypothalamic punches (1.8mm diam. from 350μm slices; McIlwain) from adult female rats were preincubated in PBS buffer for 30 min. at 37°C, washed and then re-incubated (30 min. at 37°C) in PBS buffer with or without DAGO (10^{-6}M). Tissue was then washed 3 times (5 min./wash) in ice-cold TRIS(170 mM, pH 7.4). Cell surface μ-opioid receptors were subsequently quantified with [^{3}H] DAGO, without homogenization, using a method described previously (Neuropeptides 9 357,1987; [^{3}H] DAGO concentration: 0.5-2.0 nm). When the receptors were assayed at 0°C (3hr), B_{max} was markedly reduced in DAGO-treated tissue (control: 29.6±2.7 fmoles/mg; treated: 12.5±4.4 fmoles/mg). Binding affinity (K_d) was unaffected by DAGO treatment (1.7±0.2 nm vs 1.9±0.4 nm). This implies that the reduction in B_{max} is likely not due to residually bound DAGO. This was confirmed in separate experiments by performing the binding assays at 25°C (2 hrs), conditions under which unlabelled DAGO is unlikely to remain bound to the receptor. Once again B_{max} was reduced(61.6±12.0 to 25.5±8.0 fmoles/mg) and K_d remained unchanged (2.6±0.7 nm vs 2.1±0.6 nm). Similar observations were obtained using cerebral cortex. Our results suggest that the brain slice/punch preparation may be a simple but effective technique with which to examine opioid receptor regulation.

Supported by the Canadian MRC.

229.12

ACTIVATION OF PROTEIN KINASE C IN THE PRESENCE OF OPIOID AGONISTS DOWNREGULATES δ-RECEPTORS IN NG108-15 CELLS. S. Gucker and J.M. Bidlack. Dept. of Pharmacol., Univ. of Rochester Sch. of Med. and Dent., Rochester, NY, 14642.

Phorbol 12-myristate 13-acetate (PMA), a potent activator of protein kinase C (PKC) was tested for its ability to regulate the number of opioid binding sites in NG108-15 cells. PMA and opioids were cultured with NG108-15 cells for 2 hr at 37° before preparing membranes from the cells, and subsequent measurement of [^{3}H] [D-Ala^2, D-Leu^5] enkephalin (DADLE) binding to the membranes. Treatment of cells with PMA concentrations up to 1 μM had no effect on the binding of [^{3}H]DADLE to NG108-15 membranes. However, when cells were cultured with PMA with 10 nM etorphine, a decrease in the number of [^{3}H]DADLE binding sites occurred without changing the affinity of the remaining sites. Controls consisted of culturing the cells with 10 nM etorphine alone. The loss of [^{3}H]DADLE binding sites was dependent on the PMA concentration with an IC_{50} value of 11 nM and 30 nM PMA producing a maximal inhibition of 40%. When naloxone was added at a 100-fold greater concentration than etorphine, the PMA-induced decrease in the number of binding sites was abolished. Replacing PMA with up to 1 μM 4α-phorbol, a phorbol ester that does not activate PKC, had no effect on [^{3}H]DADLE binding to cell membranes. Studies are underway to delineate the nature of PMA's effect on etorphine downregulation of δ-receptors, a receptor reportedly not linked to phosphatidylinositol turnover and hence PKC activation in NG108-15 cells. (Supported by USPHS grant DA03742 and DA07232.)

229.13

DOSE-DEPENDENCY OF DESENSITIZATION FOLLOWING CHRONIC MORPHINE EXPOSURE ON THE 7315c PITUITARY CELLS. P.S.Puttfarcken and B.M.Cox. Dept. of Pharm., USUHS, Beth., MD 20814

The 7315c pituitary tumor cells contain a homogenous population of μ opioid receptors and opiate activation of these receptors results in inhibition of adenylyl cyclase (AC) activity. Previous studies have demonstrated 100 μM morphine chronic exposure on 7315c pituitary tumor cells resulted in two time-dependent cellular adaptive processes. Desensitization (the loss in the ability of the opioid agonist, D-Ala-D-Leu-Enkephalin (DADLE), to inhibit AC activity) occurred after 5 hours of 100 μM morphine exposure while receptor number was unchanged. Membranes from cells incubated in a drug-free medium did not lose sensitivity to DADLE. Binding studies revealed down-regulation. After 48 hours of 100 μM morphine exposure, a 60% decrease in receptor density was observed. In studies designed to examine the concentration of morphine needed to observe desensitization and down-regulation the ability of the μ-selective agonist Tyr-D-Ala-Gly-N(Me)Phe-Gly-ol (DAGO), to inhibit AC activity in membranes from cells exposed to morphine concentrations from 10 nM to 300 μM for 5 hours and 48 hours was examined. DAGO no longer inhibited AC activity in a dose-dependent manner after 5 and 48 hours of 100 nM morphine exposure. Inhibition of AC by DAGO in membranes from control cells and cells treated with 10 nM morphine for 5 and 48 hours did not differ significantly. Binding studies are in progress to determine at what morphine concentration down-regulation occurs.

229.14

ENDOGENOUS OPIOID SYSTEMS AND NEURAL CANCER: SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC OBSERVATIONS. J. Conforti*, P.J. McLaughlin and I.S. Zagon (SPON: T. Lloyd). Dept. Anat., The Penn. State Univ. College of Medicine, Hershey, PA 17033.

Endogenous opioid systems (i.e., opioids and receptors) participate in regulating cancers of the nervous system. Paradigms using opioid agonists and antagonists have been effective in revealing the antitumor function of opioid agonist peptides. To study the influence of opioids on neural cancer, S20Y neuroblastoma cells grown in tissue culture were exposed to (a) methionine enkephalin (ME), a potent growth inhibitory opioid peptide, (b) ME and naloxone, which reverses opioid action, or (c) naltrexone, an extremely potent opioid antagonist which stimulates tumor cell growth. Scanning and transmission electron microscopic studies performed on cultures exposed to opioid agonists/antagonists showed no changes in morphology in comparison to control cultures. These results (a) support the hypothesis that endogenous opioid systems act as trophic factors in regulating growth, (b) reveal that opioid effects on cell growth and survival do not alter the structural biology of cells and (c) strengthen the validity of utilizing opioid agonists and antagonists in exploring the relationship of endogenous opioids and opioid receptor interactions in neural cancer. Supported by NIH grants NS-20623 and NS-20500.

230.1

RETINOTOPIC CLUSTERING IN A LOW DENSITY TECTAL PROJECTION IN GOLDFISH: EVIDENCE AGAINST ACTIVITY DEPENDENT COMPETITION. M.D. Olson and R.L. Meyer, Developmental and Cell Biology, Developmental Biology Center, University of California, Irvine, California 92717

When the optic nerve of a goldfish is severed, regenerating fibers from one small retinal region aggregate into several small clusters as revealed by spot injections of WGA-HRP into the retina. This clustering is inhibited by impulse blockade. Since the number of synapses is regulated during regeneration, this clustering may represent an activity-dependent competition for limited synaptic sites.

To test this, we created a low density projection by rerouting regenerating fibers from one retinal quadrant into an otherwise denervated tectum. It was previously shown that fibers form less than half the normal numbers of synapses under this condition. After several months, we then assayed for clustering by making a 2nl spot injection of WGA-HRP into retina. Multiple clusters were found in this low density projection. This indicates that competition for limited synaptic sites is not a prerequisite for clustering. The effect of TTX impulse blockade is being tested. If this low density clustering is found to require activity, this would further suggest that activity-dependent ordering represents a cooperative rather than a competitive process.

This work was supported in part by NIH grant 9R01 EY06746

230.2

EVIDENCE FOR THE STABILITY OF POSITIONAL MARKERS IN THE GOLDFISH TECTUM. U. Busse and C.A.O. Stuermer, Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, D-7400 Tübingen, FRG.

Regenerating retinal axons return to their retinotopic target sites in the tectum over abnormal paths and without impulse activity (Hartlieb & Stuermer '88). This supports the view that they are guided by positional cues on tectum. Such cues could be axonal remnants, transient markers imposed by the earlier axons (Schmidt '78) or longlasting markers intrinsic to tectum. To decide between these alternatives we pursued the paths of axons from a temporal hemiretina in tecta which were deprived of retinal afferents for 5 and 8 months, respectively.

The right tectum was denervated by removing the left eye. Axons from a surgically created temporal hemiretina regenerated into the denervated tectum after removal of the left tectum (T-rem). 180 days after T-rem these axons were labeled by applying HRP to the right optic nerve and visualized in tectal whole mounts.

The vast majority of temporal axons was found in the rostral tectum. In midtectal regions axons performed U-turns and returned into the rostral tectum instead of invading the vacant caudal tectal half. The obvious preference of temporal axons for the rostral tectum was substantiated by quantitative measurements. Labeled axons were counted in rostral, midtectal and caudal tectal regions (N=6). 74,1% ± 4,0 of axons were found rostrally, 25,9% ± 4,0 in midtectal and 0% in caudal regions of tectum.

Thus, in tecta denervated for up to 8 months axons from a temporal half retina occupy their retinotopically appropriate rostral tectum. We did not observe an expansion of temporal axons into caudal tectal regions. These findings argue against the view that positional cues are lost. They suggest that positional markers are intrinsic to tectum and stable, which is supported by recent data from *in vitro*-assays (Vielmetter & Stuermer '88).

230.3

PATH- AND HOMEFINDING OF REGENERATING RETINAL AXONS IN GOLDFISH IN THE ABSENCE OF NEURAL ACTIVITY

E.Hartlieb and C.A.O.Stuermer, Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, D-7400 Tuebingen, FRG

Regenerating retinal axons travel in abnormal routes and extend initially branches over inappropriate regions of tectum. They loose these branches when they establish target-directed routes and develop their terminal arbors at retinotopic sites (Stuermer,1988). To decide whether these events require neural activity we pursued the growth of TTX-blocked axons in tecta at regeneration periods of 24-80d and 120-184d.

After optic nerve section (ONS) the right eye was injected with 0.5µl 0.12mM TTX, and the left eye with Ringer. Axons from a dorso- or ventro-temporal sector in retina (2.5-6% of retinal area) were labeled with HRP and visualised in DAB reacted tectal whole mounts.

At 24-80d after ONS TTX blocked axons crossed through all regions of tectum and extended branches with growth cones like control axons. Despite their abnormal routes, the axons exhibited a preference for their retinotopically appropriate rostral hemitectum: most axons were in rostral (57.9%) and midtectal (36.3%) and fewer in caudal (7.6%) tectal regions. At 120-184d, TTX blocked as well as control axons had lost their side branches, established target directed routes and ended in terminal arbors at retinotopic sites. Axon processes in caudal tectum declined to 1.1%, and 86.6% and 12.4% were in rostral and midtectal regions, resp. The terminal arbors were confined to a distinct zone in rostral tectum, covering no more than 1.1 to 2.6% of the tectal surface.

These findings suggest that regenerating axons do not need their normal impulse activity to course preferentially through their appropriate rostral hemitectum, to extend and withdraw their branches, to develop target-directed routes and to assemble their terminal arbors in well-defined retinotopic territories.

230.4

BASKET CELLS OF THE DENTATE GYRUS ESTABLISH SYNAPTIC CONNECTIONS WITH GRANULE CELLS IN FIVE DAY OLD RATS. L. Seress and C.E. Ribak. Dept. of Anatomy and Neurobiology, Univ. of Calif., Irvine, CA 92717.

Previous studies have shown that GABAergic inhibitory neurons of the dentate gyrus are generated prenatally. In contrast, the majority of granule cells are formed postnatally. A well-characterized type of GABAergic neuron that is known to provide feedback inhibition of granule cells in adults is the basket cell. Since recent physiological studies by Swann et al. (1988) indicate that inhibition of pyramidal cells in CA3 first occurs in 5 day old rats, it was of interest to determine when a recurrent inhibitory circuitry exists in the dentate gyrus. Golgi-electron microscopic preparations were examined from 5, 10 and 16 day old rats. Basket cells in 5 day old brains displayed axons that formed symmetric synapses with somata and dendrites of granule cells. These terminals were small and contained relatively few pleomorphic synaptic vesicles. The somata of basket cells were larger than those of granule cells, and they also contained more cytoplasmic organelles, such as Golgi complex, granular endoplasmic reticulum and free ribosomes. In addition, somata and both apical and basal dendrites of basket cells were contacted at this age by relatively immature axon terminals that formed mainly symmetric synapses. Some adult features of basket cells, such as intranuclear rods, infolded nuclei and organized Nissl bodies, were first observed in 10 day old brains. By 16 days of age, most basket cells appeared to be similar to those found in adult preparations. For example, the dendrites were contacted by numerous terminals with round synaptic vesicles that formed asymmetric synapses. Such terminals have previously been identified as axon collaterals from mossy fibers. Some terminals with a similar morphology contacted the somata of basket cells at this age. These data suggest that a recurrent inhibitory circuitry is established in the 5 day old dentate gyrus even though basket cells at this age lack the typical adult ultrastructural features. The appearance of infolded nuclei and intranuclear rods in basket cell bodies following the appearance of numerous asymmetric (excitatory type) axodendritic and axosomatic synapses suggests that these structures are related to the increased metabolic activity of basket cells.

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230.5

Further Characterization of Gap Junctions and Their Messenger RNAs in Cultured Glia and Neurons. K. Spiegel, D.C. Spray, R. Dermietzel, and J.A. Kessler (Spon:D.K. Batter). Depts. Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Gap junctions and their messenger RNAs (mRNAs) were examined in cultures of glia and sympathetic neurons. Northern blot analysis of oligo-dT-cellulose fractionated RNA from cultured glia revealed a predominant 2.5 kb band and a less abundant 1.6 kb band. A previously reported 3.7 kb band was found to represent cross-hybridization to 28S ribosomal RNA. Sympathetic neurons, by contrast, expressed only the 1.6 kb mRNA. These putative gap junction mRNAs were similar in size to the mRNAs for the 21 kD gap junction protein (2.5 kb) and the 27 kD gap junction protein (1.6 kb) found in rat liver, suggesting that astrocytes express predominantly the 21 kD protein whereas neurons express the 27 kD protein. Immunocytochemical studies of cultured astrocytes confirmed the presence of the 21 kD protein and the absence of detectable 27 kD protein. By contrast, oligodendrocytes contained the 27 kD protein. Immunocytochemical stuides of cultured sympathetic neurons using the antibody to the 27 kD protein revealed punctate areas of fluorescence consistent with the presence of small soma-somatic gap junctions. Further studies are necessary to substantiate this observation.

Regulation of gap junction mRNA levels and of dye and electrotonic coupling were examined in astrocytes cultured from different brain regions. Astrocytes from different regions expressed similar levels of gap junction mRNAs and exhibited high levels of dye and electrotonic coupling. The extent of dye coupling was increased in all regions by treatment with dibutyryl cyclic AMP. However, exposure to testosterone or arachidonic acid increased coupling in astrocytes cultured from cortex or striatum, while coupling between hypothalamic astrocytes was dramatically decreased by both treatments. Thus gap junctions are regulated differently in astrocytes from different brain regions. Preliminary studies indicate that levels of the 1.6 kb mRNA in cultured sympathetic neurons are similarly regulated by microenvironmental factors, and that changes in mRNA levels are correlated with changes in electrotonic coupling.

230.6

ANALYSIS OF NEURONAL ORGANIZATION IN CELL CULTURE: USE OF LASER-ETCHING TECHNIQUE TO CREATE PATTERNED SUBSTRATE GRIDS. J.S.Whitson*, G.J.Brewer and C.W.Cotman, Dept of Psychobiology, University of California, Irvine, CA, 92717.

In vivo, neuronal organization develops in a complex environment. It appears to be directed by a number of coordinated components including substrate molecules, diffusible trophic factors, and synaptic reinforcement of appropriate connections. Knowledge of the mechanisms of neuronal organization is crucial to our understanding of the central nervous system, where neurons are highly interconnected and interdependent.

Standard explant cultures do not allow detailed examination of the process of neuronal organization because the contacts between individual neurons cannot be clearly defined at a cellular level. Thus, we have developed a system which allows examination of neuronal organization in a clear and quantifiable manner. Plastic tissue culture dishes were coated with polylysine. A square gold screen (1000 mesh) was centered in the dish and irradiated with a single pulse (15nsec, 105mJ/cm^2) from an ultraviolet laser. This etched the substrate not protected by the gold screen, forming a grid pattern of substrate lanes. Cells from well-defined brain areas (e.g. hippocampus and septum) were dissociated and seeded at low density (10,000 cells/cm^2) in etched dishes. After two days in culture, individual hippocampal neurons located on the grids extended neurites that followed the straight lanes and right angle turns of the grid pattern. The restriction of neurites to the pattern in these laser-etched dishes is superior to that obtained on grids formed by inactivation of polylysine under conventional ultraviolet-light.

This system allows neurite affinities for different substrates to be quantified. In co-cultures where one cell population is fluorescently labeled, this system permits characterization of homotypic and heterotypic neuronal connections. This work was supported under an NSF Graduate Fellowship to J.S.W.

230.7

A BASIS FOR SELECTIVE CONNECTIONS IN LEECH. X. Gu* and K.J.Muller. Dept. of Physiol. & Biophys., U. of Miami School of Med., Miami, FL 33101

In many nervous systems, neurons form connections with certain cells but not with others that appear to be suitable targets. For example, axosomatic contacts of nociceptive (N) neuron axons in the leech wrap only certain other sensory cells. The contacts are visible with the light microscope and will regenerate accurately after nerve injury, showing that mechanisms providing for precise wiring also operate in the adult. Normally, the cells wrapped by the lateral N cell are in adjacent ganglia, whereas homologues of the targets in the N cell's own ganglion are never wrapped. However, those homologues become wrapped by sprouting N cell axons if the ganglion is isolated from its neighbors by cutting the paired connectives that link them. Because of the ganglion's bilateral symmetry and the presence of targets on the left and right sides, by cutting only the connectives on the left we found that denervation of targets and not injury to the N cell could account for the sprouting. When the target homologues were selectively denervated by killing their normal presynaptic inputs(8 N cells in the 2 neighboring ganglia injected with protease), the N cells sprouted to wrap cells in their own ganglia. This indicates that normally wrappings by other cells prevent or exclude the N cell from wrapping cells in its own ganglion. (Supported by NIH Grant NS20607.)

230.8

RETROGRADE LABELING OF MOTONEURONS REINNERVATING BASEMENT MEMBRANE GHOSTS IN AXOLOTL MUSCLES. V. Boss and D.J. Wigston. Departments of Biology and Physiology, Emory University, Atlanta, GA 30322.

Selective reinnervation of axolotl limb muscles suggests the presence of muscle-specific cues that direct synaptogenesis during regeneration. These cues do not appear to be restricted to myofibers (Wigston and Donahue, 1988; J. Neurosci. in press). We are currently testing whether such cues are associated with muscle fiber basement membranes.

The left posterior iliotibialis muscle (post.ILT) was injected with the long-lasting retrograde tracer diamidino yellow (DY) which labeled the nuclei of the post.ILT motoneurons. A week later the entire right ILT was removed and damaged to destroy its myofibers but not its basement membranes. Three days later it was irradiated to prevent regeneration of new fibers from satellite cells, and sutured in place of the left ILT. After 40-45d, the fiberless post.ILT was injected with rhodamine-HRP (R-HRP). R-HRP was successfully restricted to the post.ILT in about half the animals. The spinal cords of these animals contained motoneurons labeled with both DY and R-HRP, showing that motoneurons that reinnervate basement membrane ghosts can be retrogradely labeled. We are using this strategy to test whether regenerating motoneurons can distinguish between the ant. and post. ILT in the absence of muscle fibers.

230.9

"EXPRESSION OF NERVE-MUSCLE TOPOGRAPHY DURING DEVELOPMENT". M.B. Laskowski and J.A. High. Department of Physiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Previous studies have indicated that in two muscles of the adult rat, the anterior serratus and the diaphragm, the rostrocaudal axis of the motoneuron pool projects topographically onto the rostrocaudal axis of the muscle. In the present work we have asked whether this orderly topography emerges as a function of postnatal synaptic rearrangement or whether this pattern is already established at birth. The anterior serratus muscle was studied over the period ranging from embryonic day 17 through postnatal day 30. Using two criteria of topography, average segmental innervation and average target field of cervical roots C6 and C7, we found that a topographic distribution of the motoneuron pool is already present prior to birth and is maintained throughout the postnatal period. Moreover, both C6 and C7 form an orderly map over the surface of the serratus in the embryo, and the topography is sharpened during postnatal periods. The diaphragm also is topographically innervated at birth and undergoes a comparable sharpening of the projection map postnatally. We conclude that the topographic projection of motoneurons is established prior to birth in these muscles, and postnatal synaptic rearrangement serves to sharpen this topographic map toward the adult pattern. (Supported by MDAA)

230.10

REGENERATIVE SPECIFICITY FOLLOWING HETEROCHRONIC LIMB TRANPLANTS. P.B. Farel and S.E. Bemelmans* Dept. of Physiology, Univ. N. Car. Sch. Med., Chapel Hill, NC 27599

In the bullfrog tadpole, motor axons transected prior to st. VIII regenerate to the appropriate hindlimb region. After st. VIII, target specificity is lost (JCN 254:125-132, 1986). In order to determine if the loss of regenerative specificity derives from an inability of mature motoneurons to respond to guidance cues, we replaced single hindlimbs in st. X tadpoles with hindlimbs taken from st. IV/V animals. Four weeks following the transplant, HRP was placed in the ventral thigh, and the location of retrogradely labeled motoneurons was mapped. We found specific regeneration along the dorsal-ventral axis of the hindlimb, but specificity along the proximal-distal axis was lost. When spinal nerves were severed without transplanting the limb, regeneration was not specific. These results show that the capacity for regenerative specificity is not lost in mature motoneurons, but expression of this specificity may be prevented under normal conditions.

230.11

EXCITATORY SENSORIMOTOR CONNECTIONS BETWEEN ANTAGONISTS IN THE ISOLATED CHICK EMBRYO SPINAL CORD. M. T. Lee and M. J. O'Donovan. Dept. Physiology & Biophysics, Univ. of Iowa, Iowa City, IA 52242.

In mature vertebrates, stretch-sensitive muscle afferents synapse with motoneurons in a highly specific fashion. Afferents from a given muscle monosynaptically excite motoneurons to that muscle and its synergists and disynaptically inhibit motoneurons to its antagonists. To learn more about the development of this specificity, we have examined the pattern of sensorimotor synaptic connections in the lumbosacral spinal cord of the chick embryo *in vitro*. Responses to muscle afferent stimulation were recorded intracellularly in identified motoneurons and extracellularly in hindlimb muscle nerves at 13-20 days of incubation. (Hatching begins at about 23 days under our laboratory conditions.)

The majority of the intracellular recordings were obtained from motoneurons that innervate the femorotibialis (FT), an extensor muscle in the thigh. Homonymous PSPs (produced by stimulation of the FT nerve) were detectable in all FT motoneurons, usually at stimulus intensities subthreshold for antidromic firing of the motoneurons. In addition, depolarizing PSPs could be recorded in some FT motoneurons following stimulation of the nerve to the adductor, another extensor muscle. Surprisingly, stimulation of *antagonistic* (flexor) muscle nerves, such as the sartorius and anterior iliotibialis, also elicited depolarizing synaptic potentials in some FT motoneurons. In general, non-homonymous PSPs, including those produced by antagonist stimulation, were smaller and of longer latency than homonymous PSPs. Although it is not clear whether the non-homonymous PSPs were produced monosynaptically, they persisted in the presence of APV, picrotoxin, and strychnine, which block IPSPs and eliminate much of the polysynaptic component of afferent-evoked motoneuron potentials.

These results have been corroborated and extended by extracellular recording of motoneuron synaptic potentials from muscle nerves. Such recordings, also made in saline containing APV, picrotoxin, and strychnine, reveal a pattern of widespread connectivity between afferents and motoneurons that includes excitatory connections between antagonists. It will be important to establish whether this pattern is modified as development proceeds following hatching.

230.12

SEGMENTALLY SPECIFIC MUSCLE AFFERENT PROJECTIONS TO BRACHIAL MOTONEURONS IN CHICK EMBRYOS FORM DURING BLOCKADE OF NEUROMUSCULAR ACTIVITY. B. Mendelson and E. Frank. Dept. of Neurobiology, Anatomy and Cell Science, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Neuromuscular activity was blocked with d-tubocurarine (dtc) in chick embryos from St. 28-38 to learn if segmentally specific connections between muscle sensory afferents and motoneurons would form in the absence of neurogenic muscle contractions. The segmental patterns of monosynaptic connections between muscle sensory afferents and brachial motoneurons were determined by recording motoneuronal synaptic potentials from cut ventral roots in response to the stimulation of afferent fibers in individual muscle nerves. In normal embryos stimulation of either biceps or triceps muscle afferents consistently evoked short latency, monosynaptic potentials in brachial ventral roots. The amplitudes of these synaptic potentials were greatest in the ventral roots that contained the largest number of homonymous motoneurons. Since biceps and triceps motor pools are located primarily in different segments, the synaptic potentials evoked by activity in biceps and triceps muscle afferents are segmentally specific.

In another set of embryos, neuromuscular activity was blocked by daily, *in ovo*, injections of dtc during the period when sensory-motor connections are forming (St 28-38). Ventral root recordings from these embryos treated with curare reveal that the overt pattern of segmentally specific contacts is similar to that observed in normal animals. These observations suggest that the development of specific connections between sensory and motor neurons is not entirely dependent on the normal patterns of neuromuscular activity nor the resultant afferent feedback evoked by muscle movement.

(Supported by MDA and Grass Foundation Fellowships to B.M, and by NSF BNS-8643065 to E.F.)

230.13

SELECTIVE INNERVATION OF FAST AND SLOW MUSCLE FIBERS IN NEONATAL MOUSE SOLEUS MOTOR UNITS. T. Fladby, J. K. S. Jansen. SPON: (P. Heggelund). Institute of Physiology, University of Oslo, Karl Johansgt. 47, 0162, Oslo 1, Norway.

We have examined the composition of motor units at postnatal day 5 while the polyneuronal innervation of the muscle fibers is still maximal. Identified with antibodies to F and S myosin there are about equal numbers of F and S fibers after the fiber proliferation is complete at day 2.

Individual soleus motor axons were stimulated in ventral root filaments. Muscle fibers with epps were labeled with injected Lucifer Yellow. Regularly 10 to 15 fibers (5%) were sampled from each motor unit. Alternate cross-sections of the muscle were incubated with F and S anti-myosin and a RITC labled second antibody. Lucifer labled fibers were identified and typed in the same sections.

So far more than half of all the motor units (15) contain only F or S fibers. The others contain both types with one or the other predominating.

We conclude that the F and S muscle fibers are selectively innervated before any net loss of synapses has occurred.

PROCESS OUTGROWTH, GROWTH CONES, AND GUIDANCE MECHANISMS III

231.1

GROWTH CONE ASSORTMENT IN THE OPTIC CHIASM OF FETAL MONKEYS. R.W. Williams & P. Rakic. Section of Neuroanatomy, Yale University School of Medicine, 333 Cedar Street, New Haven CT 06510

A total of 200,000 growth cones (GCs) extend through the chiasm of rhesus monkeys each day during the 2nd month of gestation. At the chiasm they assort: half cross midline and ascend in the contralateral tract; the other half grow into the ipsilateral tract. We are interested in how this sorting takes place. GCs are distributed widely in cross-sections of the distal nerve and chiasm. Densities vary up to 10-fold, and this variation corresponds with the sequence of ganglion cell genesis in this species (LaVail et al., ARVO '85). Regions that contain the fewest GCs (<10 GCs/100 μm^2) correspond to central retina while regions that contain the highest GC densities (50 GCs/100 μm^2) correspond to the edge of the retina where ganglion cells genesis is most active.

By following the trajectories of fibers backward and forward through serial thin and semithin sections we have been able to divide the distal nerve and chiasm into regions that contain heavy concentrations of GCs from the nasal and temporal sides of the eye. While we have not succeeded in distinguishing nasal and temporal GCs on the basis of differences in size, shape, or ultrastructure, we have found that the cellular environment into which these GCs grow differ markedly. Nasal GCs grow into the ventral and anterior part of the chiasm that is peppered with glial cells at early stages. In contrast, temporal GCs grow into the lateral and posterior parts in which there are virtually no glial cell bodies. This finding raises the possibility that GCs from nasal and temporal retina differ initially in their affinity for glial cell processes.

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231.2

ULTRASTRUCTURE OF CELLULAR CONTACTS MADE BY HRP LABELED GROWTH CONES IN THE HAMSTER CORPUS CALLOSUM. C.R. Norris* and K. Kalil. (SPON: E. Schweitzer) Dept. of Anatomy and Neurosciences Training Program, Univ. of Wisconsin, Madison, WI 53706.

The motile exploratory tip of a growing axon, the growth cone, responds to environmental cues by changing its shape and direction of movement. To investigate the possibility that growth cones in the mammalian CNS might be using specific cellular contacts as cues for migratory behavior, we studied at the EM level HRP labeled growth cones in the hamster corpus callosum which had been identified at the light microscopic level.

The hamster corpus callosum develops almost entirely postnatally. Thus, the relationship of the earliest growth cones to their environment could be compared with those arriving later. Hamsters ranging from newborn to 3 days after birth received small injections of HRP into the sensorimotor cortex. After a 2 hour survival time, perfusion fixed brains were processed for EM. At each stage of sectioning, growth cones were reidentified and photographed. Serial sections were collected so that growth cones could be reconstructed.

A striking feature of growth cones is the large area over which they spread, even in animals 2-3 days old when the packing density is tight and there is little extra-cellular space. The veils of lamellipodial growth cones wrap around many axons and contact diverging axon bundles. Branches of filopodial growth cones interdigitate among different bundles of axons and growth cones. Although growth cones were primarily associated with other neuronal structures, occasionally processes of growth cones were closely apposed to nonneuronal cells such as glia and blood vessels. However, specialized contacts were never observed between growth cones and these cellular elements. These observations argue strongly against a model of axonal guidance in which growth cones form obligatory contacts with nonneuronal cells or with specific axons along their trajectory.

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231.3

WAITING PERIODS AND SUBCORTICAL COLLATERALIZATION: AXON GROWTH PROGRAMS OF VISUAL AND MOTOR CORTICAL NEURONS ARE SIMILAR. T. Terashima*& D.D.M. O'Leary (Spon: R.L.Grubb), Dept Neurosurg & McDonnell Ctr for Studies of Higher Brain Function, Washington Univ Sch Med, St.Louis, MO. 63110

We find that the growth and collateralization of subcortical axons of layer V neurons of motor and visual cortex are similar despite the fact that they retain projections to different subsets of a common set of initial targets. Axons are labeled with DiI injected in visual or motor cortex of postnatal rats, or crystals of DiI placed in these areas in aldehyde fixed fetal brains. To illustrate, we describe the projection from cortex to the basilar pontine nuclei (BPN). Axons from visual cortex reach pons at birth, but continue to grow caudally past BPN, through the medulla and into the spinal cord. At P2, buds appear on these axons at a point overlying rostro-lateral BPN; collaterals develop from the buds and by P2.5 extend well into BPN. The axon segment caudal to the collateral point is later eliminated. Axons from motor cortex behave similarly but budding takes place 2 days earlier, primarily at caudal BPN, and the axon caudal to the collateral is retained. Thus, in this system, the "waiting period" is not due to axons pausing near the target and later entering it, but to a delay in formation of collaterals off of axons that have grown past the target. We find that other layer V subcortical projections also develop as interstitial collaterals in a similar way.

231.4

DEVELOPMENT OF TOPOGRAPHIC PRECISION IN CHICK RETINOTECTAL PROJECTION INVOLVES MAJOR REMODELING, H. Nakamura & D.D.M. O'Leary (SPON: J. McCasland) Dept. Neurosurgery & McDonnell Ctr Higher Brain Function, Washington Univ. Med. Sch., St. Louis, MO. 63110

Using the anterograde tracer, DiI, we have studied in E9 to E18 chicks the development of topographic order of the projection from temporal retina to rostral optic tectum. The labeled retinofugal axons course intraretinally in a compact bundle from the point of labeling to the optic fissure. Very few deviate from this bundle, but in it, much crossing over and mixing occurs. None appear to branch. The axons enter the contralateral optic tectum at its rostral edge and grow caudally, most extending well past their appropriate terminal zone in rostral tectum. After considerable elongation along the rostral-caudal tectal axis, the axons develop numerous side branches and arbors, both in and well outside of the appropriate region of tectum. Most of the overshooting axon segments and inappropriate branches and arbors appear to degenerate over the ensuing few days. A small number of overshooting axons reverse course 180^{o} and grow back to the terminal zone. Many axons enter the tectum with a positional error along the medial-lateral tectal axis; some correct this error by making dramatic alterations in their trajectory, others by extending collaterals. A proportion of the erroneous axons eventually elaborate an appropriately positioned arbor and survive. These events lead to the establishment by E16 of a discrete and highly focused zone of terminal arborization characteristic of the mature projection.

231.5

A STUDY OF THE DEVELOPING CORPUS CALLOSUM WITH A METHOD FOR STAINING NEURONAL PROCESSES IN FIXED TISSUE.
S.Catalano* and H.P.Killackey. Depts. of Anat. & Neurobio. and of Psychobio, Univ. of Calif., Irvine CA 92717

Two difficulties in tracing pathways in the developing brain are tracer diffusion and the need for a survival period post-injection during which the system continues to develop. Both of these difficulties can be overcome by the use of the fluorescent tracer DiI which diffuses through the lipid membranes of aldehyde fixed tissue (Godement,P. et al.,Develop. 101:697, 1987).

Rat pups (ages E 18 to PND 7) were sacrificed and then perfused transcardially with 4% buffered paraformaldehyde. The brains were bisected along the midsagital plane and crystals of diI placed in the corpus callosum under a dissecting microscope. The brains were then stored in the fixative at room temperature for at least one week and sectioned with a vibratome. The sections were then mounted in buffer and sealed under a coverslip, and examined with an epifluorescent microscope.

At all ages, labeled fibers and growth cones could be observed in the white matter and at later ages, they could also be observed within the cortical layers. Our preliminary observations suggest that growth cone morphology varies at different points of the pathway. In the white matter growth cones are elongate in form. Upon turning up towards cortex, they are broader with many filopodia, and on entering cortex, they exhibit a branched form tipped with many small growing ends.

231.6

THE DEVELOPMENT AND INNERVATION OF THE EPAXIAL MUSCLES.
K. W. Tosney, Biology Dept., University of Michigan, Ann Arbor, MI. 48109.

The developing epaxial muscles provide a highly specific cue that is essential for the outgrowth of the epaxial motoneurons (Tosney, *Devel. Biol.* 122: 540). I have found that the myotome originates from the cranial side of the dermamyotome shortly before axon outgrowth. Two epaxial muscles develop *in situ* from each myotome. The longissimus dorsi initially forms as a discrete series of segmental units and then reorganizes to form the long muscle of the back. The intervertebral muscles develop in each segment; they persist in thoracic and regress in lumbosacral segments. The fate of innervation during muscle reorganization and regression is under study.

The nature of the target-related cue has been partially clarified. 1) Even when the intervening sclerotome is substantially deleted, the epaxial motoneurons exhibit target-dependent and target-directed outgrowth. This suggests that the target does not act indirectly by altering the sclerotome. For instance, the target is unlikely to induce the sclerotome to form a gradient of adhesivity that orients epaxial motoneuron outgrowth. 2) The dependence of epaxial motoneurons on their target is apparantly not due to the stabilization of a few growth cones that have grown out randomly and contacted target. When the prospective target is removed from a single segment, epaxial motoneurons extend entirely within that segment and do not contact target in the more anterior segment until 2 days later. In contrast, no epaxial growth cones emerge from a spinal nerve when all epaxial target within 300 μm has been removed. These results strongly suggest that the epaxial muscle cue is relatively long-distance and may be diffusible. I am beginning to assess this possibility using a hybrid *in vivo/ in vitro* system that allows finer spatial and temporal analysis. Supported by NIH #NS21308.

231.7

NEURONAL ADHESION AND NEURITE GROWTH ON GLIA-ENRICHED SUBSTRATES FROM TARGET AND NON-TARGET BRAIN REGIONS.
E. Lieth, D.R. McClay*+ and J.M. Lauder. Dept. of Cell Biol. and Anat., UNC, Chapel Hill, NC, 27599, and +Dept. of Zool., Duke Univ., Durham, NC, 27707.

Two parameters of in vitro cell-cell interactions between serotonergic neurons and glia-enriched substrates from temporally defined target, non-target, or home brain regions were measured: strength of adhesion and extent of neurite outgrowth. Whereas the length of serotonergic neurites on home metencephalic substrates was at least 26% greater than growth on either target mesencephalic or non-target spinal cord substrates, mesencephalic substrates proved to be at least 62% more adhesive than home or non-target regions. This finding indicates that for developing serotonergic neurons, and perhaps other transmitter-identified neuronal types, specific target recognition correlates with increased adhesion, which in turn might be associated with cessation of axonal growth. Although it was previously shown that on fibroblasts or postnatal astrocytes increases in adhesive recognition can accelerate neurite growth, the current study shows that the increased adhesivity of a temporally appropriate target may cause neurite growth to slow down.

231.8

TRANSIENT EXPRESSION OF ADHERON MOLECULES AT THE EARLY STAGE OF SYNAPTOGENESIS.
H-C.T.Tsui and W.L.Klein. Dept. Neurobiology and Physiology, Northwestern University, Evanston IL 60202.

Adherons are multimolecular adhesion-promoting complexes obtained from media conditioned by cultured cells. EM immunogold studies recently have shown that adheron antigens occur in filamentous material that crosslinks pairs of adherent filopodia, suggesting that adherons may have an important role in establishing adhesive junctions such as synapses (Tsui et al., J. Cell Biol. in press). We now have found that during chick retina development in ovo, adheron immunoreactivity is transiently expressed at specific regions in the cellular and synaptic layers. At early stage only general labeling was found over the entire neural retina, but at E11 segregation of labeling started to occur. By the beginning of synaptogenesis at E14, only selected cells within the inner nuclear layer were brightly labeled. These cells were close to the inner synaptic layer. Also the inner part of the inner synaptic layer was more brightly labeled than the rest of the synaptic layer. After E17, adheron-immunoreactivity was significantly decreased and homogeneous except for the increasing labeling of the interphotoreceptor complex. This transient expression of adheron antigens at the beginning of synaptogenesis further supports the idea that they are involved in the transition from growth cones to synapses.

231.9

COMPARTMENTALIZATION OF CYTOSKELETAL AND CELL SURFACE PROTEIN EXPRESSION DURING INITIAL PROCESS OUTGROWTH OF HIPPOCAMPAL NEURONS.
K.R. Pennypacker and P. Levitt. Dept. Anatomy, Med. Coll. Pa., Philadelphia, Pa. 19129.

Neurofilament (NF) and microtubule-associated protein 2 (MAP2) have been used as protein markers of axons and dendrites, respectively. The limbic system-associated membrane protein (LAMP), which mediates target recognition by limbic axons, is expressed on developing axons in vivo. We have used antibodies to each of these proteins to investigate the molecular organization of initial process outgrowth. Monolayer cultures from E16 rat hippocampus expressed LAMP, MAP2 (antibody provided by Dr. I. Fisher, Shriver Center) and NF (200, 160kd) at each time examined. MAP2 positive cells were counted and examined for NF-positive neurites. Within 2 hr of plating, NF protein was expressed in 20% of MAP2-positive cells. By 20 hr, nearly 50% were NF-immunoreactive. Initially, nearly 60% of the NF-positive neurites exhibited MAP2 staining, but this colocalization decreased over time. At 2-4 days, many processes were stained proximally for MAP2 and distally in the same neurite for NF, suggesting an active redistribution and compartmentalization of the proteins. By 8 days, few double-stained neurites were seen. While previous studies have reported delayed (3-4 days) NF expression in culture (Shaw, G., et al, Eur. J. Cell Biol. 39:205-216, 1985), our data suggest an early and specific expression of both MAP2 and NF. The presence of LAMP during initial process outgrowth is consistent with its role as an important component of limbic connectivity. Supported by NSF BNS8519647, March of Dimes Grant 1-919 and NIH Training Grant NS-07287.

231.10

RELATIONSHIP OF LAMELLIPODIAL STRUCTURE TO RAPID LOCOMOTION IN GROWTH CONES.
N. Kleitman and M.I. Johnson+. Depts. Anatomy and +Pediatrics, Washington Univ. Sch. Med., St. Louis, MO 63110

Previous work suggested that the rate of growth cone locomotion is correlated with growth cone morphology and dependent on neuronal age (Argiro et al., J. Neurosci. 4:3051). On laminin rat sympathetic neurites elongate at rates rivalling growth in vivo and growth cone forms range from lamellipodial to entirely filopodial. We analyzed a large sample of growth cones from perinatal (E21) and postnatal (P30) rats using time lapse cinematography. As on collagen, growth cones locomote on laminin most rapidly when lamellipodial in form. Lamellipodial E21 growth cones (1 day in vitro) with no underlying filopodia move most rapidly (1.5 μm/min) compared to those with microspikes at the leading edge (0.9 μm/min) or to filopodial forms with or without small veils between adjacent filopodia (0.7 and 0.3 μm/min, respectively). When fixed and stained with fluorescent phalloidin, a probe for F-actin, 69% of E21 growth cones were predominantly lamellipodial with a meshwork of actin (in comparison to actin bundles in filopodia). Although we had never observed lamellipodial P30 growth cones on collagen, these made up 38% of the growth cones observed after 1 day in vitro on laminin, and were the fastest growing P30 neurites. In the postnatal cultures, growth cones spent less time in lamellipodial form and had slower overall growth rates. In both age groups, the proportion of lamellipodial growth cones decreased over the first week in vitro, but the rate of locomotion was more closely correlated with growth cone structure than age or length of time in vitro. (NIH NS07071, NS08069, NS21771; NSF BNS8508148.)

231.11

Growth cone-growth cone interactions in cultures of rat sympathetic neurons: A time-lapse video microscopic study. J.K. Ivins and R.N. Pittman. Dept. of Pharmacology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

The nerve growth cone is a highly motile and dynamic structure intimately involved in such diverse phenomenon as neurite outgrowth, target cell recognition, and synaptogenesis. We are interested in understanding how growth cone motility is regulated by environmental factors such as contact with other growth cones and neurites.

Sympathetic neurons from neonatal rat superior cervical ganglia were mechanically dissociated and plated on glass coverslip dishes coated with polylysine and laminin for time-lapse studies.

A variety of behaviors were observed. Most commonly, one growth cone retracted upon filopodial contact with the growth cone of another sympathetic neuron. This is often accompanied by a rapid and transient burst of lamellopodial activity 20-30 microns proximal to the retracting growth cone. On several occasions, growth cones were seen to track other growth cones, although fasciculation was relatively rare. Retraction was also occasionally seen in growth cone-neurite interactions. In general, the interactions observed between sympathetic growth cones were more complex than have previously been reported for neurons from diverse sources. (Supported by the McKnight Foundation and the PMA).

231.12

NEURITE OUTGROWTH BY AN IDENTIFIED NEURON: PROMOTION BY L-GLUTAMATE IN SITU AND IN VITRO. A.G.M. Bulloch and G. Hauser*. Dept. of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Sprouting from intact neurons of the snail Helisoma occurs in response to stress. Additionally, such stresses have been correlated with an elevation of blood-borne acidic amino acids. The present study tested the hypothesis that L-glutamate, either indirectly or directly, can induce neurite outgrowth by Helisoma neurons. Neuron B5 was examined both isolated in vitro, and also intact in situ using a newly developed preparation. L-glutamate did not induce sprouting by either intact or isolated neurons B5. We also tested whether L-glutamate can enhance conditioned medium which is known to induce neurite outgrowth. Conditioned medium is normally produced by incubating Helisoma ganglia in defined medium for 72 hrs, a procedure which releases trophic proteins; in this study medium was conditioned for only 24 hrs. Little or no neurite outgrowth occurred in such medium that lacked L-glutamate. However, the medium conditioned in the presence of L-glutamate (150-300 μM) was found to be very effective in evoking neurite outgrowth both from intact neurons in situ and also from isolated neurons in vitro. These results support the hypothesis that L-glutamate can enhance neurite outgrowth via an indirect mechanism, possibly involving either release and/or activation of proteinaceous trophic factors.

231.13

NEURITE OUTGROWTH OF AN IDENTIFIED NEURON: AN ULTRASTRUCTURAL ANALYSIS OF CELL-CELL INTERACTIONS IN SITU AND IN VITRO. R.C. Berdan, G. Hauser* and A.G.M. Bulloch. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1

We examined the ultrastructural basis of cell-cell interactions of an identified neuron following neurite outgrowth induced by axotomy. Axotomized neurons B5 from the buccal ganglion of Helisoma were compared with isolated neurons B5 in culture. Buccal ganglia containing axotomized neurons were cultured in host snails, hanging drops of blood and conditioned medium. Neuron B5 and its processes were identified in plastic sections by electron microscopy following intracellular injection with horseradish peroxidase. Putative chemical synapses were abundant on cell processes of neuron B5 prior to and following axotomy. Gap junctions were occasionally (N=3) seen between regenerating neurites and glia in situ. Small gap junctions (punctate contacts) were found to connect processes of electrically coupled pairs of neurons B5 in vitro. Furthermore, rough endoplasmic reticulum was abundant within the regenerating neurite processes. The surface features of isolated neurons were examined by scanning electron microscopy and although non-neuronal cells may associate with the giant neurons in vitro, isolated neurons were free of associated glia. This is the first study to compare cell-cell interactions of an identified neuron both in situ and in vitro and indicates that a regenerating neuron may form several types of membrane specializations with other neurons and glia.

Supported by Alberta Heritage Foundation for Medical Research.

231.14

SUCCESS OR FAILURE OF IN VIVO AXONAL REGENERATION DEPENDS ON THE AXOTOMY SITES IN AN IDENTIFIED NEURON WITH TWO AXONS. P.J. Kruk* and A.G.M. Bulloch (SPON: R.G. Lee). Dept. of Medical Physiology, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Regeneration of an identified buccal neuron (B4) after axotomy was examined in adult Helisoma. This neuron has two axons, one innervating the ipsilateral salivary gland and the other the contralateral salivary gland. Esophageal trunks (left, right or both) containing these axons were crushed very close to the buccal ganglia in situ. One week following axotomy one of the two neurons B4 was stained with Lucifer Yellow. We studied the response of the neuron to three different conditions of axotomy:

a) Contralateral proximal axotomy left an axon stump. This procedure caused neurite outgrowth restricted to the tip of this stump and resulted in the regeneration of the contralateral axon.
b) Unexpectedly, no neurite outgrowth occured after ipsilateral proximal axotomy. This procedure completely removed the ipsilateral axon, which subsequently failed to regenerate.
c) Bilateral axotomy, as in (a) and (b) together, evoked extensive sprouting from the soma and axon stump and resulted in the regeneration of both axons.

Thus, after complete removal by proximal axotomy, regeneration of the ipsilateral axon in this neuron depends upon somatic sprouting, which in turn requires proximal contralateral axotomy. So the failure of regeneration may result from a specific conditions of axotomy rather than from a general lack or loss of regenerative ability.

231.15

CALCIUM HOMEOSTASIS IN MOLLUSCAN AND MAMMALIAN NEURONS: NEURON-SELECTIVE SET-POINT OF CALCIUM REST CONCENTRATION. P. B. Guthrie, M. P. Mattson, L. R. Mills and S. B. Kater. Program in Neuronal Growth & Development and Dept of Anatomy & Neurobiology, Colorado State Univ., Ft Collins, CO 80523

Considerable evidence has recently accumulated in support of the calcium hypothesis of growth cone regulation: **growth cone structure and behavior are tightly controlled by variable levels of free cytoplasmic calcium.** Regulation of calcium levels must therefore be critical to proper behavior of growth cones. We have begun an investigation of calcium homeostasis by asking: Do individual neurons have characteristic set-points for rest calcium levels?

Intracellular calcium concentrations were measured with Fura2 using a low-light level fluorescence microscope/image-analysis system. Neurons in culture from two sources were examined: identified neurons from the buccal ganglion of the pond snail *Helisoma*, and dissociated neurons from embryonic (18 d.) and postnatal (5 d.) rat hippocampus; in all cases, different neurons had characteristic, different rest calcium concentrations. Neurons B5 and B19 from *Helisoma* had distinct calcium rest levels [B5: 221±21 nM (n=20); B19: 141±22 nM (n=13); $p<0.02$]. In embryonic hippocampal cultures, bipolar neurons had consistantly lower levels than pyramidal neurons [72±3 nM (n=15) versus 86±2 nM (n=15); $p<0.001$]. In cultures from regions of post-natal hippocampus, neurons from the dentate gyrus (presumptive granule neurons) had lower concentrations [74±2 nM (n=25)] than did pyramidal neurons from CA2 and CA3 [102±7 nM (n=11), 107±6 nM (n=13) respectively]. More striking is the observation that, even within the same class, mitotic sister neurons (neurons having undergone their final mitosis in culture) have highly correlated calcium concentrations [$r^2=0.621$; $p<0.001$]. Non-neuronal hippocampal cells had higher calcium rest levels than did any hippocampal neuron type [144±6 (n=24)].

Additional studies are now demonstrating that specific regions of single neurons will have different calcium rest levels. Thus, different neurons have the ability to set, both locally and the globally, intracellular calcium to characteristically different levels.

231.16

CALCIUM HOMEOSTASIS IN MOLLUSCAN AND MAMMALIAN NEURONS: DYNAMICS OF CALCIUM REGULATION. S. B. Kater, P. B. Guthrie, M. P. Mattson, L. R. Mills and R. S. Zucker[1]. Program in Neuronal Growth & Development and Dept Anatomy & Neurobiology, Colorado State Univ., Ft Collins, CO 80523, and [1]Dept Physiol. & Anat., Univ. of California, Berkeley, CA 94720

Since neurons can selectively set their rest calcium concentrations (preceding abstract), they must do so through regulation of influx, pumping, buffering, and/or sequestration. We are investigating the dynamics of calcium regulation by perturbing intracellular calcium levels and asking: will neurons attempt to restore calcium to original levels? Neurons in culture from two sources were examined: identified neurons from the buccal ganglion of the pond snail *Helisoma*, and dissociated neurons from embryonic rat (18 d.) hippocampus. Calcium levels were measured using Fura2.

Addition of the calcium channel blocker La^{3+} to *Helisoma* neurons B5 and B19 caused a significant decrease in intracellular calcium concentrations; within 15-30 min, levels started returning to the original levels. Similarly, removing calcium from the medium of hippocampal cultures reduced calcium concentrations in pyramidal-like neurons; even while maintained in 0mM Ca^{2+} medium, levels tended to be restored towards original concentrations.

Increasing cytoplasmic calcium concentrations with bath application of the ionophores A23187 and ionomycin raised calcium concentrations in both *Helisoma* and hippocampal neurons; again, levels were restored towards normal after 5-15 min.

A striking demonstration of active regulation of calcium levels came from experiments with the photo-labile calcium chelator NITR7. Irradiation of injected *Helisoma* neurons dramatically increased calcium levels; within minutes, calcium concentrations dropped below original levels, suggesting rapid activation of pumping or sequestration mechanisms.

These data indicate that neurons actively regulate calcium levels when perturbed. Thus, the temporal dynamics of calcium concentration changes must be considered, especially when studying the regulation of growth cone behavior in response to external stimuli both in culture (next abstract) and in situ (second abstract following).

231.17

INTRACELLULAR CALCIUM CONCENTRATIONS IN NEURONAL GROWTH CONES CHANGE WITH INDUCED TURNING AND BRANCHING BEHAVIOR. L.R. Mills, M. Murrain, P.B. Guthrie, and S.B. Kater, Program in Neuronal Growth & Development and Dept. of Anatomy & Neurobiology, Colorado State Univ., Ft Collins 80523

Physical interactions of the growth cone with its environment are among a wide variety of physiological stimuli known to influence neuronal growth cone morphology and neurite extension. We have begun to investigate these physical interactions using three paradigms that reproducibly evoke turning and branching behaviors in neuronal growth cones. Identified neurons in culture from the buccal ganglia of *Helisoma* were presented with three types of mechanical stimuli: 1) synthetic barriers were placed in front of growth cones, 2) a probe (3 micron tip) was used to touch selected sites on individual growth cones, 3) a micropipette containing medium from the culture dish was used to "puff" individual growth cones. Morphological changes in the growth cones e.g. the initiation of turning and branching were evident within minutes of growth cones reaching a barrier or within 5-10 minutes after application of the probe or puff.

Previous studies have shown that intracellular calcium levels rise in response to other stimuli (electrical activity and neurotransmitters), that inhibit growth cone motility. We are now asking: **Do physical interactions that induce turning and branching behavior also evoke changes in intracellular calcium?** Intracellular calcium concentrations were measured within growth cones using Fura2. Both touching (n=13) and puffing (n=6) evoked a rapid 2-3 fold rise in intracellular calcium level which was localized to the stimulated growth cone; after 2-10 minutes calcium levels were restored to their original rest levels, and subsequently calcium fell below rest levels. These data indicate that 1) mechanical stimuli can evoke changes in intracellular calcium in the growth cone 2) the growth cone can actively restore intracellular calcium levels following the changes induced by mechanical stimulation.

231.18

VISUALIZATION OF INTRACELLULAR CALCIUM CONCENTRATIONS IN *HELISOMA* NEURONS IN SITU. M. Murrain, L.R. Mills and S.B. Kater, Program in Neuronal Growth & Development and Dept of Anatomy & Neurobiology, Colorado State Univ., Ft Collins, CO 80523

Calcium is thought to play an important role in the control of neuronal outgrowth, as well as many other cellular and developmental processes. Previously, intracellular calcium concentrations have been visualized in cell culture, by loading individual *Helisoma* neurons with the calcium indicator dye, Fura2.

This study reports a method which allows for visualization of intracellular calcium in *Helisoma* neurons in situ. We have measured the calcium concentrations of neurons in freshly isolated buccal ganglia, as well as neurons in buccal ganglia where nerve trunks were crushed, to induce regeneration of specific neurons. It has previously been shown that application of serotonin, which regulates neurite outgrowth in isolated buccal neurons B19 in culture, results in an increase in the level of intracellular calcium from approximately 125 to 330 nM (Cohan, Conner and Kater, J. Neurosci. 7:3588-3599). We now find that the application of serotonin to buccal ganglia with Fura2 filled B19s results in an increase in the intracellular calcium concentration from approximately 90-100 nM up to 200 nM. Moreover, this rise in calcium has specific temporal and spatial patterns. Additionally, factors which are known to modulate growth and intracellular calcium concentrations in vitro have similar effects on calcium in situ. Because we can now visualize calcium concentrations in situ, we can extend our understanding of the role of calcium in neuronal outgrowth from neurons in culture to neurons in an environment that closely resembles their environment in vivo.

BIOLOGY OF NEUROGLIA

232.1

MACROPHAGE FUNCTION DURING WALLERIAN DEGENERATION OF RAT OPTIC NERVE: CLEARANCE OF DEGENERATING MYELIN AND IA EXPRESSION. Guido Stoll*, Bruce D. Trapp* and John W. Griffin. Depts. of Neurology and Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205. The role of macrophages in Wallerian degeneration in the CNS is still controversial. This study was undertaken to investigate whether macrophages are involved in the phagocytosis of myelin debris and concomitantly express Ia antigen, a product of the immune response genes, in the transected optic nerve using 1-μm and thin-frozen sections. Serial 1-μm frozen sections were stained by avidin-biotin-peroxidase complex immunocytochemistry using monoclonal antibodies to rat Ia antigen (Ox6) and two antibodies which define cell types: GFAP for astrocytes and ED1 for macrophages. After transection an increasing number of ED1 positive macrophages appeared in the injured optic nerve. A subpopulation of these macrophages expressed Ia antigen. In thin frozen sections, Ia immunoreactivity was found exclusively in cells containing multiple vacuoles and myelin debris and lacking intermediate filaments. Virtually the same cell type was labeled by ED1 antibodies. GFAP positive astrocytes did not contain myelin debris in their cytoplasm. In conclusion macrophages seem to be responsible for removal of debris and concomitantly express Ia antigen in CNS Wallerian degeneration.

232.2

ACTIVATION OF ASTROCYTIC LYSOSOMAL PROTEINASES BY FACTORS RELEASED BY MONONUCLEAR LEUKOCYTES. C.T. Bever*, D.S. Snyder, R.O. Endres*, K.D. Morgan*, A. Postlethwaite*, and J.N. Whitaker (SPON: R.F. Mayer). Res. Service, VAMC, Dept. Neurol., and Div. Rheum. U.T.M., Memphis, TN, Dept. Neurol., UMAB, Baltimore, MD, and Dept. Neurol., UAB, Birmingham, AL

Lysosomal proteinases are increased in the tissue lesions of experimental allergic encephalomyelitis (EAE) but their cellular origins are not known. To evaluate their potential as a source of these proteinases astrocytes from fetal rat brains were prepared and grown in culture by the method of McCarthy and DeVellis. Lysates of astrocytes were assayed for two lysosomal enzymes found in EAE lesions, cathepsin B (CB) and cathepsin D (CD), using established assays based on the degradation of carbobenzoxy-alanyl-arginyl-arginine-methoxy-naphthalamide for CB and [^{14}C] methemoglobin for CD. Because astrocytes are activated by inflammatory mediators in demyelinating lesions the effect of the addition of lymphokine-containing medium to cultured astrocytes was examined. Culture supernates from mononuclear leukocytes grown in the presence of phytohemagglutinin (PHA sup) induced significant increases ($p < .01$) in the astrocytic CB (8.91 ± 0.04 mU/ug protein versus 3.89 ± 0.15) and CD (49.6 ± 0.66 ng/mg protein versus 20.5 ± 0.90). Neither PHA alone, nor commercially available preparations of human interleukin-1, interleukin-2, or gamma-interferon induced significant increases when added to cultured astrocytes. PHA sup was fractionated on a Sephadex G-100 column and activity was found in fractions corresponding to a molecular weight of 45-50,000. Reactive astrocytes could make a major contribution to the increased CB and CD activity found in the lesions of EAE (Supported by the VA Research Program).

232.3

CELLULAR RESPONSE TO OPTIC TERMINAL DEGENERATION K. Rao and R. D. Lund. Dept. Neurobiol., Anat. and Cell Sci., U. Pittsburgh, Pittsburgh, PA 15261.

We have previously shown that CD1 mouse retinae transplanted to the tectal region of neonatal Sprague-Dawley rats were immunologically rejected within 3 weeks after the removal of an eye from a month old host. Here we examine whether optic terminal degeneration is associated with a sequence of cellular events, that may precipitate the rejection process.

Unilateral eye removal was done in one month-old normal Sprague-Dawley rats. Animals were perfused from 1 day to 18 days later and frozen sections of the midbrain were cut. Alternating series were stained with the following immunohistochemical stains: (1) OX-6 for class II MHC antigens, (2) OX-18 for class I MHC antigens, (3) OX-42 for microglia/macrophages and (4) GFAP for astrocytes. In normal rats, no staining of the midbrain parenchyma was seen with either OX-6 or OX-42, but 3-4 days after eye removal, both antibodies stain cells in the region of the superior colliculus in which terminal degeneration is found. There is also a simultaneous and progressive increase in the number of stained astrocytes and macrophages/microglia in the same area.

It is therefore possible, given the induction by the degeneration process of MHC expression in tectal cells, that this may be an important factor in the instability of neural xenografts lying in the vicinity of regions in which neural degeneration is in progress. Supported by grants from Winters Foundation and NIH (EYO5283 and EYO5308).

232.4

STRUCTURAL SIMILARITY OF GLIAL HYALURONATE BINDING PROTEIN (GHAP) AND HA-BINDING REGION OF CARTILAGE PROTEINS. G. Perides*, W.S. Lane*D. Andrews*D. Dahl and A. Bignami. Harvard Medical School, and VA Medical Center, Boston, MA 02132 and The Biological Laboratories, Harvard University, Cambridge, MA 02138.

We previously reported on the localization in white matter astrocytes of GHAP (Bignami and Dahl, J. Neurocytol. 15:671, 1986). Here we report on the partial characterization of this protein. GHAP with an isoelectric point 4.3-4.4 was isolated from human brain white matter, the yield being 4 mg protein/50 g wet tissue. Comparison with GHAP from a viable glioma removed at surgery showed that the 60 kDa protein isolated from autopsy material is not a degradation product resulting from postmortem autolysis. After enzymatic deglycosylation an immunoreactive 47 kDa polypeptide was obtained. Proteolytic degradation with carboxypeptidase Y showed that the binding sites to HA are in the carboxy-terminal half of the protein. A 48 amino acid sequence of GHAP showed a striking similarity (up to 89%) with the HA binding region of cartilage proteins (bovine nasal cartilage proteoglycan, rat and chicken link protein). However, the amino acid composition and other amino acid sequences suggested that there are also major differences between brain specific GHAP and cartilage proteins. Supported by NIH grant NS 13034 and by the VA.

232.5

RETINAL GLIAL CELLS POSSESS FUNCTIONAL $GABA_A$ RECEPTORS. R. P. Malchow, H. Qian*, and H. Ripps, Depts. Ophthalmology and Anatomy & Cell Biology, Univ. Illinois College of Medicine, Chicago, Il. 60612.

Recently we reported that the radial glia of the retina (Muller cells) were depolarized by GABA and that GABA could induce large currents in these cells (Invest. Ophthal. Vis. Sci. suppl. 29, 1988). We describe here experiments suggesting that the effects of GABA are due to activation of $GABA_a$ receptors. Muller cells were dissociated from skate retina and responses to GABA examined using the whole-cell voltage clamp technique. When clamped at -70 mV, micromolar concentrations of GABA and the $GABA_a$ agonist muscimol elicited large inward currents (typically 1 nA); application of the $GABA_b$ agonist baclofen was without effect. Both bicuculline and picrotoxin were capable of antagonizing the effects of GABA. Further, the currents generated by GABA were found to depend on chloride, with the reversal potential of the GABA response varying linearly as a function of the log of the chloride concentration.

Preliminary experiments have also shown that B-alanine and taurine are also potent depolarizers of Muller cells. These experiments demonstrate that glia have a wider repertoire for responding to changes in the extracellular milieu than was previously suspected.

Supported by a research grant (EY-06516) from the N.E.I., and unrestricted awards to HR from Research to Prevent Blindness, Inc., and the Retina Foundation of Houston, Texas.

232.6

IDENTIFICATION AND PARTIAL CHARACTERIZATION OF A GLIAL-DERIVED CYTOPLASMIC ANTIGEN GA-1 BY MONOCLONAL ANTIBODY. N.C. Chang*, Y.H. Lin*, R.L. Chou* and A.C. Chang* (SPON: B. Azzarelli). Inst. Neurosci. & Inst. Microbiol. & Immunol., National Yang-Ming Med. Coll., Taipei, Taiwan, Republic of China.

The advent of hybridoma technique has paved the way that spectrum of powerful monoclonal antibodies (MAb) thus obtained would allow the identification of various glial-derived macromolecules of potential functional significance in glia-neuron interaction, glial differentiation and neoplastic transformation. Production of MAb against glia was carried out by the standard methodology. After preliminary specificity screening, one MAb, an IgG2a, kappa secretor was selected to further characterize its antigen, designated as GA-1. Basic polyacrylamide gel electrophoresis (PAGE)-Western blot revealed GA-1 as a single band (Rf=0.09) which may be further resolved into 2 subunits of molecular weight 200 and 78 kDa by SDS-PAGE and radioimmunoprecipitation under non-reducing conditions. Subcellular localization by immunocytochemistry revealed its cytoplasmic presence with a punctate pattern perinuclearly. While significant expression of GA-1 may be detected in two rat glioma and two glial cell lines derived from neonatal rat brain, no expression may be detected in various tissues and brain regions of adult normal rat (Sprague-Dawley). Nor can GA-1 be detected in 19 mammalian cell lines of different species and cell types. The above data suggest that GA-1 is a species (rat)-specific, cytoplasmic glial antigen. (Supported by R.O.C.-N.S.C. grant # B010-24)

232.7

GOMORI-POSITIVE PERIVENTRICULAR GLIA IN THE HUMAN BRAIN. H.M. Schipper, E.G. Stopa and T. Wheelock. Tufts Univ. Sch. of Med., Dept. of Endocrin. and Path., Boston, MA and Human Brain Tissue Resource Center, McLean Hosp., Belmont, MA.

A unique subpopulation of periventricular astrocytes has been described in amphibia and rodents. The cells contain thiol-rich cytoplasmic granules exhibiting an affinity for Gomori's chrome alum hematoxylin (CAHP) stain peroxidatic activity, and auto-fluorescence in the porphyrin range. In rodents, Gomori glia increase with age. Estrogen accelerates, and gonodectomy retards, age-related granule accumulation in the rodent female hypothalamus. Estrogen may stimulate peroxidase and porphyrin synthesis by these cells.

In the present study, sections of human diencephalon were serially stained with either DAB medium for peroxidase activity or CAHP. Topographically superimposable CAHP-and DAB-positive astrocytes were noted in the OVLT, anterior periventricular area, infundibular region, and capsule of the mammillary body. Female and aged brains appeared to contain greater numbers of these cells. The distribution and tinctorial properties of this human periventricular glial system suggest that these cells may be homologous to the Gomori-positive glia observed in lower vertebrates.

232.8

GLIA MATURATION FACTOR (GMF) REDUCES BEHAVIORAL DEFICITS AFTER CAUDATE NUCLEUS (CN) INJURY IN RATS. C.M. Palatucci, D. Charbonnier*, R. Lim# and D.G. Stein. Brain Research Lab., Clark Univ., Worc., MA 01610 #Dept. of Neurol., Univ. Iowa, Iowa City, IA 52242

GMF is an acidic protein derived from brain tissue (Lim and Miller, *Methods in Enzymology* 147 225-235 (1987)) of about 14 kDal. which has the ability to promote phenotypic expression of brain cells *in vitro*. It is chemically distinct from Nerve Growth Factor and Fibroblast Growth Factors. We now present the results of intracaudate injections of GMF in adult rats after electrolytic CN lesions.

Fifty adult male Sprague Dawley rats (about 70 days old at the time of surgery) were randomly assigned to one of five experimental groups (n=10/gp.). Sham rats (S) received superficial retraction of scalp tissue. All other rats received bilateral electrolytic lesions of the CN. Group B rats were then injected with 200 ng Bovine Serum Albumin (BSA) as control, H (high dose) rats with 200 ng GMF, M (middle dose) rats with 100 ng GMF and L (low dose) rats with 50 ng GMF. Both GMF and BSA were diluted in lactated Ringer's solution to 5 μL (2.5 μL/side), and BSA was used as the vehicle for the GMF. Three days after surgery all animals began testing on an active avoidance/spatial reversal task, ten trials/day for the following twenty days. One day after testing was completed, all animals were trans-cardially perfused by the Karnovsky's method.

A one-way ANOVA indicated a significant lesion effect, and H rats were indistinguishible from B rats on many behavioral measures. Importantly, on number of failures/reversal, L rats performed at sham level while all other lesioned groups were signifigantly worse than shams. Also, L rats were better than H and B rats on a number of other behavioral measures. These data are consistent with e.g. those of Kesslak et al. (*Exptl. Neurol.* 92, 377-390 (1986)) which indicate that exogenously administered substances which manifest their effects principally on glial cells are capable of promoting behavioral recovery. Cresyl violet staining and Glial Fibrillary Acidic Protein immunohistochemistry are underway and will be discussed.

232.9

Further Characterization of the Myelin-Like Membranes from Earthworms (Lumbricus terrestris L.) Pereyra*, P.M. and Roots, B.I. Univ. of Toronto.

Initial characterization of the myelin-like membranes from earthworm nerve cords by SDS-PAGE and immunoblotting showed that the composition of these membranes is substantially different from the structural component of vertebrate myelin (Pereyra and Roots, Neurochem. Res. in press (1988)).

Comparative studies were also undertaken using epifluorescent light microscopy to determine autofluorescence (due mostly to NAD(P)H and FAD's and tetracycline-metal interactions in myelin and myelin-like membranes (Pereyra and Roots, Brain Res., under rev.).

It became clear from these studies that the similarities between the myelin-like ensheathments of invertebrates and myelin, might derive their common structure as a result of similar underlying metabolic processes despite variations in their structural composition.

As a first step, this hypothesis is being tested by determining the activities of various Oxidoreductases and ATPases in earthworm giant axon preparations and purified myelin-like membranes. Also, the susceptibility of the autofluorescence to various uncouplers and ionophores is being used to determine the source of the redox potential in these membranes.

The result of these two approaches will be presented and discussed in relation to similar observations made on vertebrate myelin.

232.10

AUTOFLUORESCENT EXTRACELLULAR PRODUCTS OF GLIAL ACTIVATION FOLLOWING SENSORY AXON DEGENERATION ARE ELICITED TRANSNEURONALLY IN AN INSECT *ACHETA DOMESTICUS*. J.S. Edwards, P. Brunner, M.R. Meyer and S. Raj. Zoology Dept., Univ. of Wash., Seattle, WA 98195.

Sensory axons of insects degenerate rapidly when severed from their cell bodies. Ultrastructural changes which are evident at terminals within four hours, lead to the appearance of the well known subterminal osmiophilic degeneration figures used in mapping. As degeneration proceeds rapidly throughout the severed axon, glial cells are activated and extracellular material increases in lacunae left by collapsing axons. Activated glial cells contain extensive arrays of tubular structures associated with dense plaques and superficially similar to hemidesmosomes. Matrix material accumulates outside the plaques and appears to arise from them.

We report here a previously unrecognised response to sensory axon degeneration. Autofluorescent extracellular material appears distally in the severed cercal sensory nerve less than 2 days after axotomy and proceeds centripetally throughout the central projection, reaching a peak at 4-5 days and persisting for at least two weeks. We postulate that the autofluorescent material is a component of the extra cellular matrix described above. Autofluorescent material appears in the ipsilateral connective in layers surrounding giant interneurons. Its initiation must be signalled transneuronally and may reflect altered metabolic activity in giant interneurons that receive cercal sensory input. The autofluorescent glial product is currently being characterised.

Supported by NIH grant NS-07778.

232.11

EVIDENCE FOR ELECTROGENIC $Na^+/HCO3^-$ COTRANSPORT IN GLIAL CELLS OF MUDPUPPY (NECTURUS MACULOSUS).

M.L. Astion* and R.K. Orkand (SPON: I.D. Hentall) Institute of Neurobiology, University of Puerto Rico Medical Sciences Campus, Old San Juan, PR 00901.

In the presence of Ba^{++} (2-5 mM), the K^+ conductance of mudpuppy glial cells is blocked and other membrane permeabilities can be examined. We have used this fact to reveal a hyperpolarizing effect of HCO_3^- (Astion et al., Neurosci. Lett., 76:47, 1987). The experiments reported here investigate the mechanism of the HCO_3^- effect.

In experiments using microelectrodes and done in the presence of 2 mM Ba^{++} we found the following: 1)In low Na^+ solutions (12 mM, Na^+ replaced with N-methyl-D-glucamine), the hyperpolarizing effect of 10 mM HCO_3^- was reduced by about 80%., 2)SITS reduced the HCO_3^- effect by more than 50%., and 3)In the presence of HCO_3^-, reduction of Na^+ from 112 to 12 mM caused a depolarization which was much greater than that seen in HCO_3^--free solutions. These observations suggest the presence in the glial membrane of an electrogenic Na^+/HCO_3^- cotransporter in which the stoichiometry of HCO_3^- to Na^+ is greater than 1. Electrogenic Na^+/HCO_3^- cotransport has been described in a variety of tissues (e.g. Boron and Boulpaep, J. Gen. Physiol., 81:53, 1983), but to our knowledge, this is the first report of its presence in the vertebrate nervous system. Supported by NIH grants NSO7464, NS12253, GM 07170 (MSTP Univ. Pennsylvania), and RII8705802 (NSF).

232.12

A POSSIBLE MECHANISM FOR LONG TERM SURVIVAL OF SEVERED MEDIAL GIANT AXONS IN CRAYFISH.

R.A. Sheller, M.M. Curie*, M.E. Smevers*, S.L. Tanner*, and G.D. Bittner. Department of Zoology and Institute for Neurological Science, University of Texas, Austin, TX 78712.

A crayfish Medial Giant Axon (MGA) severed from its cell body remains morphologically and physiologically intact for 50-300 days. A possible mechanism for the long term survival (LTS) of an MGA is intercellular protein transfer from adaxonal glia to the MGA.

Large portions of a severed distal stump of an MGA or a nonsevered MGA can be microdissected from the ventral nerve cord (VNC), homogenized, and prepared for protein analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). We also obtain diluted axoplasm from a severed distal stump or nonsevered MGA by perfusion of the axon with microcapillary pipettes. This "axoperfusate" is analyzed for protein content by SDS-PAGE. During the perfusion, the MGA is isolated from its own cell body and extracellular fluids in order to obtain only axonal protein. Similar protein bands (200-30 kD) are seen in axoperfusate samples from both severed and nonsevered MGA's. When labelled amino acids are placed in the bath surrounding the VNC, labelled protein can be collected in a 3-hour axoperfusate from severed and nonsevered MGA's. This indicates that there is another trophic source for the MGA other than its cell body. Protein synthesis, which might be responsible for the LTS of the MGA, could be occurring within other cells of the VNC which then transport proteins to the MGA. The adaxonal glia of the MGA are a likely candidate for an MGA trophic source because they are in close proximity to the axon, they have a specialized morphology which might allow for transfer, and they respond to the severence of the MGA by increasing in both size and number. Suppported by TATP grant from Texas.

232.13

LONG TERM SURVIVAL OF ENUCLEATED MAUTHNER AXONS

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We have observed that severed distal segments of myelinated Mauthner axons (M-axons) in goldfish (Carassius auratus) survive morphologically and physiologically intact for 160-190 days at 10°C, 30-60 days at 20°C, and 20-30 days at 30°C. Cancalon (J.Cell Bio.97:6-14,1983) has reported that unmyelinated axons in garfish olfactory nerve show temperature dependent degeneration in a proximal to distal sequence. This degeneration proceeds at a rate equal to that of slow axonal transport, which is also temperature dependent.

We are now collecting detailed light and electron microscopical data on the possible existence of a proximo-distal sequence of degeneration of severed M-axons in animals kept at 10-30°C. In particular, we are examining whether the degenerative sequence involves a loss of integrity of cytoskeletal elements (e.g. neurofilament and microtubules), an increase in number of vacuoles and lysosomes, and alterations of the myelin sheath surrounding these axons.

We are also examining whether long term survival of severed M-axons might be due to axoplasmic protein synthesis or transfer of proteins from adjacent glia.

Supported by a TATP grant from Texas.

232.14

GLIOTOXIC ACTIVITY OF ACIDIC AMINO ACIDS.

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The gliotoxic action of L-α-aminoadipic acid has been known for several years. Little, however, is known about the mechanisms or pharmacological specificity of this toxic effect. In the present investigation we have identified several acidic amino acids that mimic the gliotoxic action of L-α-aminoadipic acid.

Primary cultures of astrocytes were prepared from four day old rat cortex and purified as described by McCarthy and de Vellis (J. Cell Biol., 85, 890, 1980). The cells were grown in Dulbeccos modified Eagle's medium and Ham's F12 (1:1) supplemented with 15 mM HEPES buffer and 10% fetal calf serum. Potential gliotoxic amino acids were included in the cultures with normal media and their effects on the astrocytes followed by phase contrast microscopy for 96 hours.

L-Homocysteic acid, L-cysteine sulfinic acid and serine- O-sulphate were found to exhibit the most potent gliotoxic action. Analogues that exhibited little or no activity included: D- α-aminoadipic acid, α-ketoglutarate, α-ketoadipate, kainate, NMDA, and serine-O-phosphate. Upon exposure to the gliotoxins, the astrocytes appeared to go through a series of morphological changes that eventually ended with massive cellular destruction. These changes, which included nuclear swelling and prominent nuclear rims and nucleoli, were suggestive of Alzheimer's type II astrocyte morphology. Interestingly, a number of compounds, including L-glutamate, appeared to initiate this sequence of events, but proved to be non-toxic, as the cells returned to an apparently more normal morphology without cellular degeneration. Supported by MH-19691 and the PEW Foundation.

232.15

ENDFEET OF RETINAL GLIAL (MULLER) CELLS HAVE A NON-RECTIFYING K+ CONDUCTANCE.

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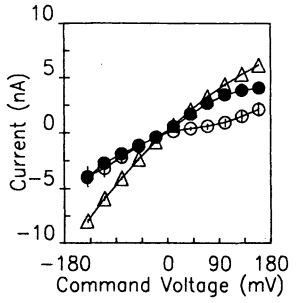
Retinal Muller cells are almost exclusively permeable to K+. In amphibians, these cells shunt extracellular K+ produced during neuronal activity into the vitreous humor by having 95% of their K+ conductance in their endfoot processes which lie adjacent to the vitreous. To further characterize this nonuniform distribution of K+ conductance, we voltage-clamped intact and endfootless Muller cells from the salamander (*Ambystoma tigrinum*) with the whole-cell patch technique. Isolated cells were prepared by enzymatic dissociation and perfused with HEPES-buffered saline containing 80 mM K+. Current response amplitudes to 1 sec voltage steps from a -10 mV holding potential are shown (mean ± SD for n cells). Cells which have lost their endfeet (o, n=5) show less conductance and more inward rectification than do cells with endfeet (Δ,n=2). Subtracting current responses of endfootless cells from those of intact cells approximates the current-voltage (I-V) relationship for the endfoot conductance alone. The resulting nearly linear I-V relationship (●) indicates that the conductance of the endfoot is non-rectifying and may be mediated by a different type of K+ channel than those found elsewhere on the cell.



Supported by NIH training grant EY07074-07 to A.R.M. and NIH grant EY04077 to E.A.N.

232.16

SEPARATE POPULATIONS OF ENDOGENOUS NON-NEURONAL CELLS PROLIFERATE AND DIFFERENTIATE IN ADULT EXPLANTS OF SENSORY GANGLIA,

C. M. Morshead, T. P. O'Connor, and D. van der Kooy. Neurobiology Research Group, Department of Anatomy, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

An extensive gliotic response is elicited in adult rat trigeminal ganglia after axotomy and explant culture. The absence of blood-born cells in the culture enables us to study the response of endogenous non-neuronal cells within the structually intact trigeminal ganglia. Cellular responses to axotomy and culture may elucidate the lineages of the endogenous non-neuronal cells within sensory ganglia.

On the basis of biochemical and proliferative studies we observed four distinct populations of cells. (1) A small proliferative population of cells were found in both the fibre tract regions and neuronal cell body areas of the ganglia. These cells were vimentin (Vim)-'ve, glial fibrillary acidic protein (GFAP)-'ve, and S-100-'ve. (2) A small population of GFAP+'ve, Vim-'ve and S-100-'ve cells turned off GFAP synthesis or died after axotomy and culture. (3) A large S-100+'ve, GFAP-'ve, and Vim-'ve population did not alter protein synthesis in culture and (4) a subpopulation of S-100+'ve cells started to synthesize Vim in response to axotomy. This subpopulation is located within the neuronal cell body areas and thus the turn on of Vim may be due to a local cue from neuronal perikarya. These four subpopulations of peripheral non-neuronal cells appear to cut across the previous subdivisions of peripheral glial (satellite versus fiber tract cells or myelin versus non-myelin forming cells) that were based on descriptive morphological criteria in normal tissue. We suggest that the peripheral glial cell population may consist of at least four cell lineages.

232.17

A STAGE-SPECIFIC CELL SURFACE ANTIGEN OF SCHWANN CELLS, C4, IS ALSO EXPRESSED BY CELLS IN THE CENTRAL NERVOUS SYSTEM. Cornbrooks, C.J. and Neuberger, T.J., Dept. of Anat. and Neurobiol., Univ. of Vt., Burlington, Vt., 05405.

Schwann cell (Sc) differentiation, a necessary requirement for normal development and regeneration, is induced by extracellular cues. It is therefore hypothesized that molecules in the Sc plasmalemmae are required to mediate and promote normal interactions with neurons. Immunohistochemical methods were used to identify and localize a trypsin sensitive epitope, C4, associated with a 75kD protein located on the Sc membrane. During development, most if not all, Sc expressed the C4 antigen. As Sc differentiation ensued, C4 immunoreactivity began to segregate with a portion of the Sc. In the normal adult animal, the C4 antigen was expressed on the surface of ensheathing Sc, but not on the surface of myelinating Sc. When the normal nerve was transected, Sc associated with the injured axons in the distal stump ceased to express C4 on their surface. However, upon contact with regenerating axons, C4 was transiently associated with the Sc cytoskeleton and reappeared on the plasmalemmae of nonmyelinating Sc. In the normal CNS, the C4 antigen was associated with astrocytes in the optic nerve and tanicytes but not with the majority of glia in the parenchyma. Upon injury to the CNS, C4 is also expressed by reactive astrocytes that grew in and near the lesion cavity.

Current evidence suggests the C4 antigen is involved with Sc polarization; however, its function in the CNS remains unknown. Supported by PHS RO1 20189.

232.18

MORPHOLOGICAL STUDIES FOLLOWING KAINIC ACID INJECTION INTO THE RAT HIPPOCAMPUS. C.M. Severin, C.L. Bowman, D.J. McFarland, and J.W. Swann. (SPON: F. Sansone). School of Medicine, SUNY-Buffalo, Buffalo, NY 14214 and Wadsworth Center, NYS Department of Health, Albany, NY 12201.

The cytology of scar tissue was examined several months following injection of 4 nmol of kainic acid into the CA3 part of the dorsal region of the rat hippocampus. Gross observation showed considerale variation in the degree of damage. Two animals displayed a total unilateral loss of the hippocampus while in four others it was present but significantly reduced in size. In these animals, light and electron microscopic analyses confirmed an anisomorphic astrocytosis. Under light microscopy numerous spherical pale staining structures (1-3μm in diameter) with a dense central core were observed after staining with toluidine blue. A few large cells (10-20μm) with light or dark staining nuclei were scattered throughtout the scar. Electron microscopic examination showed the structures to be astrocytic processes while the cells were tentatively identified as reactive astrocytes. No neuronal cell bodies were present, but several neuronal processes were observed. In vitro electrophysiological studies of this preparation have shown inexcitable cells with large processes (Bowman et. al. The Biochemical Pathology of Astrocytes, pp. 135-136, 1988, Alan R. Liss, Inc.). The fimbrial area adjacent to the scar appeared normal.

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232.19

EFFECTS OF POTASSIUM AND CALCIUM CHANNEL BLOCKERS ON THE PROLIFERATIVE RESPONSE OF EXPLANTED RAT OPTIC NERVES. Hsu, P.* and S.Y. Chiu. (SPON: P. H. Smith) Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Recent studies have shown that the proliferative response of explanted PNS rabbit sciatic nerves was inhibited *in vitro* by K channel blockers and that K channels in Schwann cells might be functionally important for proliferation. We have now extended these studies to the CNS. Undesheathed optic nerves from adult rats (5 and 12 weeks) were cut into 2 mm segments and placed in culture medium (DMEM+10%FBS). Proliferative response, as measured by scintillation counts of ^{3}H-thymidine incorporation in the segments, increased following explant, reached a peak at day 4 and declined afterwards. The proliferative response was virtually competely inhibited when K channel blockers (quinine, 0.35 mM; 4-AP, 9 mM; TEA, 10 mM) or Ca channel blocker (Cd, 0.03 mM) was present in the culture medium. Compared to TEA (10 mM), TMA (10 mM) produced no significant inhibition. Washing away the blockers after 2-4 days of treatment led to resumption of a normal proliferative response for TEA and 4-AP, but not for cadium or quinine. From the dose response curves for proliferation block measured after 4 days of treatment, the half-block concentrations are (mM): 0.0035 (Cd), 0.18 (quinine), 3.5 (4-AP) and 6.5 (TEA).

Since astrocytic proliferation presumably contributed to the proliferative response, one possible explanation is that K and Ca channels that are known to be present on astrocytes could be functionally important for proliferation.

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232.20

A NOVEL MARKER FOR A SUBSET OF PRIMATE ASTROGLIA: IMMUNOCYTOCHEMICAL CHARACTERIZATION. C.T. McDonald and J.M. Allman. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

We have employed a modification of the immunosuppression protocol of Matthew and Patterson (CSHSQB, **XLVIII:** 625, 1983) to generate monoclonal antibody (mAb) markers against elements of the newborn and adult primate cerebral cortex. In our most recent fusion, newborn (P0) macaque striate cortex was used as the primary immunogen to generate a novel glial marker, mAb 13C3.

MAb 13C3 recognizes in newborn and adult macaque cerebral cortex an antigen associated with the membranes of astrocytes in the underlying white matter as well as cells and fibers in cortical layer 1. In the newborn macaque cortex, numerous transitional astrocytic cells can be seen in white matter, probably corresponding to a population first described by Schmechel and Rakic (Anat. Embryol., **152:** 115, 1979). Unlike anti-GFAP (Boehringer), mAb 13C3 does not appear to recognize the numerous astrocytes present within the cortical plate. MAb 13C3 does, however, stain a vimentin-positive, GFAP-negative population of lepto-meningeal cells and fibers. In the new born macaque cerebellum, Bergmann glia are positive for all three markers. MAb 13C3 stains GFAP-positive, vimentin-negative astrocytes in the underlying white matter in addition to a few scattered astrocytes in the granular layer.

A previous fusion, using adult macaque striate cortex as the primary immunogen, demonstrated the existence of an antigen (mAb 6F2) present only in glial fibers of layer 1 in the adult primate cerebral cortex (McDonald, C.T. *et al.*, Soc. Neurosci. Abstr., **12:** 131, 1986). A second population of fibers, closely apposed to a transient zone of germinal cells underlying striate cortex, is also recognized by mAb 6F2 in the infant (P7) marmoset monkey.

With specificity to a subset of astrocytes, mAb 13C3 further underscores the biochemical heterogeneity of primate neuroglia. MAb 13C3 may serve as a developmental probe to assay that subset of glial progenitors destined to become the astrocytes of layer 1 and the white matter of primate cerebral cortex.

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TRANSPLANTATION: HIPPOCAMPUS

233.1

TRANSPLANTATION OF A HIPPOCAMPALLY-DERIVED, IMMORTAL, TEMPERATURE-SENSITIVE, NEURONAL CELL LINE INTO ADULT RAT CNS. S.R. Whittemore, V.R. Holets, M. Gonzalez-Carvajal* and D. Levy*. Dept. Neurological Surgery, Univ. Miami, Miami, FL 33136

Primary cultures of dissociated E18 hippocampus were infected with a retrovirus encoding the temperature-sensitive mutant of the SV40 large T antigen to generate an immortal cell line, HT4, which differentiates at non-permissive temperature (39 C) in vitro with neuronal phenotype and expresses neuron-specific surface antigens and nerve growth factor (NGF) mRNA (Levy et al., submitted).

HT4 cells, transplanted into a lesion cavity which interrupted the fimbria-fornix, either prelabelled with the vital dye DiI or identified by residual T antigen, were observed up to 8 weeks in vivo, the longest time examined. HT4 cells appear to differentiate, extending processes and are non-tumorigenic at rat body temperature.

In situ hybridization demonstrated that HT4 cells make NGF mRNA in vivo. Preliminary results suggest that transplanting HT4 cells, but not another similarly-derived cerebellar cell line, subsequent to unilateral fimbria-fornix transection, partially spare septal cholinergic neurons.

233.2

FETAL CA1 NEURONS GRAFTED TO ISCHEMIC LESIONS CONNECT WITH THE ADULT HOST HIPPOCAMPUS. J. Zimmer*, N. Tønder*, T. Sørensen* and F.F. Johansen* (Spon. M. West). PharmaBiotec Res. Cent., Institute of Neurobiology, University of Aarhus, DK-8000 Aarhus, Denmark.

Ischemic damage to the dorsal CA1 of adult rats was induced by transient four-vessel occlusion, followed one week later by grafting of a fetal, E19 CA1 cell suspension. After 6 weeks graft connections were examined by - a) retrograde axonal transport of Fluorogold injected into the ipsilateral host hippocampus and subiculum, - and b) EM of anterograde degeneration of host commissural fibers 3 days after contralateral fimbria transections.

About 80% of the grafts survived and had grown well in the lesioned area. By EM degenerating host commissural terminals were found even deep in the CA1 graft neuropil. The terminals made synaptic contacts with dendritic spines and shafts. Graft efferent projections to the ipsilateral host CA1 and subiculum were demonstrated by retrograde labelling of graft pyramidal cells after Fluorogold injections into these areas which normally are innervated by CA1. The presence of possible graft projections to the septum (normal) and the contralateral hippocampus (abnormal) are being examined.

We conclude that fetal CA1 neurons can exchange connections of normal type with the adult host brain after grafting to ischemic CA1 lesions.

233.3

A HUMAN NEUROFILAMENT TRANSGENE AS A GENOTYPE MARKER FOR MOUSE EMBRYONIC SEPTAL NEURONS TRANSPLANTED INTO ADULT RAT HIPPOCAMPUS. M. Vidal-Sanz*[1], M.P. Villegas-Pérez*[1], D.A. Carter*[1], I. Tretjakoff*[2], J.P. Julien*[3], A. Peterson[2] and A.J. Aguayo[1]. [1]Neuroscience Unit, The Montreal General Hospital and McGill University; [2]Ludwig Institute; [3]Institut du Cancer; Montreal, Quebec, Canada.

To identify the projections of neurons transplanted into the adult host CNS, we grafted embryonic septal cells from transgenic mice that express the neurofilament light gene (Julien et al. Genes Dev. 1:1085, 1987). After transecting the right fimbria-fornix in adult immunosuppressed rats we injected a septal cell suspension from E14-15 transgenic embryos into the right hippocampus using standard grafting techniques (Bjorklund et al. Acta Physiol. Scand. Suppl 522:49, 1983). After intervals of 3 weeks to 3 months, the host rats were investigated immunohistochemically with a monoclonal antibody that recognizes the transgene product (Julien et al. 1987).

Anti-human neurofilament immunoreactivity was observed exclusively in the transplanted neurons, primarily within fine neuronal processes and occasionally in neuronal somata. Our findings indicate that nerve cells from these transgenic mice express the human neurofilament light gene after transplantation. The visualization of neuronal processes obtained with this technique provides a promising tool for the identification of the projections originating from the grafted neurons.

233.4

SEPTAL CELL DEATH FOLLOWING FIMBRIA/FORNIX TRANSECTION, AND HIPPOCAMPAL CHOLINERGIC REGENERATION FOLLOWING NERVE GROWTH FACTOR INFUSION PLUS GRAFTING OF SYNTHETIC AND NEURONAL BRIDGES. M. H. Tuszynski, G. Buzsaki, G. Stearns* and F.H. Gage. Dept. Neurosci. M-024, UCSD School of Medicine, La Jolla, CA. 92093.

Following fimbria/fornix (F/F) transection in the rat, cholinergic neurons of the medial septum degenerate as measured by cholineacetyltransferase (ChAT), nerve growth factor-receptor (NGF-r)(courtesy E. Johnson), acetylcholinesterase (AChE) and Nissl stains, and extrinsic hippocampal (HC) cholinergic innervation is lost. NGF infusion will prevent septal cholinergic cell loss but will not restore HC innervation. To address whether loss of septal cellular staining might represent cell *dysfunction* rather than *death*, rats were injected with rhodamine fluorescent microspheres (FM's) that are retrogradely transported to the cellular cytoplasmic component, and are lost after cell disruption. 25 rats received unilateral FM injections followed 5 days later by either no lesion, unilateral, or bilateral F/F lesions, and sacrifice 4 weeks later. An additional group received bilateral F/F lesions plus 4 weeks of NGF infusion and placement of Matrigel "bridges" mixed with fetal HC neuronal grafts in an attempt to restore HC innervation. Compared to controls, animals that received either unilateral or bilateral F/F lesions showed extensive loss of FM's (87% and 93% respectively, $p<.01$), ChAT (82% and 76% respectively, $p<.01$), and NGF-r (75% and 68% respectively, $p<.01$). Thus, cells appear to die after F/F transection, and this correlates with ChAT and NGF-r loss. Some cells were double-labelled for FM's and NGF-r or ChAT after F/F lesions, indicating that some septal cells projecting to the HC derive NGF support from another source, or are not NGF-dependent despite possessing NGF receptors. Some animals with lesions followed by NGF and "bridges" regained AChE-positive HC fibers.

233.5

FETAL HIPPOCAMPAL CELL IMPLANTS ALLEVIATE A WORKING-MEMORY DEFICIT RESULTING FROM NMDA-INDUCED HIPPOCAMPAL PYRAMIDAL CELL LOSS. Yvonne Wilsdon[1], Pushpa Tandon[2], Ronnie L. McLamb[2], Richard A. King[1], Hugh A. Tilson.[2] [1]Dept. of Psychology, Univ. N. Carolina, Chapel Hill 27514. [2]Lab. Molec. Integrative Neurosci., NIEHS, Research Triangle Park, N.C. 27709.

Behavioral recovery of memory function after hippocampal lesions has been demonstrated using implants of fetal cells taken from locus coeruleus or medial septum, but little work has been done on memory recovery after implantation of fetal hippocampal cells.

Pyramidal cell lesions were made in both dorsal and ventral CA2/CA3 hippocampal areas of male Fischer 344 rats. Lesions were made with 10 µg. per site of N-methyl-D-aspartate (NMDA). Three weeks after lesioning, a suspension of cells obtained from 17-18 day old fetuses of the same rat strain were implanted into the site of the original lesion. A 2x2 design was used, in which rats received either NMDA or vehicle, and either implants or vehicle. Beginning a week after the implant, rats were trained in a standard T-maze forced-choice alternation paradigm for food reward. There was a 20-second delay between the forced trial, in which a block of wood in one randomly determined arm prevented entry, and the choice trial, in which subjects were reinforced for entering the arm not previously entered.

Subjects with a combination of pyramidal cell lesions and viable cell implants performed significantly better than those with lesions alone. Furthermore, the performance of the lesioned implanted animals did not differ significantly from unlesioned controls with or without fetal cell implants.

Following behavioral studies, subjects were transcardially perfused and brain slices were stained with cresyl violet and counterstained with luxol fast blue. Cells from the implant which stained normally were seen adjacent to the lesion site, either within the hippocampus proper or in a nearby area of the ventricle.

233.6

A MONOCLONAL ANTIBODY MARKER FOR IDENTIFICATION OF TRANSPLANTED RAT HIPPOCAMPAL CA3 PYRAMIDAL CELLS. (SPON: G. Raisman) P. L. Woodhams*, P. J. Seeley*, M. Webb**, & D. J. Atkinson*. Norman and Sadie Lee Research Centre, Nat. Inst. for Medical Research, London NW7 1AA, and *Sandoz Institute, London WC1E 6BN, U. K.

A monoclonal antibody was raised against glycoproteins isolated from 21 day old rat hippocampus using a lentil lectin column followed by an antibody affinity column to remove the immunogenic protein Thy-1. Hybridomas were first screened by ELISA and then by immunoperoxidase staining of frozen 21 day old rat forebrain. Antibodies from clone 11A4 strongly stained large pyramids in hippocampal field CA3, whilst small pyramids in CA1 and dentate granule cells were unstained in polyester wax sections. This specificity was retained during development, antigen becoming detectable on CA3 pyramids postnatally and increasing markedly during the second week. In mature grafts of sub-dissected embryonic hippocampal fragments transplanted to adult hippocampus antibody 11A4 provided a useful means of distinguishing the neuronal cell types present. The antigen was detected on western blots as a single band of apparent MW >200 kDa.

233.7

NON-SPECIFIC EFFECTS OF FETAL BRAIN GRAFTS ON RESTORATION OF A CHOLINERGIC EPSP IN HIPPOCAMPAL SLICES FROM FORNIX LESIONED RATS. W.D. Knowles and E. Korfali*. Cleveland Clinic Foundation, Cleveland, OH 44195 and Uludag University, Bursa, Turkey.

Ten to 16 days following fornix lesion, 6 "septal graft" rats received a graft of fetal septal tissue into the fornix lesion cavity. Three "cerebellar graft" rats received a graft of fetal cerebellar cortex. Ten to 27 weeks after the fornix lesion, cholinergic function was evaluated by the presence of a slow EPSP in intracellular recordings from CA1 pyramidal cells using in vitro hippocampal slices.

In 7 non-lesioned animals, 62% of 24 cells had the slow EPSP (2.4±1.5 mV amplitude; mean±S.D.). In a "lesion only" group (no graft), much fewer, 30% of 10 cells, had the slow EPSP (1.2±0.3 mV). In the "septal graft" group, there was a large increase in the number, 86% of 7 cells, that had the slow EPSP (2.0±2.0 mV). This increase was also seen in the "cerebellar graft" group in which 80% of 5 cells had the slow EPSP (2.7±1.0 mV).

Thus, grafts of both septal and cerebellar tissue produced an increase in slow EPSPs in the hippocampus of fornix lesioned rats. We conclude that the grafts had nonspecific enhancing effects, possibly on the sprouting and reinnervation by host cholinergic fibers.

Supported by the State of Ohio Department of Aging.

233.8

VIABILITY OF GABAERGIC STRIATAL NEURONS GRAFTED INTO NORMAL HIPPOCAMPUS. P.A. Schwartzkroin and D.D. Kunkel. Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

The transplantation of neuronal populations from fetal central nervous system to mature host brain has become a well accepted technique in neurobiology. In most studies, successful grafting is carried out against the background of a lesioned or damaged host CNS. However, it is not always clear that gross morphological changes underlie, or accompany, neurological deficits (e.g., in many models of epileptiform activity). We have been interested in the possibility of using transplanted inhibitory neurons (i.e., cells that contain GABA) to modulate CNS hyperexcitability, and have previously shown that GABAergic striatal cells can be successfully grafted into the hippocampus of rats treated with the epileptogenic agent, kainic acid. It is unclear, however, if graft maintenance in this environment is dependent on the effects of the kainate-induced lesion. We now report that striatal cells transplanted into normal hippocampus (with no lesion) also survive and maintain GABA expression (as seen with GABA and GAD immunocytochemistry). As was the case in kainate-lesioned animals, however, the cells of the striatal graft appear to cluster in a well-circumscribed area (often at the ventral border of hippocampus). GABAergic fibers and terminals from the graft also appear to be maintained within the confines of the graft, and do not extend significantly into host hippocampal regions. Studies are currently underway to assay the electrophysiological effects of graft stimulation on host excitability.

These studies are supported by a Klingenstein Foundation Fellowship (P.A.S.).

233.9

ALTERATIONS IN MUSCARINIC BINDING IN RAT HIPPOCAMPUS PROVIDES EVIDENCE FOR FUNCTIONAL REINNERVATION OF FIMBRIA-FORNIX LESIONS BY FETAL SEPTAL TRANSPLANTS. V.L. Dawson, F.H. Gage[1], and J.K. Wamsley. Depts. of Pharm. and Psych. Univ. of Utah Sch. of Med., Salt Lake City, UT. 84132, Dept. of Neuroscience, UCSD, La Jolla, CA. 92093

A unilateral fimbria-fornix lesion by micropunch was performed on male Sprague-Dawley rats, half of which received fetal septal transplants. Control rats had sham operations. After six months the animals were prepared for study. Acetylcholinesterase staining in the hippocampus of lesioned animals was absent, but staining was present in control levels in animals with transplants. Muscarinic receptors labeled with [^{3}H]-QNB showed up-regulation in areas CA2 and CA3 of stratum oriens and radiatum, and the dentate gyrus in animals with lesions. The receptor up-regulation was determined to be of the M1 subtype in stratum radiatum of areas CA2 and CA3 and area CA3 stratum oriens, and of the M2 subtype in stratum oriens of areas CA2 and CA3 through the use of [^{3}H]-pirenzepine (PZ) and [^{3}H]-QNB + 100nM PZ respectively. In transplant animals the density of muscarinic receptors was present at control levels. In lesioned animals the density of [^{3}H] hemicholinium-3 binding was below background levels. The binding in transplant animals was present at control values in the dentate gyrus molecular layer, the strata oriens and radiatum. These data indicate the transplants are providing a source of cholinergic activity.

233.10

RESTORATION OF EXTRAHIPPOCAMPAL PROJECTIONS BY HIPPOCAMPAL NEURONS TRANSPLANTED INTO THE ISCHEMICALLY LESIONED HIPPOCAMPUS. L.A. Mudrick & K.G. Baimbridge. Dept. of Physiology, University of British Columbia, Vancouver, B.C., V6T 1W5.

Immature neuronal tissue may have the capacity to repair acute lesions in the CA1 region of the *adult* rat hippocampal formation (HF). Electrophysiological analyses have shown that afferent synaptic contacts and efferent projections, within the HF, can be reinstated by the transplanted neurons. We have now examined the ability of the transplanted neurons to project neurites along the normal efferent pathway to the lateral septal nucleus (LSN): the chief extra-hippocampal target for HF pyramidal cells.

Severe forebrain ischemia was used to lesion the dorsal CA1 region of the HF. One week later this region was repopulated with suspensions of 18 day old fetal hippocampal tissue. After 6-14 months Fluoro-gold (FG) was pressure injected into the dorsomedial area of the LSN. Following an 8-10 day survival period the brain tissue was fixed and 30*um* cryostat sections were made. Retrogradely transported FG could be observed in transplanted neurons up to 4.0mm caudal to the injection site. The number of labelled cells decreased from 85% to <10% in a rostro-caudal direction. In animals where neurons had been transplanted too deeply, few FG positive neurons were observed.

Guidance for the neurite projections from the transplanted neurons to extrahippocampal targets may be provided by the release of neurite promoting factors along degenerating CA1 efferent pathways. The target specificity of these projections are now being investigated by electrophysiological and anterograde tracing techniques.(Supported by the B.C. Heart Foundation and an MRC Program Grant)

233.11

CONNECTION SPECIFICITY BETWEEN HOST HIPPOCAMPUS AND GRAFTED SEPTAL NEURONS. O.G. Nilsson*, D.J. Clarke*, P. Brundin* and A. Björklund (SPON: G. Doucet). Dept. of Medical Cell Research, Univ. of Lund, Biskopsg. 5, S-223 62 Lund, Sweden.

To understand the basis for the effects mediated by intracerebral neural grafts, it is important to have an idea about the specificity by which host areas are innervated by the graft and to what extent different neuronal elements in the graft and host can establish connections. The present experiments were performed in order to study (1) the specificity by which cholinergic grafts of different origins reinnervate the denervated hippocampus, and (2) the afferent and efferent connections made between a septal graft and the host hippocampus. Cholinergic rich tissue from the septum, nc basalis, striatum, brain stem and spinal cord was grafted into the cholinergically denervated hippocampus. Three to 4 months after transplantation the brains were analyzed using acetylcholinesterase (AChE) histochemistry and immunocytochemistry, and the septal grafted rats were in addition injected with neuronal tracers. There were great differences between the graft types in the degree and lamination of the AChE-positive reinnervation of the hippocampus, and in the ability to form synaptic contacts with host neurons. The septal grafts produced the most normal connections, whereas the grafts of brain stem and spinal cord origin were most abnormal. In the tracing experiment we combined retrograde tracing with immunocytochemistry and preliminary observations show that not only cholinergic cells innervate the hippocampus, but that also at least substance P-positive cells do. More results from the tracing experiments will be presented.

CYTOSKELETON AND AXONAL TRANSPORT II

234.1

CHARACTERIZATION OF A MICROTUBULE-ASSOCIATED COFACTOR INDEPENDENT PROTEIN KINASE. Clay W Scott*, Claudia B. Caputo* & Andre I. Salama. (SPON: R. Krell) Pharmacology Dept., ICI Pharmaceuticals Group, Wilmington, DE 19897.

We have identified a cofactor independent protein kinase which copurifies with microtubules (MT) through repetitive cycles of MT assembly/disassembly. The MT-associated protein kinase (MTAK) from pig brain was not stimulated by cyclic nucleotides, polyamines, Ca2+/calmodulin, or Ca2+/phospholipids. MTAK activity was unaffected by a synthetic peptide inhibitor of cAMP-dependent protein kinase (cADPK), suggesting that MTAK is not the active catalytic subunit of cADPK. MTAK phosphorylates various MT-associated proteins and can be inhibited with trifluoperazine (IC_{50} = 300 µM) in a Ca2+-independent fashion. MTAK was maximally active with 3.0 mM MgCl2, showed a broad pH maxima from 6.4 to 8.4 and had a Km for ATP of 18 µM. Histone IIIS was a suitable exogenous substrate for MTAK (Km = 0.3 mg/ml), while synapsin I, tubulin, neurofilaments, histone IIA and VIIIS, casein, phosvitin and glycogen synthase were not phosphate acceptors. MTAK did not phosphorylate synthetic peptides which are substrates for cADPK, cGMP-dependent protein kinase, smooth muscle myosin light chain kinase, or protein kinase C. Together, these data confirm that MTAK has a substrate specificity which is distinct from the known cofactor-dependent kinases and casein kinases. MTAK is a unique protein kinase which copurifies with microtubule structures.

234.2

AN UNIQUE EPITOPE MEDIATING THE ATTACHMENT OF ASSOCIATED MOTOR MOLECULES TO MICROTUBULES MAPS TO THE ß-SUBUNIT OF THE TUBULIN HETERODIMER. M.Goldsmith, L.A.Connolly, N.Kumar* and D.van der Kooy, Dept. of Anatomy, University of Toronto, Toronto (Ontario), Canada, M5S 1A8.

Microtubule based, ATP-dependent motility systems include the dynein ATPase of cilia and flagella and vesicle transporters in neurons. We asked if homologous stuctural elements underlie the motility in these different microtubule based systems. Previous experiments showed that the unique antitubulin sera, NS20, could inhibit axonal transport in vivo and in vitro. Furthermore, we have shown that NS20 inhibits motility in demembranated sea urchin sperm whereas a control antiserum against tubulin, PC13, does not. Western blot analysis with NS20 revealed only reactivity against tubulin in whole brain, twice cycled tubulin, and crude dynein (provided by Dr.I.Gibbons) preps. Moreover, radioimmunoassays confirmed that the intriguing functional effects of NS20 relative to PC13 did not result from differences in the overall antitubulin activity of the two sera. We now report that preincubation of NS20 with recombinant beta, but not alpha, tubulin (provided by Drs.K.Yarbrough and J.Wu) abrogates the inhibitory effect of the antiserum in the sperm motility assay. As this tubulin is expressed in E.Coli (and thus completely devoid of microtubule associated proteins), an IgG activity against an epitope of ß-tubulin must account for the biological effect of the NS20 antiserum.We propose that this unique epitope of ß-tubulin mediates the attachment of tubulin associated motors. Moreover, the results suggest a fundamental structural homology in the microtubule based mechanisms underlying sperm motility and axonal transport.

234.3

ROLE OF EXTRACELLULAR Ca^{++} IN NORMAL AND PARATHYROID HORMONE STIMULATED FAST AXONAL TRANSPORT. A.C. Breuer, M.B. Atkinson* and C.M. Ferrario. Depts. of Neurology and Brain and Vascular Research, Cleveland Clinic Foundation, Cleveland, OH 44195

Parathyroid Hormone (PTH - rat, synthetic fragment 1-34) has previously been demonstrated to increase the rate of fast axonal transport (FAT) both in the anterograde (ANTERO) and retrograde (RETRO) directions (Breuer, A.C. & Atkinson, M.A., Soc. for Neuroscience Abs, 13 (Part 1):123, 1987). The intracellular mediator of this effect is of great interest as a potential regulator of axonal transport speeds. Ca^{++} has been implicated in the PTH response (Breuer, A.C. & Atkinson, M.A., Ann Neurol, 1988, in press) and the current study further explores the validity of this hypothesis.

Axonal preparations from male, Spraque-Dawley rats were treated with PTH in normal (with Ca^{++}) or Ca^{++} free buffer (+ 1 mM EGTA) and video-recorded under DIC optics for later analysis by computer methods. ANTERO and RETRO FAT speeds were decreased by 31.1 ± 2.5% ($p < 0.001$) and 14.3 ± 1.6% ($p < 0.001$) respectively in Ca^{++} free buffer (controls in normal buffer). PTH in normal buffer increased ANTERO FAT by 13.0 ± 2.5% ($p < 0.05$) and RETRO FAT by 18.0 ± 1.1% ($p < 0.001$) (controls in normal buffer). PTH in Ca^{++} free buffer failed to increase either ANTERO or RETRO FAT (controls in Ca^{++} free buffer). These results substantiate the importance of Ca^{++} as an intracellular mediator of transport speed alterations and its potential role in regulating fast axonal transport speeds in mammalian axons.

234.4

IDENTIFICATION OF A PHOSPHORYLATION SITE IN THE 68 KD NEUROFILAMENT POLYPEPTIDE L. Zuo-Shang Xu* and Mark Willard, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

Each of the three polypeptides, H, M, and L that compose neurofilaments, is phosphorylated at multiple sites. The average phosphate content of the L polypeptide from rat has been estimated to be 3 moles per mole of L (Julien, J.-P., & Mushynski, W. E., J. Biol. Chem. 257:1046, 1982). We have identified one site on the L polypeptide that is phosphorylated in vitro by an endogenous protein kinase activity. Rat spinal cord was extracted with Triton X-100, and the insoluble material was incubated with 32P-ATP to label substrates of endogenous protein kinases. L was separated from other proteins by HPLC, first on a DEAE matrix, and then on a reverse phase C-4 matrix. The purified L was digested sequentially with trypsin and S. aureus V8 protease. The resulting peptides were separated by HPLC on a reverse phase C-18 matrix. One peak contained approximately 40% of the total eluted radioactivity - four times more than the next largest radioactive peak. This peak contained two peptides of overlapping sequences: AKDEPPSEGE and DEPPSEGE. We conclude that the serine in these peptides, which is located sixty seven amino acids from the carboxy terminal end of the reported mouse L sequence (Julien, J.-P. et al., Mol. Brain Res. 1:243, 1986), is the major phosphorylation site for the endogenous kinase activity in this preparation.

234.5

A CELL CULTURE MODEL FOR VIRUS-HOST CELL TARGETING AND FUSION SJ Morris(1,2)*, DP Sarkar(2)*, JM White(3)*, JJ Zimmerberg(4)*, O Eidelman(2)* and R Blumenthal(2)* (SPON: BM Chronwall). (1)Div. of Mol. Biol. and Biochem., School of Basic Life Sciences, Univ. of Missouri at Kansas City, MO. 64110; (2)Sect. on Membrane Structure and Function, LTB, NCI and (3)Sect on Biol. Physics, LBM, NIDDK, NIH, Bethesda, MD, 20892; (4)Dept of Pharm. and Cell Biol. Program, Univ. of California San Francisco, CA, 94143-0450.

Most envelope viruses invade by attachment to cell surface receptors followed by fusion to the host membrane, either at neutral pH (eg, Sendai virus), or at low pH in an endocytic vesicle (eg, influenza virus). Fusion is catalysed by viral spike glycoproteins, the hemagglutinin (HA) of influenza being the best-characterized.

3T3 fibroblasts were transfected to express HA on their extraplasmic cell surface. Human red blood cells (RBC) were labelled either with a fluorescent rhodamine membrane probe, a soluble fluorescent NBD probe, or both. After attachment of the RBC to the 3T3, fusion was monitored by spectrofluorometry as dequenching of the probe(s). Upon lowering the pH below 5.4, the fluorescence increased after a delay of about 30 s at 37°C, and leveled off within 2 min. In control experiments where RBCs bound to 3T3 cells expressing the uncleaved precursor hemagglutinin (HAo) were incubated at 37°C and low pH, no fluorescence increase was observed, indicating that dequenching occurred as a result of HA-induced fusion of plasma membranes. Fusion showed a very steep pH-dependence with a threshold at pH 5.4, similar to that for HA-induced fusion seen previously. The fusion rate increased and the delay for the onset of fusion decreased as the temperature was raised above 20 °C. The delay had a breakpoint at about 27 °C. Low pH-activation of the fusion process at 37°C could be partially arrested by raising the pH after 2-10 s, but not after 15 s, indicating that the irreversible pH-activated conformational change of HA necessary for fusion was complete within about 15 s. Hill analysis of the data indicates that the pH-induced membrane fusion activity of HA is a highly cooperative event, similar to calcium dependence of neurotransmitter exocytosis.

Fusion also could be seen by fluorescence microscopy. Image-enhanced fluroescence videomicroscopy has allowed time resolution of single fusion events, which are amenable to stochastic modelling. A video tape demonstration of the technique will be presented.

234.6

NEUROFILAMENT SPACING AND CROSS-LINKING INTERACTIONS. G. Shaw*, T. McCabe, H. Zhao-Chen and J. Harris. (SPON. G. Freund). Department of Neuroscience, JHMHC Box J-244, Gainesville, Florida, 32610.

We have recently developed a simple method for the extraction of neurofilamentous cytoskeletons from mammalian peripheral nerve and spinal cord. Our experiments show that this material forms well defined bundles, each bundle apparently consisting of the neurofilamentous complement of one axon. This finding also points to the existence of a physiologically stable neurofilament bundle forming interaction. We have examined the stability of these bundles to a variety of agents, such as extremes of pH and salt concentration. Bundles of neurofilaments are extremely resistant to these treatments. We could find no evidence for a breakdown of neurofilaments to single filaments prior to solubilization of the neurofilament bundles. We conclude that the interaction which holds neurofilaments into bundles, possibly localized in the neurofilament "tail" domains, is an extremely strong one, and seems to be comparable in strength to the interaction that holds individual neurofilament subunits into neurofilaments. Enzymatic dephosphorylation does not result in breakdown of the neurofilament bundles. The spacing of filaments in bundles can be altered by changes in salt concentration in a manner suggesting that filament spacing is the result of an electrostatic repulsion phenomena. We are currently looking at fusion proteins coded for by cDNA sequences specifying neurofilament tail sequences to try to localize the regions responsible for neurofilament spacing and cross-linking.

234.7

VIRAL COAT AND LIPOSOMAL INFUSION AND BINDING TO PRIMARY NEURONAL CULTURES AND SYNAPTOSOMES FROM RAT BRAIN. E.M. Meyer, S. MacKay*, D.H. Otero*, J.H. Judkins*, D. Bottiglieri*, and M.-T.T. Nguyen*. Department of Pharmacology, U.Florida, Gainesville, 32610.

The introduction or infusion of large or charged macromolecules into neurons, with or without selectivity, remains one of the obstacles in studying intracellular processes and modulating neurotransmission presynaptically. We therefore began two lines of study to develop this capability in vitro, each with the potential to introduce substances selectively into selected neuron-types.

The first involved liposomes consisting of 95% dipalmitoylphosphatidylcholine and 5% phosphatidylethanolamine. Although these liposomes were found to infuse calcium ions into cholinergic synaptosomes at high concentrations and to encapsulate significant amounts of a large enzyme, acetylcholinesterase, we were only able to demonstrate modest infusion of this enzyme into nerve terminals or neuronal cultures. Attachment of an antibody against the NGF receptor (generously provided by Dr. Eugene Johnson) increased binding to synaptosomes at low liposome concentrations but did not appear to increase infusion of the enzyme into cholinergic terminals.

We therefore recently turned to Sendai viral coats, which are devoid of indigenous nucleotides and which we find to be very potent with respect to infusing substances into cholinergic, GABAergic, and noradrenergic terminals.

234.8

ARREST OF ORGANELLE TRANSPORT IN AXONS REGENERATING IN VITRO BY SIALIC ACID-SPECIFIC LECTINS IS REVERSED BY CYTOCHALASIN D. B. Edmonds* and E. Koenig. Dept. of Physiology, State Univ. of New York, Buffalo, NY 14214.

Goldfish retinal ganglion cell (RGC) axons regenerating in vitro contain several classes of organelles and aggregate structures (e.g., varicosities) that undergo translocation, as viewed by videomicroscopy. Lectins, which bind sialic acid residues, partially or completely inhibit transport. Wheat germ agglutinin (WGA; 300 ug/ul), not specific of sialic acid, maximally reduces organelle transport frequency by 60-70%, while Limax Flavus agglutinin (LFA; 100 ug/ul), specific for sialic acid, arrests transport completely. Sialic acid included with the lectin blocks lectin-induced arrest or it reverses it after it is induced. Focal application of LFA to a single varicosity blocks all transport reversibly. Pretreatment with cytochalasin D (CD; 10 uM) blocks LFA-induced arrest of transport. CD also reverses the arrest after it is induced. FITC-phalloidin labels F-actin in the cortex of varicosities and in intervening segments after LFA binding, and CD pretreatment prevents the labeling. The results indicate that the arrest of transport caused by an apparent cross-linking of an axonal surface sialoglycoconjugate may involve a polymerization and/or reorganization of axoplasmic actin. Supported by NIH grant NS21843.

234.9

MOLECULAR CLONING OF RAT OLFACTORY NEURON-SPECIFIC cDNAs BY DIFFERENTIAL SCREENING. E. Barbosa*, B. Paven§*, and R.R. Reed§* (SPON: D.L. Price). Neuropathology Lab. and §Howard Hughes Med. Lab. of Genetics, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182.

Unlike other neuronal tissue, the vertebrate olfactory neuroepithelium retains the capacity to form functional neurons from residing stem cells throughout adult life. Pulse labeling of animals with [^{3}H] thymidine following unilateral bulbectomy led to uptake in stem cells with their subsequent division, migration, and differentiation into typical bipolar neurons. In an effort to identify and characterize genes that are neuron specific and developmentally regulated, a normal rat olfactory cDNA library was prepared in λgt-10. Replicate plaque lifts of this cDNA library were probed with [^{32}P] cDNA prepared from polyA+ RNA from either untreated animals or animals eight days following bulbectomy (time of maximal neuronal cell loss). In this manner, we have identified several olfactory neuron-specific cDNAs as well as a cDNA common to both olfactory and brain neurons. We will describe the characterization of a neuron-specific cDNA that is regulated developmentally and present both in olfactory and brain neurons. Finally, by differential screening, libraries made at times of division, migration, and differentiation may provide a source in which to identify cDNAs that are essential to processes of neuronal differentiation in mammals.

234.10

EVIDENCE THAT PROTEINS OF THE SYNAPTIC MEMBRANE ARE SYNTHESIZED LOCALLY WITHIN DENDRITES: STUDIES OF SYNAPTOSOMAL PROTEIN SYNTHESIS. A. Rao*, E. R. Torre* and O. Steward, Dept of Neuroscience, Univ. of VA., Charlottesville, VA., 22908.

Recent studies have revealed that protein synthetic machinery (polyribosomes and associated membranous organelles) is selectively localized beneath postsynaptic sites on CNS neurons. This machinery is particularly prominent during periods of synaptogenesis, suggesting that it may synthesize constituents of the postsynaptic membrane specialization. The present study evaluated this hypothesis by determining whether proteins synthesized in synaptosome preparations (containing pre and postsynaptic components and contaminant mitochondria) are synaptic plasma membrane proteins.

Synaptosomes were isolated from developing (10-20d) rat forebrain. Comparision of protein synthesis in the presence of cycloheximide (CHX), chloramphenicol (CAP) or RNase confirmed previous studies showing that approximately 70-80% of the synthetic activity was due to a eucaryotic membrane-sequestered component. Labelled synaptosomes were fractionated into total membrane, synaptic plasma membrane (SPM) and synaptic junctional complex (SJC) preparations and subjected to SDS-PAGE and fluorography. In total membrane preparations, 12 labelled bands were apparent, 4 of which were faintly labelled. Labelling of 8 of these bands was CHX-sensitive and CAP-insensitive. In SPM fractions 10 labelled bands remained and two minor CAP-sensitive bands were no longer apparent. In SJC preparations only 9 of these bands were seen, among which the single remaining CAP-sensitive protein was very faintly labelled. These results are consistent with the hypothesis that certain proteins of the synaptic junction are locally synthesized. Supported by NIH NS12333.

234.11

LOCAL PROTEIN SYNTHESIS WITHIN DENDRITES OF CULTURED HIPPOCAMPAL NEURONS. L. Davis, G.A. Banker[1], and O. Steward. Dept. of Neuroscience and the Neuroscience Program, Univ. of VA, Charlottesville, VA 22908. [1]Dept. of Anatomy, Albany Medical College, Albany, NY, 12208.

Previous studies have demonstrated that polyribosomes, consisting of mRNA and ribosomes, are selectively localized in the cytoplasm beneath postsynaptic sites on CNS neurons (Steward and Levy, (1982) J. Neurosci. 2; 284-291). To determine whether dendritic polyribosomes are translation-competent we pulse-labeled low density cultures of hippocampal neurons with ^{3}H-leucine (125uCi/ml) for 10, 30, 60, or 90 minutes prior to fixation and processing for autoradiography. Radiolabeling at the 10 minute interval will reveal the extent of protein synthesis at particular sites, since 10 minutes is too short a time for there to be significant transport of recently synthesized protein from the site of synthesis. The pattern of labeling at longer intervals will reveal the transport of newly synthesized proteins.

Cultures prepared after a 10 minute pulse exhibited heavy labeling over cell bodies, and substantial labeling of dendrites. Axons were unlabeled. There was no labeling when the cultures were pulse-labeled with ^{3}H-leucine in the presence of puromycin (10^{-5}M), a protein synthesis inhibitor. This indicates that the labeling reflects protein synthesis and not uptake and/or binding of the precursor. At later time points dendritic labeling increased and radiolabel first appeared over axons extending several hundred micrometers from the cell body. This suggests that recently synthesized proteins are transported into axons only after a delay. Taken together, these results indicate that dendrites, like somata, contain functional protein synthetic machinery. Supported by NIH NS23094 to GB and OS. LD was the recipient of a predoctoral fellowship from NIH NS07199.

234.12

MICROTUBULE POLARITY IS UNIFORM IN THE AXONS BUT MIXED IN THE DENDRITES OF HIPPOCAMPAL NEURONS IN VITRO. J.S. Deitch, P.W. Baas*, M.M. Black*, and G.A. Banker. Dept. of Anatomy, Albany Med. Coll., Albany, NY, 12208 and Dept. of Anatomy, Temple Univ. Sch. of Med., Philadelphia, PA 19140.

We have compared the polarity orientation of microtubules in the axons and dendrites of rat hippocampal neurons growing in vitro. Microtubules are polar structures—subunits are preferentially added to the [+] end and lost from the [-] end. In many non-neuronal cell types the [-] end is stabilized by association with a microtubule organizing center (MTOC). Microtubule polarity was assayed by determining the orientation of hooks formed on the microtubules by polymerization of exogenous tubulin.

Axonal microtubules were uniformly oriented, with their [+] ends pointing toward the tip (95 ± 8%; $\bar{x}$ ± s.d.), as reported for axons of other neurons. In the dendrites, however, nearly half of the microtubules (43 ± 6%) were oriented with [+] ends pointing toward the soma. Microtubules of opposite polarities were interspersed within the dendrites. At the tip of the dendrite the microtubules were uniform in polarity, their [+] ends pointing distally. No MTOC was identified for microtubules of either polarity.

These results show a fundamental difference between axons and dendrites in the organization of their microtubules; this difference may form the basis for differential sorting and transport in axons and dendrites.

Supported by NIH grants NS 17681 & NS 23580 to MMB, and NS 23094 & NS 17112 and NSF grant BNS 8607114 to GAB.

234.13

ISOLATION AND CHARACTERIZATION OF FAST AXONALLY TRANSPORTED PROTEINS: AN IMMUNOCHEMICAL APPROACH. R.L. Rotundo, D.L. Wilson and G.W. Perry. University of Miami School of Medicine, Miami, Florida.

Due to their low abundance in nerves, fast axonally transported proteins (FTPs) remain a difficult family of proteins to isolate, identify and characterize. Consequently, the functions of the vast majority of FTPs, as well as the molecular mechanisms underlying their sorting and localization remain unknown. Acetylcholinesterase (AChE), one of the most thoroughly studied FTPs, is packaged together with other FTPs into vesicles for transport along the axon. We have now developed a method based on the AChE density-shift technique of Porter-Jordan et al. (J. Neurosci. 6:3112, 1986) to prepare enriched fractions of FTP-containing vesicles from frog sciatic nerve. Monoclonal antibodies generated against this fraction recognize two general classes of antigens. The first, designated ECM antibodies, recognize antigens associated with the Schwann cell extracellular matrix and/ or nerve sheaths. The second class of antibodies recognizes antigens localized within the axoplasm or on the axonal plasma membrane. These antigens can be metabolically labelled with 35S-methionine and are rapidly transported along the axons of organ-cultured dorsal root ganglia. Eight monoclonal antibodies recognizing ECM and FTP antigens were chosen for initial characterization. Immunofluorescence localization studies show that all eight antibodies recognize epitopes surrounding or associated with motor and sensory fibres in the sciatic nerve. In addition, a few of the antibodies also recognize epitopes in the frogs optic nerve. The presence of the FTP antigens along both branches of the dorsal root ganglia processes shows they are not transported specifically in either branch.

Supported by grants from the NIH to RLR, DLW and GWP, and The National Parkinson Foundation to GWP.

234.14

ELECTRICAL STIMULATION OF DORSAL ROOT GANGLIA INHIBITS FAST AXONAL TRANSPORT. R. Hammerschlag, J. Bobinski*, D. McKenna* and D. McAfee. Div. of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte CA 91010 and Dept. of Pharmacology, Nelson Res. Ctr., Univ. California, Irvine CA 92715.

Fast axonal transport of newly-synthesized protein was examined *in vitro* using bullfrog dorsal root ganglia (DRG) with roots and nerves attached. This preparation was placed in a compartmentalized chamber permitting selective pulse-labeling of DRG ([3H]leucine, 1 h) and subsequent washing with medium containing the protein synthesis inhibitor, cycloheximide. The spinal nerve or DRG was then supramaximally stimulated (0.1-10Hz) through suction electrodes for 1-4 h. The contralateral nerve or ganglion served as a sham stimulated control. Stimulation produced a frequency dependent decrease in the amount of acid-insoluble radioactivity detected after 16 h at a nerve ligature 20 mm from the DRG. Stimulation of the nerve inhibited transport by 23% (10Hz/4h), while stimulation of the DRG inhibited transport by 62% (10Hz/1h) or 38% (1Hz/1h). The effects of stimulation were inhibited by TTX and were not mimicked by incubation of DRG in 50 mM K+. Stimulation reduced the amount but not the rate of transported protein in non-ligated nerves. We hypothesize that afferent stimulation may alter the amount of protein that is "loaded" onto the axonal transport system. Supported by BNS 8417380 and NS 22470.

234.15

BETA-TUBULIN AND SEVERAL INTERMEDIATE FILAMENT PROTEINS SHARE A COMMON EPITOPE IDENTIFIED BY MONOCLONAL ANTIBODY G6. C.E.Miller and V.M.Ingram. Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Microtubules and intermediate filaments are two of three major components of the neuronal cytoskeleton.

Monoclonal antibodies G6 and G2 were raised against a growth cone preparation from embryonic rat brain. They recognize several proteins in a Triton-X100-insoluble rat brain extract. These include the smallest neurofilament protein, NF68, a 66kD protein, vimentin and GFAP. This cross-reactivity may reflect recognition of an epitope within the α-helical rod region conserved among these intermediate filament proteins. Interestingly, neurofilament proteins NF150 and NF200, which also contain the rod region, are not recognized by either antibody. G6 also recognizes a soluble 53kD protein, beta-tubulin. G6 recognizes four mouse beta-tubulin isotypes expressed from cDNA clones(JCB 101:852,1985) in E. coli. Immunocytochemical staining patterns are consistent with immunoblot results.

G6 and G2 recognize NF68 protein expressed from a cDNA which lacks only the first 22 amino acids of NF68(Mol.Cell Biol.6:1529,1986). We are precisely mapping the epitopes for G6 and G2 on NF68 subclones generated by Exonuclease III deletion mutagenesis. Positively-reacting subclones will define the minimum epitope. This knowledge will help to define functional sites and aid the investigation of intermediate filament and microtubule functions.

234.16

TUBULIN REMAINING BEHIND THE PEAK OF SLOW AXONAL TRANSPORT IS PREFERENTIALLY ASSOCIATED WITH STABLE MICROTUBULES. D.F. Watson, P.N. Hoffman and J.W. Griffin. Department of Neurology, Johns Hopkins Hospital, Baltimore, MD 21205

We have investigated the relationship between cold-stable microtubules (MT) and the pool of axonally transported tubulin. Rat L5 dorsal root ganglia were labeled with ^{35}S methionine, and segments of sciatic nerve 20-30 mm from the ganglion were analyzed at times indicated below. Nerves were fractionated into soluble tubulin, cold-labile MT, and stable MT fractions. The radioactivity in tubulin bands from SDS gels of each fraction was determined and expressed as a percentage of the total tubulin recovered:

Age at labeling	Transport interval	N	Labile MT % (SEM)	Stable MT % (SEM)
3 weeks	7 days	8	36.3 (2.7)	22.2 (1.7)
3 weeks	20 days	7	30.2 (2.1)	47.0 (2.3)
3 weeks	40 days	8	3.9 (1.4)	55.1 (2.9)
7 weeks	10 days	8	9.2 (1.3)	43.0 (4.1)

The "tail" of labeled tubulin remaining after the peak of transport had passed (3 wk\40 day group) was more highly associated with stable MT than the peak in animals which were the same age at labeling (3wk\7day) or the same age at sacrifice (7wk\10day). These results suggest that some tubulin in maturing axons becomes associated with a pool of stable microtubules which is stationary or very slowly transported.

234.17

Distribution of Microtubules in the Soma of NGF Activated PC12 Cells: The Nuclear Microtubular Basket. J.K. Stevens, J. Trogadis*. Playfair Unit, Toronto Western Hospital, and University of Toronto, Toronto, Ontario, M5T2S8, Canada.

PC12 cells have become a widely adopted model of the developing neuron. The undifferentiated cells are grown in tissue culture, and, when nerve growth factor (NGF) is added, produce neurites within a few days. We have serially reconstructed the soma at the EM level in a total of seven NGF activated PC12 cells. We have carried out complete somal reconstructions as well as sample MT density at selected regions within the soma. MT density is given as the total length of MT's contained within a cubic micron box of cytoplasm.

Unexpectedly, we find that the nucleus is surrounded by a "basket" of long, dense microtubules (20 MT microns/cubic somal micron). Both serial EM and immunofluorescence show that the centrioles are always found adjacent to the nucleus in pairs and are surrounded by many randomly oriented, short segment MT's (2.5 MT microns/cubic somal micron). It should be emphasized that these short centriolar MT's are only a few microns away from the longer MT's surrounding the nucleus. Any random sample from the somal cytoplasm reveals short segment MT's of varying density similar to those adjacent to the centrioles. Finally, the MT's, found in the initial segment and neurites, attached to the same cell, are longer and packed at a high density (47 MT microns/cubic somal micron).

These data suggest that NGF activated PC12 cells may contain many sites where MT's can be either *polymerized* or *annealed*. Specifically, it appears that both the nuclear surface and the neurite itself play a special role. In contrast, the region around the centriole appears to be no different than any random somal sample. It is interesting to speculate that the dense basket of microtubules around the nucleus might be the "motor" responsible for nuclear rotation often reported in these and other cells. This work supported by MRC, Canada.

NUTRITIONAL AND PERINATAL FACTORS IN DEVELOPMENT

235.1

LIMB DEFECTS IN GRAY OPOSSUMS FOLLOWING POSTNATAL INJECTION WITH ETHANOL OR SALINE. B. Fadem-Chenal*, R. A. Tassava* and S.I. Miller*, (SPON: A. Beyer-Mears) Dept. Psychiat., UMDNJ-New Jersey Medical School, Newark and Dept. Anat., Ohio State Univ., Columbus.

Limb abnormalities have been described in mammalian fetuses following maternal exposure to alcohol. Newborn marsupials are useful for studying the teratogenic effects of alcohol on limb development since their hindlimbs at birth are in an embryonic "paddle" stage of development.

In this study, 62 newborn gray opossums were injected with 10 μl 25% ethanol (ETOH)/physiologic saline (SAL) (v/v) (n=29) or 10 μl SAL only (n=33) in the right or left hindquarter on days 2 and 4 of postnatal life. Forty-four percent (n=11) of surviving animals in the ETOH group and 16% (n=4) of animals in the SAL group had limb defects in the limb associated with the hindquarter that was injected. Impairment of gait, reduced limb size and clubbing of the foot were seen in the SAL group. In addition these defects, absent digits, fused digits and absent limbs were seen in animals in the ETOH group.

It is likely that damage to the developing sciatic nerve by injection or introduction of fluid was involved in these defects since caudal neural lesions in neonatal gray opossums produce defects similar to those seen here. Presence of significant digital defects in ETOH but not SAL animals indicates that alcohol itself may have a further teratogenic effect on limb development.

235.2

SEROTONIN AND 5-HYDROXYINDOLEACETIC ACID ARE DECREASED IN THE MOTOR AND SOMATOSENSORY CORTEX IN RATS EXPOSED TO ETHANOL IN UTERO. M.J. Druse-Manteuffel and N. Tajuddin*. Department of Biochemistry, Loyola U. Stritch School of Medicine, Maywood, IL 60153.

This laboratory previously demonstrated a cortical deficiency of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), decreased 5-HT uptake in the motor cortex, and decreased 5-HT_1 sites in the motor and somatosensory cortices of the developing offspring of rats, fed a 6.6% (v/v) ethanol liquid diet on a chronic basis prior to parturition. This study extends previous work by assessing the concentrations of 5-HT and 5-HIAA in the motor and somatosensory regions of the cerebral cortex. Samples were separated by HPLC, using electrochemical detection.

The levels of both 5-HT and 5-HIAA were consistently lower in the motor and somatosensory regions of the cerebral cortices from the 19- and 35-day-old offspring of control and ethanol-fed rats. There was a significant ($P < 0.05$) 50% decrease in the concentration of 5-HT in the motor cortices of 19- and 35-day-old ethanol-exposed rats. In addition, the concentration of 5-HIAA was decreased by 30-50% in this region. Within the somatosensory cortex, there was a 20-40% deficiency of 5-HT and 5-HIAA in the 19- and 35-day-old offspring of ethanol-fed rats. These results emphasize the sensitivity of the developing serotonergic system to ethanol.

This work was supported by the USPHS - AA0390.

235.3

FETAL EXPOSURE TO ALCOHOL ALTERS POSTNATAL DEVELOPMENT IN RATS: AN EXAMINATION OF NEUROBIOCHEMICAL PARAMETERS. F. Chiappelli, A.N. Taylor, A. de los M. Espinosa*, J. de Vellis and H. Weiner. Depts. of Microbiology & Immunology, Anatomy, and Psychiatry, UCLA, and West Los Angeles VA Medical Center, Brentwood Div., Los Angeles, CA 90024.

Fetal exposure to alcohol is associated with neurobehavioral dysfunction both in animal models and in humans. Sprague-Dawley rats subjected to 5% ethanol (EtOH) during the last 15 days of gestation give birth to pups who exhibit alterations in the ontogeny of the brain-pituitary-adrenal axis (Taylor et al, 1986). Using the same animal model, we present data outlining the early ontogeny of neurobiochemical parameters in vitro. By initiating mixed whole brain neonatal cultures, maintaining them under standard culture conditions, and fixing and immunostaining them at given time points for up to 28 days approximate brain age, we found that prenatal EtOH exposure retards the appearance of selected markers. The expression of specific oligodendrocytic markers, such as glycerol phosphate dehydrogenase, cyclic nucleotide phosphohydrolase, and to a major extent myelin-basic protein and transferrin, is delayed in prenatal EtOH pups compared to controls. These data suggest that fetal exposure to alcohol may lead to profound alterations in the process of myelinogenesis. Astrocytic (e.g., glutamine synthase, glial fibrillary acidic protein) and neuronal (e.g., neuro-specific enolase, neurofilament) markers seem less severely affected by prenatal alcohol exposure.

235.4

PRENATAL ETHANOL EXPOSURE AFFECTS METHIONINE-ENKEPHALIN (m-ENK) FIBERS IN THE MEDIAL PREOPTIC AREA (MPOA) OF RATS. D.E. ADAM, C.A. FOX, J. HANNIGAN, E.P. RILEY AND C.D. JACOBSON. Dept. Veterinary Anatomy, Iowa State University, Ames, IA. 50011 and Center of Behavioral Teratology, SUNY, Albany, NY 12222.

Prenatal ethanol exposure alters the adult morphology of the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA) in male rats. In this study, we examined the effects of in utero alcohol exposure on the immunohistochemical localization of m-ENK in the MPOA. Adult offspring of dams fed liquid diets containing either 35% or 0% ethanol derived calories (EDC) on gestation days 6-20 were used. Offspring of dams given lab chow ad libitum served as another control group. m-ENK staining was obtained using the avidin-biotin-nickel-enhanced immunocytochemical technique. The m-ENK-like immunoreactive fiber plexus in the MPOA was markedly reduced in 35% EDC males and somewhat reduced in 35% EDC females as compared to both control groups. There also appeared to be more immunoreactive fibers in the female MPOA, including the area encompassing the SDN-POA, than in the male, regardless of prenatal treatment condition. In addition, the sexually dimorphic m-ENKergic periventricular band located in the MPOA was not altered by prenatal alcohol exposure. Further studies are in progress in order to investigate effects of prenatal ethanol exposure on sexual differentiation of the brain.

235.5

Prenatal alcohol ingestion and brain development:neurochemical and morphologic observations. M.C. Yu, Z.Y. Zang*, Z. Cherf and C.I. Giambelluo. Dept. of Anatomy, N.J. Med. Sch., Newark, NJ 07103 and Rhode Island Psychiat. Res. and Training Center, RI 02920 (CTG).

The purpose of this study was to make biochemical determination of brain neurotransmitter metabolism, and to observe morphological changes of rat pups exposed to alcohol in utero during gestation. Female Sprague-Dawley rats were fed an isocalonic liquid ethanol diet of 6.6% (vol/vol) for two weeks prior to mating. These dams, rendered pregnant on the first day (positive vaginal smear) were maintained on the diet for the duration of gestation, and after birth of the pups. A control group of dams was fed a regular rat chow diet. At postnatal days 7, 17 and 30, both groups of pups were sacrificed. Tissues from the corpus striatum and hippocampus were dissected out and frozen at -80°C until use. Tissues were homogenized in 10 volumes of 0.1N perchloric acid containing 1nq/ul DHBA as internal standard, centrifuges and filtered. The supernatent was used for quantitative assay by high pressure liquid chromatography while the lower solid phase was used for protein analysis.

In the corpus striatum, the dopamine concentration of the alcohol pups were significantly greater that the control group at 7 and 16 postnatal days, but was slightly more by 30 days. The concentrations of the two metabolites, DOPAC and HVA, however, were significantly higher in the control than the alcoholic group at the corresponding periods. In the hippocampus, the concentration of serotonin was similar at day 7, but greater in the alcoholic pups at postnatal days 17 and 30. The levels of 5-hydroxyndoleacetic acid were consistently higher in the control than the alcoholic pups. Preliminary electron microscopic observation of the caudate nucleus revealed the presence of edema, a less developed complement of organelles in the neurons of alcoholic pups at 7-day of age. By days 17 and 30, there was glycogen accumulations in astrocytes but no other changes.

235.6

INTERACTIVE EFFECTS ON BRAIN DEVELOPMENT IN MICE OF FETAL ETHANOL EXPOSURE AND DIETARY ESSENTIAL FATTY ACIDS. P.E.Wainwright,Y.S.Yuang*,D.E.Mills*,G.R.Ward*,R.P.Ward* and D.McCutcheon*.Dept. of Health Studies, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada.

Polyunsaturated fatty acids (PUFA),particularly arachidonic acid(AA,20:4 n-6)and docosahexaenoic acid(DHA,22:6 n-3)accrue in the developing brain.Ethanol affects fatty acid metabolism by reducing the conversion to longer chain derivatives.Thus ethanol may affect brain development by altering PUFA availability.This study examined whether dietary supplementation with PUFA's would ameliorate the effects of ethanol on brain and behavioral development.Pregnant mice were fed a liquid diet with 20% (Cal.)ethanol and 20% either safflower oil (S) or a combination of S with a fish oil concentrate (F,containing preformed,long chain n-3 PUFA).Pair-fed controls has maltose-dextrin substituted isocalorically for ethanol and a group fed chow ad-libitum served as a nutritional control.Treatment ceased prior to parturition.Blood alcohol concentrations did not differ between the groups.Two days after birth there was an effect of nutrition only on pup body and brain weight.Ten days later there was a significant ethanol by EFA interaction on brain weight,with the F animals receiving ethanol having heavier brains than controls,whereas the reverse was true in the S group.Ethanol retarded behavioral development,but PUFA supplementation had no effect.Fatty acid profiles of the brain membrane phospholipids will also be presented.

235.7

BEHAVIORAL AND NEUROANATOMICAL RECOVERY AFTER PRENATAL ETHANOL EXPOSURE IN THE RAT. N.J. Lobaugh, T. Wigal, P. Greene, J. Diaz-Granados, and A. Amsel. Department of Psychology, University of Texas, Austin, TX 78712.

We have recently demonstrated that prenatal ethanol exposure eliminated the Partial Reinforcement Extinction Effect (PREE) in 15-day-old rats (Wigal et al., 1988). This absence was due to equally rapid extinction after both partial (PRF) and continuous (CRF) reinforcement training. The present experiment demonstrates that, in animals prenatally exposed to ethanol, persistent responding after PRF training recovers with age. Three prenatal treatments, ETOH, PAIR-FED, and LAB CHOW, were combined factorially with PRF or CRF training at 21 days or 6 months of age. The results at 21 days replicated those of Wigal et al. for 15-day-olds: The ETOH-PRF and ETOH-CRF animals extinguished at the same rate, both faster than PRF controls. ETOH-CRF animals did not differ from CRF controls. However, when tested as adults, the ETOH-exposed animals showed complete recovery of function: a strong PREE, with no deficit relative to controls.

Analysis of hippocampal CA1 pyramidal cell number and density was made in horizontal, midtemporal, 3μ sections. We found that at 21 days, the prenatal ETOH treatment resulted in 18% fewer CA1 cells, and significantly reduced cell density. However, the adults exposed to ethanol prenatally did not differ from controls in either measure.

235.8

EFFECTS OF PRENATAL EXPOSURE TO ETHANOL ON HEART AND HIPPOCAMPAL MUSCARINIC AND BETA-ADRENERGIC RECEPTORS IN INFANT RATS. S.B. Wigal, R.E. Wilcox and A. Amsel. University of Texas, Austin, TX 78712.

The present work assessed the development of myocardial and hippocampal muscarinic and beta-adrenergic receptors in 8- and 17-day-old rat pups following prenatal exposure to ethanol.

Multiparous rats were intubated with either 6 g/kg ethanol or isocaloric dextrose twice daily from gestational Days 10 to 16. At birth, offspring were fostered to untreated mothers. Hearts were removed and the hippocampus from the right half of each brain was dissected from 8- and 17-day-old animals. Two concentrations of each ligand were used: [^{3}H]-quiuclidinyl benzilate (QNB): 300 and 1000 pM ± 300 nM atropine; and [^{125}I] -pindolol (IPIN): 300 and 600 pM ± 1 μM propranolol.

Prenatal alcohol exposure did not affect either beta-adrenergic or muscarinic binding in the heart. In the hippocampus, an ontogenetic increase in beta-adrenergic and muscarinic binding was apparent from 8 to 17 days of age. In addition, 17-day-old animals exposed to ethanol in utero showed a smaller increase in beta-adrenergic binding than the same-aged control animals. However, analyses of total protein yielded no significant differences between offspring of ethanol- and control-intubated mothers. Results suggest an ethanol-associated attenuation of beta-adrenergic receptor development.

235.9

THE NUMBER OF NEURONS AND GLIA IN SOMATOSENSORY CORTEX ARE AFFECTED BY PRENATAL EXPOSURE TO ETHANOL. G. Potempa* and M.W. Miller (SPON: M.E. Friedlander). Department of Anatomy, School of Osteopathic Medicine and Robert Wood Johnson Medical School, UMDNJ, Piscataway, NJ 08854.

Microcephaly is one of the charactersitic features of fetal alcohol syndrome. The number of cells in each layer of the somatosensory cortex of rats prenatally exposed to ethanol was determined using stereological methods. Pregnant rats were fed a liquid diet containing 6.7% (v/v) ethanol (E) or pair-fed a nutritionally matched, isocaloric diet (C). The offspring of these rats were examined on postnatal day 30. Prenatal exposure to ethanol affected the size and number of cells in each layer of cortex. The somatic diameter of neurons in each layer of somatosensory cortex, except layer V, was smaller ($p<0.01$) in E-treated rats. In contrast, the size of glia in each cortical layer was significantly greater ($p<0.01$) in the E-treated rats. Despite these changes, the cell packing density was similar in C- and E-treated rats. The volume of each layer was 27-41% less in E-treated rats. Overall, the estimated total numbers of neurons and glia in each layer of the somatosensory cortex of E-treated rats was 35% less than in pair-fed controls. The total volume occupied by neuronal cell bodies was less ($p<0.01$) in E-treated rats; consequently the neuropil occupied relatively more space in E-treated rats. Thus, ethanol-induced microcephaly is associated with structural changes in each layer of cortex. Funded by AA 06916, AA 07568, and DE 07734.

235.10

EFFECTS OF PRENATAL EXPOSURE TO BENZODIAZEPINES ON EXPLORATORY BEHAVIOR AND MESOLIMBIC DOPAMINE TURNOVER. R.J. Gruen, A.Y. Deutch, and R.H. Roth. Department of Pharmacology, Yale Univ. School of Medicine, New Haven, CT

Prenatal exposure to benzodiazepines (BZD) has been shown to lead to a variety of behavioral changes including learning deficits and alterations in motor and exploratory behavior. These latter deficits suggest that prenatal BZD exposure may impact on mesotelencephalic dopamine (DA) systems. We have therefore examined the behavioral and neurochemical sequelae of prenatal exposure to diazepam (gestational days 8 through 21) in rats. BZD-treated animals exhibited a significant decline in exploratory behavior (but not spontaneous locomotor behavior) at 90, but not 60 days after birth compared to vehicle-pellet implant and control subjects. These changes were paralleled by a significant decrease in DA turnover (DOPAC/DA) in the nucleus accumbens (NAS), but not in the other DA terminal fields examined (amygdala, olfactory tubercles, striatum, prefrontal and cingulate cortices). Treated animals had the lowest turnover in the NAS, followed by vehicle-pellet implant and control subjects. In addition, DA turnover in the ventral tegmental area was decreased in the BZD treated subjects, but not controls. These results suggest that alterations in exploratory behavior induced by prenatal exposure to diazepam are associated with significant changes in DA turnover in the NAS, a site previously shown to subserve exploratory behavior.

235.11

NUTRITIONAL INFLUENCES ON DEVELOPMENT OF CENTRAL AND PERIPHERAL NEUROTRANSMITTER SYSTEMS. C. Lau, NSI-ES/US EPA, RTP, NC, J.M. Bell, H.A. Navarro*, F.J. Seidler* and T.A. Slotkin, Dept. Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710

Maturation of the adrenal medulla and development of splanchnic innervation were evaluated in over- or undernourished rats (enlarged or diminished litter size), and compared to ontogeny of the central cholinergic and catecholaminergic neurons. Activities of choline acetyltransferase (ChAT) and tyrosine hydroxylase (TH) in the brain were monitored. Adrenal catecholamine (CA) stores, activities of TH and phenylethanolamine N-methyltransferase (PNMT) were measured, along with ChAT as a marker for the preganglionic neurons innervating the chromaffin cells. Neonatal nutritional deprivation slowed body weight gain and retarded development of the chromaffin cells, as evidenced by subnormal CA stores, TH and PNMT activities. These effects persisted despite recovery of body weights post-weaning. Neonatal nutritional enrichment promoted body weight gain but failed to enhance adrenomedullary development. In contrast, maturation of neurons in the brain was unaffected by nutritional status and only minor effects were seen in development of neurons innervating the chromaffin cells. These results suggest that development of central transmitter systems is spared from nutritional changes, but these protective mechanisms are not extended to similar tissues in the periphery: ontogeny of chromaffin cells is adversely, and perhaps permanently, affected by neonatal nutritional deprivation. (Supported by EPA-68-02-4450 and USPHS HD-09713)

235.12

PRENATAL PROTEIN MALNUTRITION ALTERS DEVELOPMENT OF PERFORANT PATH KINDLING IN THE RAT. **J.D. Bronzino, R.J. Austin-LaFrance* and P.J. Morgane. Dept. of ECS, Trinity College, Hartford, CT 06106 and The Worcester Found. for Exp. Biol., Shrewsbury, MA 01545.**

Nutritional insults have been shown to have profound effects on the developing CNS. In the present study pups born to dams maintained on either a 25% or 6% protein diet during gestation were fostered to dams on the 25% diet at birth, creating two experimental groups: 25%-25% (control) and 6%-25% (test). At 90 days of age these pups were tested for seizure susceptibility by daily application of a kindling stimulus to the medial perforant pathway during the vigilance state of quiet waking (QW). The kindling train was delivered at the minimum intensity required to elicit an afterdischarge within the granule cell field of the ipsilateral dentate gyrus. Preliminary data indicate that animals of the 6%-25% diet group require: 1) a significantly lower stimulus intensity to evoke afterdischarge activity, and; 2) significantly more daily stimulations to achieve the motor seizure state (stage 5) indicative of complete kindling. These results agree with previously published findings of this laboratory in which kindling stimulations were applied to region CA1 during QW. This study provides evidence for the fact that the prenatal dietary insult results in higher seizure susceptibility and retardation in the development of kindling even in the face of dietary rehabilitation at birth. **Suppoted by NIH Grant # 1 P01 HD22539-01**

235.13

THE EFFECTS OF KINDLING ON PAIRED-PULSE RESPONSE IN THE DENTATE GYRUS. **R.J. Austin-LaFrance*, P.J. Morgane and J.D. Bronzino (SPON: O. Resnick) Dept. of ECS, Trinity College, Hartford, CT 06106 and The Worcester Found. for Exp. Biol., Shrewsbury, MA 01545.**

This study was designed to investigate the effects of kindling on inhibitory activity within the dentate gyrus of the rat. Paired-pulse stimulations using inter-pulse intervals (IPIs) ranging from 20msec. - 1sec. were applied to the medial perforant pathway. Field potential responses to these stimuli were recorded from the granule cell field of the ipsilateral dentate gyrus before, during and after daily application of kindling stimulation to the same pathway. Paired-pulse stimulations were applied at intensities determined to elicit a population response to the first pulse equal to 75% of the maximal population response. Prelimnary data indicate that kindling enhances depression of the evoked action potential (EAP) response to the second pulse of a pulse pair at all IPIs tested, with the most significant enhancement occurring between the first and second afterdischarge. This enhanced depression is most marked at both the lower and upper end of the range of IPIs tested, suggesting that kindling alters mechanisms associated with both intrinsic recurrent and feed-forward inhibition within the dentate gyrus. Results of these studies will be used as control data to assess the effects of prenatal protein malnutrition on these inhibitory mechanisms during the development of kindling. **Supported by NIH Grant # 1 P01 HD22539-01**

235.14

VIGILANCE STATE MODULATION OF PAIRED PULSE RESPONSES IN RAT HIPPOCAMPUS. C. Beiswanger*, K. Austin*, J. Bronzino, and P.J.Morgane (SPON: J. McKearney). Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Recently we found that the level of inhibition and/or facilitation in the dentate gyrus (DG) is dependent on vigilance state (Austin et al., in press). In the current study, the paired pulse technique was used to establish an index of inhibition/facilitation in the DG by determining the ratio of inhibitory to facilitory responses to perforant path stimulation in four behavioral states (immobile waking (IW), active waking (AW), slow wave sleep (SWS) and REM sleep). In rats 90 to 120 days of age, a stimulus-response curve was determined in each behavioral state before paired pulse testing was done at IPIs between 20 and 1000 msec. Paired pulse tests provide evidence of a changing ratio of inhibition to facilitation of the population spike in DG at several IPIs. The ratio of facilitory to inhibitory responses at short IPIs is significantly greater during AW than during IW, SWS and REM, suggesting that inhibition (recurrent or feed-forward) may be suppressed during AW. Since prenatal nutritional insults to the developing CNS have effects persisting into adulthood, especially in highly plastic structures such as the hippocampus (Morgane et al., 1986; Austin et al., 1986; Bronzino et al., 1986), we are assessing how prenatal protein malnutrition affects vigilance state modulation of the index of inhibition/facilitation (Supported by NIH grants HD23338 and HD22539).

235.15

ASPARTAME(ASM)-INDUCED CHANGES IN PLASMA AND BRAIN AMINO ACIDS IN THE ABSENCE OF EFFECTS ON SEIZURE SEVERITY. S.M. Lasley, P.C. Jobe, R.L. Burger*, A.F. Bettendorf* and J.W. Dailey. U. of Ill. Coll. of Med., Peoria, IL 61656.

It has been hypothesized that the dipeptide sweetener ASM (aspartyl-L-phenylalanine methyl ester) facilitates seizures in animals and man by an action of its metabolite phenylalanine (PHE) on the synthesis of monoamine neurotransmitters in brain (e.g., norepinephrine (NE)). Previous studies have demonstrated that seizure predisposition and severity in genetically epilepsy-prone rats (GEPR) are partially determined by, and inversely related to, brain noradrenergic deficits, thus offering an excellent model by which to test this hypothesis. Dose-response relationships were determined for ASM and seizure severity and plasma and brain neutral amino acid concentrations, employing both moderate seizure (GEPR-3) and severe seizure (GEPR-9) animals. ASM was suspended in 0.5% methylcellulose and 0.1% polysorbate-80 and administered by gavage in the range of 0-2000 mg/kg. One hour later half the animals were tested for audiogenic seizure severity and the other half were sacrificed for plasma and brain amino acid determinations. At 2000 mg/kg ASM produced substantial increases in PHE in plasma and brain (maximum increase 276%) and in tyrosine in plasma and brain (maximum increase 122%), but decreased brain tryptophan by 30%. ASM did not alter audiogenic seizure severity in either GEPR-3s or GEPR-9s at any dose. These findings indicate that ASM does not facilitate seizures in animals whose seizure predisposition is at least partially dependent on noradrenergic deficits. (Supported in part by the NutraSweet Co.)

235.16

NEUROBEHAVIORAL EFFECTS OF PRENATAL EXPOSURE TO ANTIADRENERGIC ANTIHYPERTENSIVE DRUGS (AHD'S). C. L. Ryan* and B. A. Pappas. Dept. of Psychology, Carleton Univ., Ottawa, Canada, K1S 5B6.

AHD's are prescribed to treat hypertensive disorders of pregnancy. Since little is known concerning the long-term consequences of fetal exposure to these drugs, we investigated the effects of prenatal administration of three AHD's. Wistar dams were orally administered throughout gestation, one of clonidine (20 or 100 µg/kg), propranolol (5 or 20 mg/kg) or atenolol (5 or 20 mg/kg). Analysis of physical and behavioral development using parts of the Cincinnati Test Battery revealed no differences among the groups. At weaning, half the pups were housed in impoverished (I) and half in enriched (E) conditions for 40 days. They were then tested in a modified Hebb-Williams maze consisting of 5 training problems followed by 11 test problems (one/day). The E-reared animals made fewer errors than the I-reared rats. Exposure to propranolol, but not clonidine or atenolol, impaired maze performance. Furthermore, there was a high mortality rate during maze testing, but only from the E-reared females prenatally exposed to the high dose of clonidine. This was associated with gastric ulceration, thymic atrophy, and adrenal hypertrophy.

In summary, prenatal exposure to propranolol impaired problem-solving ability while clonidine had a latent and lethal effect in females which was precipitated by environmental factors.

235.17

ADULT-ONSET CALORIC RESTRICTION: LACK OF EFFECT ON HIPPOCAMPAL CA3 PYRAMIDAL CELL MORPHOMETRY. A. Andrews*, R. Rountree*, W. Nowak, R. Hart*, and A.C. Scallet. Natl. Ctr. for Tox. Res., Jefferson, AR 72079 and Univ. of Ark. for Med. Sci., Little Rock, AR 72205.

Previous analyses of the dendritic trees of rodent cortical neurons suggest nutritional and age-related factors as well as various neurotoxic exposures might produce reductions in the extent of dendritic arborization. Caloric restriction (CR) has been extensively used to delay the appearance of various age-related parameters. The aim of the present study was to identify several measures of hippocampal CA3 dendrites potentially useful in such studies. We measured 6 Golgi-Cox impregnated CA3 cells/rat from 4 calorically retricted and 4 control 18-month old male rats using a motorized microscope stage interfaced to a personal computer. No differences in CR vs control soma areas (453 ± 89 vs 403 ± 29 square microns), number of dendrites per cell (4.8 ± 0.5 vs 4.9 ± 0.3), or total length of dendrites per cell (1183 ± 195 vs 1187 ± 206 microns) were measured. The data suggest CR beginning in young adult rats (at 14 wk) is without effect on several morphometric parameters of the 18-month old rat hippocampus.

235.18

PRENATAL EXPOSURE TO MORPHINE: EFFECTS ON TOLERANCE AND WITHDRAWAL OR NOCICEPTION AND ACTIVITY? C. P. Cramer, R. A. Fite, and M. S. Fanselow*. Dartmouth College, Hanover, NH 03755.

Many authors have described the postnatal sequelae of prenatal opiate exposure in terms of addiction. Because, in previous studies, we have been unable to demonstrate tolerance and withdrawal in infant rats younger than 15 days postpartum, in the present experiments we analyzed these addiction-related behaviors in neonates that were prenatally exposed to morphine.

Rat dams were injected daily with either isotonic saline, 10 mg/kg morphine, or 20 mg/kg morphine at least 3 weeks prior to conception and throughout gestation. After birth and 24 hr after the last morphine injection, pups were randomly assigned to 1 of 5 drug groups: saline, 1, 2, or 4 mg/kg morphine, or 10 mg/kg naloxone. Pups were tested at 30 min intervals for nociception using a modified hotplate (52°C) technique; pawlift latency was measured. Pups receiving naloxone were also weighed at hourly intervals and were viddeotaped for 2 hr after injection. Videotapes were analyzed both for general activity (squares entered) and for specific behaviors.

Pups exposed prenatally to morphine responded more quickly to the nociceptive stimulus, however they did not show reduced responsivity to morphine. That is, they were not tolerant. Similarly, they were more active following naloxone injection, but their behavioral profiles were not clearly suggestive of withdrawal.

235.19

NERVE DEVELOPMENT IN GROWTH RETARDED FETAL SHEEP S. Rees*, R. Harding*, U. Proske* (SPON: W. Webster) Department of Physiology, Monash University, Clayton, Victoria, 3168, Australia.

We have studied the effects of experimental growth retardation on the development of both the peripheral and central nervous systems of fetal sheep. Growth retardation was produced by restriction of placental growth in 7 ewes. At 140 days of gestation (term = 145 days) three different nerves were examined, the optic, trochlear and peroneal. Morphometric analysis of myelinated fibres in the optic nerve showed that in growth retarded animals the myelin sheath was abnormally thin. In the trochlear nerve the normal myelin to fibre ratio was maintained but there was an abnormally large number of small axons. Measurements of whole-nerve conduction velocity in the peroneal nerve showed, for the most rapidly conducting fibres, a significant slowing from the normal value of 48.9±2.8 to 33.8±3.3 m/s ($p<0.05$).

Hence we have shown in these nerves that conduction velocity, or factors which are known to affect it, are altered in the growth retarded fetus at birth. The mechanisms which interfere with the normal development of nerves may differ according to location in the nervous system. Supported by the National Health and Medical Research Council of Australia.

235.20

NORMAL RAT DEVELOPMENT FROM WEANING TO ADULT M. Sakuma, L.M. Hryhorczuk,* Dept. of Biol. Sci., Wayne State Univ., Detroit, MI 48201: Lafayette Clinic, Detroit, Mi 48207

Sprague-Dawley rats born to dams fed a diet differing in tryptophan (TRP) content during the second half of gestation were investigated in a series of studies of development. This report deals only with the long-term developmental changes (from week 3 to week 15) observed in rats born to dams on a regular diet (Purina Lab Chow). The animals, segregated by sex (12 male, 14 female), were evaluated weekly by body weight and in 5 min open field tests. The animals were scored on the basis of rearing counts, latency of rearing, face washing counts, latency of face washing, and defecation. Two way analysis of variance with repeated measures revealed that all variables showed an age dependency at $p<0.01$. With respect to sex differentation, males had a greater weight gain ($p<0.01$) and lower number of rearings ($p<0.01$). These differences will be examined with results on animals born to dams on diets high and low in TRP content. This study was in compliance with the NIH Guide for the Care and Use of Laboratory Animals.

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WORKSHOP: HIPPOCAMPAL CELLULAR ACTIVITY AND SPATIAL-COGNITIVE PROCESSING. P.J. Best, U. New Orleans and J.B. Ranck Jr. SUNY Med. Ctr. Brooklyn, (Chairpersons); J. Kubie, SUNY Med. Ctr., B. McNaughton, U. Colorado, S. Deadwyler, Bowman Gray, H. Eichenbaum, Wellesley, D. Olton, Johns Hopkins.

Activity of hippocampal pyramidal cells is highly related to the animal's position or place in the environment. Each neuron fires in a small place field in the test arena. The workshop examines the nature of the information required for place field activity and the subsequent processing of that information. Place field activity is normally stable for extremely long periods of time, and is very dependant on distal environmental cues. Yet place cells are not merely sensory cells. The fields are affected by the animal's motion, and the fields can be brought under the control of local, biologically relevant stimuli. Further, the same cells can show a remarkable degree of plasticity under appropriate conditions. Their place fields can be modified by varying stimuli or past experience, and they show phasic responses to discrete nonspatial stimuli, which can be modified by experience. The implications of the new data for the interpretation of behavioral-cognitive correlates of hippocampal cellular activity and for theories of hippocampal function will be discussed.

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SYMPOSIUM. SEX DIFFERENCES AND HORMON/ INFLUENCES ON COGNITIVE BRAIN FUNCTION. R. Lewis, Pomona Coll.(Chair); B. Kolb, U. Lethbridge; C. Williams, Barnard Coll.; M. Hines, UCLA; E. Hampson, U. Western Ont.; D. Kimura, U. Western Ont. (Organizer) Men generally excel on extrapersonal spatial tasks, women on intrapersonal motor/ articulatory skills. Previous studies have shown greater dependence on left anterior regions in women, left parietal in men, for motor programming function. Kolb- anterior/posterior differences exist also in male and female rats, with females more affected on locomotor orientation tasks by frontal, males by parietal, lesions. Williams- early sex hormone treatment in rats alters the preference of males and females, respectively, for geometry vs. landmark cues. Hines- in adult humans, cognitive, brain asymmetry and social effects are associated with early anomalous sex hormone exposure. Hampson- menstrual cycle hormone fluctuations in women alter spatial and motor skills in reciprocal ways, predictable from the known sex differences. Kimura- post-menopausal women show enhanced motor programming skills during phases of estrogen therapy.

PROCESS OUTGROWTH, GROWTH CONES, AND GUIDANCE MECHANISMS IV

240.1

DAMAGE OF A MIDLINE BOUNDARY AND FORMATION OF A TISSUE BRIDGE ALLOWS THE MISGUIDANCE OF OPTIC AXONS ACROSS THE MIDLINE IN HAMSTERS. M. Poston*, S. Jhaveri*, G. Schneider and J. Silver. Case Western Reserve Univ., Cleveland, OH 44106. Massachusetts Institute of Technology, Cambridge, MA 02139.

We are interested in the events which control axonal guidance and misguidance in the vertebrate superior colliculus (SC). In this study we examined, at light microscopic and TEM levels, the morphology of the midline between the two halves of the SC in normal and lesioned hamsters. Postnatal day one (P1; PO=day of birth) hamsters were lesioned by placing a heated pin onto the cartilage overlaying the right SC and part of the SC midline. The midline of newborn unlesioned hamsters, which optic axons do not normally cross, consists of cells whose processes form a wedge-shaped structure which serves as an axon-refractory boundary between the two halves of the SC. In contrast, in the lesioned hamsters the midline is drastically changed. No wedge-shaped cellular boundary separating the SC halves is present. Rather, a tissue bridge spanning the lesion-induced cavity is contiguous from the lateral aspects of the right SC to the medial aspects of the left SC. Optic axons grow along this bridge, cross the altered midline and enter the left SC. Cells of the tissue bridge possess radial processes containing intermediate filaments and glial endfeet covered with an intact external limiting membrane. Thus, the tissue bridge presents the crossing axons with a terrain that is morphologically similar to the normal marginal zone where optic axons readily grow. We propose that at least three events occur which allow optic axons to cross the SC midline in lesioned hamsters: damage of an axon-refractory boundary at the midline, rehealing of the external limiting membrane and marginal zone of injured brain, and formation of a suitable substrate for the misguidance of optic axons. Supported by NIH EY05952.

240.2

EVIDENCE FOR A DIFFUSIBLE FLOOR PLATE-DERIVED FACTOR THAT CONTRIBUTES TO AXONAL GUIDANCE IN THE EMBRYONIC RAT SPINAL CORD. M. Tessier-Lavigne*[1,2], M. Placzek*[1], A. Lumsden*[3], J. Dodd[1], and T. Jessell[1,2]; [1]Center for Neurobiology and [2]Howard Hughes Medical Institute, Columbia University, New York, NY 10032, and [3]Department of Anatomy, Guy's Hospital Medical School, London, England.

Two classes of projection neurons differentiate concomitantly in the dorsal region of the embryonic rat neural tube. Association (A) neurons send axons ipsilaterally to form the lateral funiculus, whereas the axons of commissural (C) neurons project ventrally and medially, cross the midline of the neural tube at the floor plate (a specialized set of epithelial cells), and turn longitudinally to form the contralateral ventral funiculus. To determine the mechanisms underlying the selection of distinct pathways by the axons of A and C neurons, we have investigated whether floor plate cells release a diffusible factor(s) that contributes to the guidance of C axons towards the ventral midline.

Evidence for the existence of a diffusible floor plate-derived factor was obtained by examining the effect of the floor plate on axon extension from the dorsal neural tube, when explants of both regions were cultured 100-300 μm apart in a collagen gel matrix. Explants were taken from embryonic day 11 neural tube, when C neurons are beginning to extend axons. Axons did not project from dorsal neural tube explants that were cultured alone for 36 hrs. In the presence of floor plate, however, axons emerged from such explants within 20-24 hrs, and projected towards the floor plate explant in thick fascicles. These axons appear to derive from C neurons since they express the TAG-1 antigen, a cell-surface glycoprotein expressed on C but not A axons during their initial extension. The presence of floor plate also appears to have an orienting effect on the trajectory of C axon growth, since in dorsal explants that were cultured with the original dorso-ventral axis parallel to a floor plate explant, a large majority of the emerging axons grew towards the floor plate, deviating significantly from their normal trajectory. These findings suggest that floor plate cells release a diffusible factor(s) that may influence the guidance of C axons in vivo.

240.3

TISSUE SPECIFICITY AND PROPERTIES OF A FLOOR PLATE-DERIVED FACTOR THAT INFLUENCES COMMISSURAL AXON OUTGROWTH. M. Placzek*, M. Tessier-Lavigne*[1], T. Jessell[1] and J. Dodd. Center for Neurobiology and [1]Howard Hughes Medical Institute, Columbia University, New York, NY 10032.

Floor plate cells of the embryonic rat spinal cord may play a role in guiding the axons of commissural neurons to the ventral midline of the neural tube by releasing a diffusible tropic factor(s) (Tessier-Lavigne et al., companion abstract). To determine whether this factor is restricted to floor plate cells, a number of embryonic tissues were assayed for their ability to substitute for floor plate in the co-culture bioassay. Embryonic notochord, gut, brain, ventral spinal cord, somite, mandibular process, kidney, adrenal and lung did not mimic the effect of floor plate on commissural axon outgrowth. Moreover, the ability of floor plate to induce axon outgrowth is selective with respect to neuronal subtype. Embryonic trigeminal ganglion, dorsal root ganglion, ventral spinal cord and retinal neurons did not respond to floor plate explants with enhanced axonal outgrowth.

To characterize further the properties of the floor plate-derived factor, we examined the activity of defined medium conditioned in vitro by exposure to floor plate explants. Axon extension from dorsal neural tube explants cultured with conditioned medium was comparable to that observed when explants were cultured with floor plate. The active component in floor plate conditioned medium was retained by an ultrafiltration membrane with a 30 kDa exclusion limit and was inactivated by trypsin. The fact that most neurons did not respond to the presence of floor plate and that laminin is not detectable in the rat floor plate suggests that laminin is not the active component. Since the floor plate is located in the neural tube, but shares biochemical properties with the notochord, an axial mesodermal structure, we tested known neurotrophic and mesodermally-derived growth factors for their ability to mimic floor plate conditioned medium. The effects of conditioned medium were not mimicked by EGF, aFGF, bFGF, PDGF, TGFβ, NGF, CNTF, BDNF or by medium conditioned by the mesodermally-derived XTC cell line. The floor plate may therefore release a factor distinct from those examined or a combination of factors that act in concert to influence axon outgrowth.

240.4

Role of Glia in the Formation of Axon Pathways in the Drosophila CNS Revealed by Cell Lineage Markers and Developmental Mutants J.R. Jacobs and C.S. Goodman. Dept. of Biochemistry, Univ. Calif. Berkeley, Berkeley, CA 94720 .

Each segment of the Drosophila CNS contains a bilateral pair of longitudinal tracts, two major commissures which bridge these tracts, and two major peripheral nerve roots. Prior to growth cone extension, the location of the longitudinal and commissural tracts, and the intersegmental nerve, are prefigured by different classes of glial cells positioned under the basement membrane on the dorsal surface of the neuroepithelium. We characterized the origin and development of two of these classes of glia with electron microscopy, and using a P-element lineage marker (provided by Yash Hiromi) which directs the expression of B-galactosidase in a specific glial lineage. Using this B-gal marker, we find that the three pairs of glial cells which prefigure the longitudinal connectives in each hemisegment arise from the division and subsequent migration of progeny from a single glioblast. The two commissures are prefigured by a different class of glia which originate from the midline neuroepithelial cells. We hypothesize (i) these different classes of glia interact with one another to establish their characteristic patterns, and (ii) the first growth cones interact with these glia to establish their characteristic axon pathways. We have begun using these and other probes to examine mutants which are abnormal in these events. For example, in the single-minded mutant (Thomas et al., 1988; Crew et al., 1988) and the slit mutant, the commissures do not properly form. The longitudinal connectives fail to properly form in the midline mutant. The commissures develop abnormally in the Star mutant and in deficiencies which remove the fasciclin III gene. Analysis of the ontogeny of the phenotypes of these mutants will help reveal the role of cell interactions in the patterning of these early glia and of the axon pathways which form along their surfaces.

240.5

MUTATIONS AFFECTING GROWTH AND GUIDANCE OF MECHANOSENSORY NEURONS IN THE NEMATODE CAENORHABDITIS ELEGANS.
S. S. Siddiqui. Laboratory of Molecular Biology, Toyohashi University of Technology, Toyohashi 440, Japan.

Six mechanosensory receptor neurons called microtubule cells mediate touch sensitivity in C. elegans(Chalfie, M. Sulston, J. Develop Biol. 82, 278, 1981). Each of the six receptor cells has a long process which is apposed to the cuticle of the animal. We have previously shown that a monoclonal antibody 6-11B-1 raised against Sea Urchin axonemes and specific for acetylated alpha tubulin(Piperno G. and Fuller, M, J.C.Biol 101, 2085, 1985) stains touch cells in C. elegans(Siddiqui, S. S., Aamodt, E., Rastinejad, F. and Culotti, J. G. 1988). Here we have analysed the morphology of touch cells in uncoordinated mutants of C. elegans representing 90 unc genes, immuocytochemically, using 6-11B-1 on wholemount animals. In mutants of 15 genes(unc-5, unc-6, unc-13, unc-33, unc-34, unc-44, unc-49, unc-51, unc-55, unc-61, unc-71, unc-73, unc-76, unc-98, and unc-102) a variety of axonal guidance errors were seen in a fraction of stained animals. Most frequently the stained receptor processes ran in abnormal dorsal or ventral positions, sometime reaching the ventral nerve cord or the dorsal nerve cord in abnormal positions. In addition, we observed abnormal bifurcation of axonal processes and multipolar touch cells. Mutants of unc-59 and unc-85 exhibit binucleate touch cells with multiple axon processes. The touch sensitivity data will be shown.

240.6

NERVE GUIDANCE GENES OF C. ELEGANS. J.Culotti*[1], N. Ishi*[2], C.Leung-Hagesteijn*[1], B.Stern*[3], E.Hedgecock*[3], and D.Hall[4] (1)Mt. Sinai Hosp. Res. Inst., Toronto, M5G 1X5, Canada (2)Tokai U., Isehara, Japan (3)Johns Hopkins U:, Baltimore, Md 21218, (4)A. Einstein Coll., Bronx, N.Y. 10461

The nematode epidermis is a cylindrical neuroepithelium which supports neurons, precursors, a basal lamina, and attached mesodermal cells on its basal surface. Mutations in 3 genes disrupt the circumferential migrations along the epidermis of both pioneer axons and mesodermal cells. unc-5 mutations disrupt dorsalward migrations, unc-40 mutations primarily disrupt ventralward migrations, and unc-6 mutations disrupt migrations in both directions. These genes identify a circumferential guidance system acting throughout the animal.

We have cloned the unc-5 and unc-6 genes by transposon tagging. The predicted unc-5 protein contains an exon of 122 amino acids comprising a tandemly arranged pair of homologous domains each beginning with the tryptophan rich sequence DGGWSXWSXW. Similar domains are found in apparently unrelated proteins involved in cell adhesion, including the platelet protein thrombospondin, complement components C8 alpha, C8 beta, and C9 and the circumsporozite proteins of malarial parasites. In thrombospondin, 3 tandemly arranged copies of this domain are present in a region of the protein which is believed to bind to various extracellular matrix components including laminin. Therefore, this domain may be adapted for binding to the extracellular matrix. This possibility is particularly appealing since, based on its amino terminal sequence, unc-6 appears to be a nematode B2 laminin.

240.7

REDUPLICATION OF SPECIFIC BRANCHING PATTERNS EXPRESSED IN THE CNS OF THE LEECH BY ISOLATED IDENTIFIED NEURONS IN CULTURE. S.E. Acklin and J.G. Nicholls, Biocenter, Basel, Switzerland, 4056.

Two identified interneurons (designated, alas, as 61 and 21) exhibit a series of well-defined physiological and morphological properties in situ. These cells, initiate swimming, secrete 5-HT, are electrically coupled to one another, and display rhythmical bursts of impulses. Within the ganglion, they branch in a stereotyped and distinctive manner (Lent, C.M., J.Exp.Zool., 216:311-361, 1981). In both cells the initial process divides to form two principal elongated neurites. In one (21) the arborization resembles the letter Y, in the other the letter T. The cells were isolated and plated on Con A in L-15 medium. Their resting and action potentials remained normal and they became coupled electrically. One novel finding was that Lucifer Yellow spread from cell to cell. Another was that a single cell could continually procuce bursts of impulses similar to those arising in the ganglion. Quite unexpectedly, each cell in culture faithfully reproduced the configuration Y or T. In the absence of any external cues the first sprouts grew in specific directions, with subsequent fasciculation. Other types of leech neurons growing in culture have not reproduced patterns formed during development. These two cells with their regular and simplified conformations provide favorable preparations for analysing intrinsic and external factors involved in directed growth. (Supported by grants from the Swiss National Fonds and the U.S. Navy.)

240.8

A GROWTH CONE COLLAPSING ACTIVITY FROM EMBRYONIC CHICK BRAIN MEMBRANES. J.A. Raper and J.P. Kapfhammer*. Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen, FRG.

It is often assumed that growth cones are guided to their destinations by localized neurite promoting activities. Less attention has been paid to the possibility that specific, localized, growth inhibiting activities control growth cone behavior. We recently found that particular types of growth cones collapse and consequently fail to locomote on contact with specific axons in culture (J. Neurosci. 6:2527-34; 7:201-12; 7:1595-1600). We proposed that growth cones collapse when they recognize signals associated with specific axon membranes.

We have found that growth cones which are always in contact with a permissive laminin substratum can be induced to collapse when exposed to a suspension of embryonic chick brain membranes. Time lapse studies show that collapse is reversed if the membrane suspension is removed, suggesting that the cells are healthy and that the substratum is not damaged by the membranes. Similar preparations from liver or fibroblasts are ineffective.

The collapsing activity is lost if the particulate portion of the membrane suspension is removed by centrifugation, suggesting that it is an insoluble, membrane associated activity. The activity is lost after heat or trypsin treatment, suggesting that one or more proteins are involved. The activity can be solubilized with detergent and reconstituted by its removal. We are currently purifying the collapsing activity.

The results support the hypothesis that growth cone collapse can be triggered by contact with membrane proteins, and provide us with a practical assay for purifying at least one causal agent.

240.9

CELL SURFACE BINDING SITES FOR PLASMINOGEN ACTIVATORS. R.N. Pittman. Dept. of Pharmacology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

Cultured rat sympathetic neurons release both urokinase plasminogen activator (UK) and tissue plasminogen activator (t-PA). Inhibition of UK and/or t-PA results in increased neurite outgrowth. In addition to being present in the culture medium, both t-PA and UK are present on the surface of neurons. At least two and probably three independent binding sites are present on the surface of sympathetic neurons. One cell surface binding site is selective for t-PA, binds with high affinity to a region of t-PA distinct from the active site, and is present in high concentrations (>200,000 sites/neuron). Immunohistochemical localization of surface bound t-PA indicates fairly smooth homogenous binding at the cell body and along the processes. At the growth cone, more surface bound t-PA appears to be associated with filopodia than lamellipodia. A second cell surface binding site has a MW of ca. 50 kDa, binds to the active site of UK and t-PA and appears to be associated with cell surface heparan sulfate proteoglycan. A third binding site appears selective for UK and binds to a region of UK distinct from the active site. Localization of surface bound UK has been somewhat inconsistant but appears to be punctate, heterogenous, and on the bottom surface of neurons next to the substrate. Supported by grants from the NIH (NS 22663) and McKnight Foundation.

240.10

MONOCLONAL ANTIBODIES THAT DEFINE ROSTRO-CAUDAL POSITION IN THE MAMMALIAN NERVOUS SYSTEM. T. Suzue*, J. Imrich* and P. H. Patterson. Biology Div., 216-76, California Institute of Technology, Pasadena, CA 91125.

Transplantation experiments have shown that spinal cord neurons can distinguish rostral vs. caudal sympathetic ganglia, as well as rostral vs. caudal intercostal muscles, when the muscles are transplanted in place of the ganglia. This raises the possibility that the ganglia and the muscles share a molecule(s) that has a differential distribution along the rostro-caudal axis, and that the molecule is involved in specific synapse formation or axon guidance. In an attempt to identify molecules with such a distribution, we employed the immunosuppression technique. Mice were first injected with membranes from newborn, caudal sympathetic or dorsal root ganglia, followed by injection of the immunosuppressant drug, cyclophosphamide. After injection of membranes from rostral ganglia, hybridomas were generated. Many mAbs were produced which preferentially bind to rostral ganglia, and some of these also stain the nerves that innervate intercostal muscles. The staining of one of the mAbs is highest in the rostral nerve and it declines in a graded manner in the caudal segments. Surprisingly, this mAb appears to stain glial cell surfaces rather than axons. Since glial surfaces can promote axon growth, this antigen could play a role in the rostral preference displayed by rostral spinal cord axons.

240.11

ULTRASTRUCTURAL LOCALIZATION OF STAGE-SPECIFIC NEURITE-ASSOCIATED PROTEIN (SNAP) IN DEVELOPING RAT CORTEX AND CEREBELLUM. M. Yamamoto, J.E. Crandall and L. Hassinger* Dept. of Developmental Neurobiology, E.K. Shriver Center, 200 Trapelo Rd., Waltham MA 02254

SNAP (or TAG) antigen recognized by 4D7 MAb is expressed on the plasma membrane of growing axons in the embryonic rodent nervous system. Light microscopic study demonstrated that this glycoprotein is expressed in a subset of axons during narrow temporal window of development (Yamamoto et al. 1986 J. Neurosci. 6:3576). The ultrastructural localization of the SNAP antigen was studied in embryonic (E15-17) cerebral cortex and postnatal (P4) cerebellum using HRP conjugated secondary antibody. Immunoreactivity was localized predominantly in the intermediate zone (IZ) of the cortex where afferent and efferent axons course. Some immunoreactivity was also seen in the cortical plate (CP) and subplate. Immunoreactivity was associated with the plasma membranes of neuritic processes, neural somata and selective groups of fasciculated axons. In the IZ SNAP positive axons could be found in distinct bundles. In the CP SNAP immunoreactivity was detected on only some portions of individual neuronal somata. Along stained membranes SNAP appeared to be localized in a regularly spaced, punctate fashion with a periodicity of 50-100 nm. The distinctive distribution of SNAP antigen along developing neuronal membranes suggests a possible role for this molecule in selective axon-axon interactions. Supported by grants NS24746 to M.Y. and NS24386 to J.C.

240.12

DEVELOPMENTAL REGULATION OF $pp60^{c-src}$ AND TYROSINE PHOSPHORYLATION OF A 53-55 KD PROTEIN IN NERVE GROWTH CONES. Patricia F. Maness and Muriel Aubry*. Dept. Biochemistry, Univ. North Carolina, Chapel Hill, NC 27514.

Differentiating neurons express high levels of the proto-oncogene product $pp60^{c-src}$, a tyrosine kinase of unknown function. $pp60^{c-src}$ is concentrated approximately 10-fold in membranes from a subcellular fraction from fetal rat brain that is enriched in nerve growth cones. Indirect immunofluorescence staining showed $pp60^{c-src}$ in neuronal growth cones and processes, with a concentration in growth cones of long neurites. $pp60^{c-src}$ was present at lower levels in subcellular fractions from mature brain but was not enriched in synaptosomal membranes. Using antibodies directed against phosphotyrosine, we have identified a 53-55 kD protein in the growth cone membrane fraction as the major protein phosphorylated at tyrosine in vivo. This protein was highly enriched in growth cone membranes relative to fetal brain homogenate or synaptosomal membranes. In protein kinase reactions with isolated growth cone membranes, the major protein phosphorylated at tyrosine is also a 53-55 kD protein identified as α- and β-tubulin. These results indicate that $pp60^{c-src}$ and tubulin tyrosine phosphorylation may be important determinants of growth cone-mediated neurite extension and synaptic plasticity.

ION CHANNELS: SODIUM CHANNELS I

241.1

EXPRESSION OF Na CHANNEL SUBTYPES DIFFERS IN TWO POPULATIONS OF VERTEBRATE SENSORY NEURONS. D.T. Campbell. Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

Dorsal root ganglion cells from garter snake (T. sirtalis) and frog (R. temporaria) were enzymatically dissociated and maintained in culture media for 1-3 weeks. Ionic currents were measured using the whole-cell voltage clamp technique. Test solutions contained 5-10 μM La, 1 mM Ca and 9 mM Mg to minimize current through Ca channels. When TEA·Cl replaced all or part of the NaCl in test solutions the reversal potential of the early transient current followed the Na equilibrium potential, suggesting that this current was due to Na ions flowing through Na channels. Cell capacitance ranged from 4-40 pF (snake) and from 15-110 pF (frog). The Na current of the largest cells of both species (>23 pF, snake; >50 pF, frog) was virtually completely blocked by 1 μM TTX. By contrast, in the majority of smaller cells of both species, 10-90% of the Na current was extremely resistant to block by TTX. TTX concentrations up to 100 μM failed to elicit appreciable block of the resistant channels. In both species, the TTX-resistant channels activate at more negative potentials and exhibit ~3-times slower inactivation than the TTX-sensitive channels seen in the larger cells. The TTX-resistant channels of snake were not appreciably blocked by 1 μM STX, whereas the TTX-resistant channels of frog were 90-95% blocked by 1 μM STX. The differential expression of Na-channel subtypes in large and small DRG cells suggests that their kinetics may play a role in determining the distinct action potential characteristics of large and small sensory neurons.

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241.2

PROTON-GATED SODIUM CURRENTS IN MAMMALIAN CENTRAL NEURONS R. Grantyn, M. Perouansky and H.D. Lux+. Max Planck Inst. for Psychiatry, 8033 Martinsried, F. R. G.

A transient proton-activated sodium current ($I_{Na(H)}$) is described for chick dorsal root ganglion neurons (Konnerth, Lux and Morad 1987) and assigned to transformed Ca channels (Davies, Lux and Morad 1988). Cultured neurons from the tectum of E12 rat embryos were now used to characterize pharmacological properties and development of $I_{Na(H)}$ in a defined population of neurons from the mammalian brain.

After two weeks in vitro, all tectal neurons generate $I_{Na(H)}$. Under optimal activation conditions (low Ca and Mg, holding pH 7.9, fast application) $I_{Na(H)}$ exceeds in amplitude all other inward currents, the EC_{50} for $/H^+/_o$ being 0.14 uM. Comparing the effect of Ca channel antagonists on $I_{Na(H)}$ and on two types of voltage-activated Ca-currents ($I_{Ca(LV)}$ and $I_{Ca(HV)}$) it was found that $I_{Na(H)}$ is pharmacologically close to $I_{Ca(LV)}$. Both currents are blocked by low doses of 5-amino-diethylamiloride (IC_{50}: 7.5 uM), while $I_{Ca(HV)}$ was unaffected by this compound. Suppression of $I_{Ca(LV)}$ for the duration of $I_{Na(H)}$ suggests that these currents are, in fact, carried through the same channels.

During early differentiation (first day in vitro), $I_{Na(H)}$ and $I_{Ca(LV)}$ appear roughly in parallel, provided that laminin is used as a growth substrate. The absence of this extracellular matrix protein results in dissociated expression of $I_{Na(H)}$ and $I_{Ca(LV)}$, indicating that the proton- and voltage-gating property of Ca channels may evolve independently.

241.3

PHARMACOLOGY OF INSECT SODIUM CHANNELS, Sarah C.R. Lummis* and David B. Sattelle, AFRC Research Unit, Dept of Zoology, Downing Street, Cambridge, CB2 3EJ, UK.

Saxitoxin (STX) has proved extremely useful in the isolation and characterization of sodium channels. Voltage-clamp experiments on isolated giant axons of the cockroach (Periplaneta americana) show that STX specifically and reversibly blocks transient inward sodium currents. From the concentration dependence, it appears that individual sodium channels are blocked by single molecules of STX, which bind reversibly to the channel with a K_d of 3nM. Membrane extracts from the cockroach nervous system contain a saturable component of specific [^{3}H]STX binding. Scatchard analyses yielded a K_d of 2nM, similar to that determined from electrophysiological studies. The specific binding component was blocked by STX and TTX, but not by veratridine or deltamethrin, which act at different sites on the channel molecule. Unlabelled STX samples prepared from different sources (Mytilus, Saxidomus and Gonyaulax) were all effective as inhibitors of [^{3}H]STX binding. Specific, saturable binding sites for [^{3}H]batrachotoxin, have also been identified in a cockroach CNS membrane preparation, although experimental conditions are distinct from those required to optimise binding of this ligand to vertebrate CNS membranes. The results demonstrate that, using the nervous system of the cockroach, a multidisciplinary experimental approach can be applied to the characterization of insect sodium channels - major molecular targets of pyrethroid insecticides.

241.4

BREVETOXIN ACTIONS ON SINGLE SODIUM CHANNELS IN NG108-15 CELLS. **R. E. Sheridan and M. Adler. Neurotox. Br., USAMRICD, APG, MD 21010 and Dept. of Pharm., Georgetown Univ., Washington, DC 20007.**

The actions of the marine toxin brevetoxin (PbTX-3) were studied on voltage-dependent sodium channels in clonal NG108-15 neuroblastoma x glioma cells. Single sodium channels were recorded in cell-attached and in excised inside-out or outside-out membrane patches at 21-25°C. PbTX-3 (20 μM) shifted the activation of sodium channels to membrane potentials more negative than normal, but did not increase the maximum probability of channel opening near 0 mV. The effect was equivalent to a shift in the voltage-dependence of activation by -8 mV. PbTX-3 did not change the single channel mean open lifetime, which was approximately 330 μsec for all potentials between -50 and -20 mV. This lack of change in mean lifetime suggests that PbTX-3 did not alter the inactivation rate of sodium channels. There was also no change in single channel conductance. These results suggest that brevetoxin increases the probability that voltage-dependent sodium channels will open at rest and that the resulting depolarization is self-limiting due to the presence of normal inactivation. Preservation of normal inactivation distinguishes brevetoxin from other activators of the sodium channel, such as batrachotoxin.

241.5

MOLECULAR PROPERTIES OF SINGLE SODIUM CHANNELS FROM DISEASED AND NON-DISEASED HUMAN BRAIN IN BILAYERS.
C. Frenkel*, D.S. Duch*, E. Recio-Pinto* and B.W. Urban. Depts. of Anesthesiology and Physiology, Cornell Univ. Medical College, New York, N.Y. 10021.

With approval of our Committee on Human Rights in Research, cortical tissue samples were obtained from patients undergoing craniotomies for removal of diseased (tumorous) and non-diseased (aneurysm and hydrocephalus operations, etc.) tissues, and synaptosomes prepared. Channels were incorporated into lipid bilayers in the presence of batrachotoxin and characterized under our standard conditions (500mM NaCl). The single channel slope conductances were 26.3 ± 0.7 pS (S.D.; n=37 membranes, diseased) and 26.2 ± 0.5 pS (n=30 membranes, non-diseased); averaged steady state activation midpoints were -87.0 ± 10.4 mV (S.D.; n=16 membranes, diseased), and -85.1 ± 7.8 mV (n=7, non-diseased); fractional open times outside the gating region were 0.95 ± 0.03 (S.D.; n=14 membranes, diseased, and 0.94 ± 0.06 (n=11, non-diseased). Subconductance states of 5-6 pS as well as similar channel incorporation rates were observed in both preparations. These results indicate that single sodium channels, originating from either diseased or non-diseased human brain tissue, do not differ significantly in their measured characteristics. Preparations from either tissue may be used as viable sources for future bilayer studies of sodium channels and their electrophysiological and pharmacological properties.

241.6

VERATRIDINE MODIFICATION OF PURIFIED SODIUM CHANNELS FROM EEL ELECTROPLAX IN PLANAR BILAYERS.
D.S. Duch,* E. Recio-Pinto* and B.W. Urban (SPON: W.F. Riker). Depts. of Anesthesiology and Physiology, Cornell Univ. Med. Coll., New York, N.Y. 10021.

Highly purified, reconstituted sodium channels from the electric organ of the electric eel were incorporated into planar bilayers in the presence of veratridine. In symmetrical 500 mM NaCl, veratridine activated channels in a voltage-dependent manner, with depolarizing potentials increasing both the probability of channel opening and mean open times. Fractional open time reached half its maximum at -25 mV (physiological potential, at least 26 channels in 6 different membranes). I/V curves were linear with an average slope of the predominant single channel conductance of 11.3 ± 0.94 pS (sd;n=139). Smaller conductances ranging between 2 and 9 pS were also present in many membranes, representing approximately 25% of measured transitions. These smaller conductances were rarely found in the absence of larger conductances. TTX blocked channels in a voltage-dependent manner similar to BTX, with a $K_{\frac{1}{2}}$ value of 17.6 ± 1.7 nM (sem;n=6) at 0 mV. In contrast to the results with eel sodium channels, no 10-13 pS sodium channels were recorded when either dog (21 membranes with channel activity from 4 preparations) or human (3 membranes, 1 preparation) brain synaptosomes were fused with bilayers in the presence of veratridine. Instead, the primary conductances were 3-6 pS.

241.7

THE DROSOPHILA PARA LOCUS IS A SODIUM CHANNEL STRUCTURAL GENE
B. Ganetzky and K. Loughney*. Laboratory of Genetics, University of Wisconsin, Madison, WI 53706

The $para^{ts}$ mutation in Drosophila was previously shown to block conduction of nerve action potentials in a temperature-dependent fashion. Based on this and other observations it was proposed that the para locus affected sodium channels in some way (Wu and Ganetzky, Nature 286:814, 1980). To elucidate the molecular nature of the para gene product we cloned the para region by means of P-element transposon tagging (Loughney and Ganetzky, Soc. Neurosci. Abstr. 11:782, 1985; Ganetzky et al., Ann. New York Acad. Sci. 479:325, 1986). We mapped the extent of the para gene by localizing the molecular lesion associated with a number of mutant para alleles and found that it spans a region of at least 70 kb. cDNAs that derive from this region have now been isolated and their nucleotide sequence determined. The sequence analysis reveals that the para gene product shares extensive amino acid similarity with the alpha subunit of sodium channels from rat brain. The para amino acid sequence can be aligned with each of the four internally homologous domains of the rat channel. Furthermore, in each domain, the para gene product conserves the S1-S6 membrane spanning regions of the rat polypeptide (Noda et al. 320:188, 1986). This structural similarity between the rat sodium channel together with the patch-clamp evidence that para mutations disrupt sodium currents (O'Dowd et al. Soc. Neurosci. Abstr. 17: 577) argues strongly that para is a sodium channel structural gene. Although other sodium channel genes have been cloned from Drosophila by homology to the vertebrate gene, (Salkoff et al. Science 237:744, 1987), so far para is the only one of these for which mutations exist. Therefore, the para locus provides a unique opportunity for the detailed mutational analysis of sodium channel function, regulation and expression in the Drosophila nervous system.

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241.8

ISOLATION OF cDNA CLONES ENCODING A FULL LENGTH RAT SKELETAL MUSCLE SODIUM CHANNEL.
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To generate a cDNA clone encoding a full length rat skeletal muscle sodium channel, we have screened a denervated muscle cDNA library with two probes, a 1.2 kb cDNA encoding for the carboxyl terminus of the rat brain type II sodium channel and a 120 base cDNA encoding the first 40 amino acids of the rat brain type I sodium channel. Twelve cDNA clones were isolated and characterized. DNA sequence analysis of these clones reveals large regions of homology with the rat brain type II sodium channel. However, the predicted amino acid sequence of the muscle sodium channel differs from the brain and electroplax channels in several regions. These cDNA clones hybridize to a 9.5 kb mRNA on Northern blots of RNA isolated from neonatal, innervated adult and denervated adult rat skeletal muscle. A full length cDNA clone encoding a skeletal muscle α-subunit has been constructed from three overlapping cDNA clones. Expression of mRNA transcribed *in vitro* from this cDNA is being examined in a number of heterologous expression systems. Our results indicate that skeletal muscle sodium channels represent distinctive members of the mammalian sodium channel multigene family. A comparison of the predicted amino acid sequences between muscle and brain sodium channel proteins indicates regions which may be responsible for the observed differences in pharmacological and functional properties of these two channel classes.

241.9

A RAT BRAIN Na CHANNEL α SUBUNIT WITH ALTERED INACTIVATION PROPERTIES.
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We have investigated the macroscopic and single-channel properties of Na channels induced in *Xenopus* oocytes by *in vitro* transcripts of the rat brain IIA α subunit cDNA clone. Our data show that the steady-state voltage dependence of inactivation is shifted by 10 mV in the depolarizing direction relative to that for Na currents induced by injection of rat brain poly(A) RNA. In addition, the rate of recovery from inactivation is altered for rat IIA channels. When a train of depolarizing pulses is given from a holding potential of -100 mV to +10 mV at 10 Hz there is no change in the peak amplitude of Na current in oocytes injected with poly(A) RNA. In oocytes injected with rat IIA RNA there is a marked reduction in current amplitude during the train. Pulsing at 10 Hz reveals two kinetic components in the rat IIA currents. One component has a slow inactivation rate and is completely eliminated at this frequency while the other inactivates rapidly and is not removed at 10 Hz. The slow component is attenuated even for stimulation frequencies as slow as 1 Hz. The rate of recovery from inactivation of this slow component is voltage dependent. There is less attenuation of current if the holding potential is -120 mV as opposed to -80 mV. We have begun a quantitative characterization of the single-channel properties of Na channels induced by rat IIA RNA. At the single-channel level we see a decreased open time relative to Na channels from poly(A) RNA and an increased probability of reopening at a given potential. Supported by NS-11756, GM-10991, and fellowships from NIH and the Markey Trust.

241.10

LOW MW RNA ENCODED PROTEIN(S) MODIFIES THE FUNCTIONAL PROPERTIES OF THE RAT BRAIN Na CHANNEL.
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We have investigated the inactivation properties of Na channels induced in *Xenopus* oocytes by co-injection of low molecular weight (mw) RNA and RNA transcribed from the rat brain IIA α subunit cDNA clone. Na channels induced by injection of rat IIA RNA alone demonstrate 2-4 fold slower inactivation, a 10 mV shift in the steady-state voltage dependence, and a 20-25 mV shift in the voltage at which maximum inward current occurs compared to channels induced by rat brain poly(A) RNA. A low mw sucrose gradient fraction of rabbit brain RNA from 1-2.5 kb or a fraction < 4 kb from rat brain restores the rate and steady-state voltage dependence of inactivation to normal. These changes render the currents induced by rat IIA plus low mw RNA essentially indistinguishable from those induced by injection of poly(A) rat brain RNA with respect to inactivation. Although normal rapid inactivation is restored, the voltage for maximum inward current is not. This may have interesting implications for state models of the Na channel. We have begun single-channel recording from Na channels induced by injection of rat IIA RNA plus low mw rat brain RNA. Preliminary results indicate that the probability of re-opening is returned to values similar to those measured for Na channels induced by rat brain poly(A) RNA. We are currently employing a functional cDNA cloning strategy using the low mw RNA fractions to isolate a specific cDNA clone which modifies the rat brain α subunit. Supported by NS-11756, GM-10991, and fellowships from NIH and the Markey Trust.

242.1

DIFFERENCES IN THE ACTIVITY AND cAMP-DEPENDENT REGULATION OF NEW AND OLD ACETYLCHOLINE RECEPTORS ON BOVINE ADRENAL CHROMAFFIN CELLS. L.S. Higgins and D.K. Berg. Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093.

Activation of nicotinic acetylcholine receptors (AChRs) on bovine adrenal chromaffin cells leads to the release of endogenous catecholamines. Here we report that AChRs newly inserted into the plasma membrane appear to be more active than are older receptors. In addition, cAMP analogs substantially enhance the nicotinic response of older AChRs but have little effect on new AChRs.

cAMP analogs increased nicotine-induced ^{3}H-norepinephrine (^{3}H-NE) release from the cells 66±15% and increased the nicotine-induced membrane conductance (g_{nic}) 3-fold. No change was observed in K^+-induced ^{3}H-NE release or in the current-voltage relationship of the membrane, confirming that the increases represented effects on AChRs. The effect was specific for a cAMP-dependent process and acted on existing AChRs in the plasma membrane.

To compare the properties of new and old AChRs, cells with only new AChRs were obtained by allowing new AChRs to accumulate on the cells for 3-6 hours (ca. 0.2 AChR half lives) after either blocking existing AChRs with affinity alkylation or removing them with antigenic modulation. Cells with old AChRs were obtained by blocking the appearance of new receptors for 24 hours (ca. 1 AChR half life) with tunicamycin or puromycin, allowing existing receptors to age. Cells with new AChRs had 2-3 times more nicotinic response per equivalent number of AChRs than did control cells, assayed either by ^{3}H-NE release or by g_{nic}. The nicotinic response of cells with new AChRs was not increased by treatment with a cAMP analog. Cells with old AChRs had about the same nicotinic response per AChR as did control cells and the response was tripled by treatment with a cAMP analog. The results indicate that AChRs and/or associated components undergo a maturation in the plasma membrane that alters their function and regulation by second messengers. (Supported by NS12601 and NS25916)

242.2

CYCLIC AMP MODULATES ACETYLCHOLINE RESPONSES IN OLD BUT NOT YOUNG CHICK CILIARY GANGLION NEURONS. D. Gurantz*, V.E. Dionne*, D.K. Berg, and J.F. Margiotta. Depts. of Pharmacology and Biology, UCSD, La Jolla, CA 92093.

In ciliary ganglion neurons grown in culture, cyclic AMP (cAMP) analogues increase the peak whole-cell acetylcholine (ACh) current by 2-3 fold (Margiotta et al., PNAS 84:8155, 1987). The enhanced response probably reflects a cAMP-dependent activation of non-functional ACh receptors (AChRs) since neither the properties of functional AChRs, nor the total number of surface AChRs are altered by the treatment. To ask whether the cAMP-dependent AChR modulation is developmentally regulated, neurons isolated from young and older ganglia were incubated in saline ± 8-bromo-cAMP and IBMX for 6h. Treated and untreated neurons from young ganglia (E8,9) displayed similar whole-cell currents in response to microperfusion with 500 uM ACh. In contrast, treated neurons isolated from older ganglia (E13,14) displayed about 2-fold larger ACh currents (n=12) than did untreated controls (n=12), as was seen in neurons grown in culture for 5-8 d. The cAMP-dependent increase in ACh response observed in acutely isolated older neurons could not be accounted for by a proportional change in the total number of AChRs detected with ^{125}I-mAb 35. The results demonstrate that cAMP-dependent modulation of AChRs on ciliary ganglion neurons is developmentally regulated, and parallels other developmental changes in receptor function such as increased channel open time and reduced apparent affinity for ACh. Supported by NIH grants NS24417 and NS12601.

242.3

SUBUNIT COMPOSITION AND PHOSPHORYLATION OF NEURONAL ACETYLCHOLINE RECEPTORS FROM CHICK CILIARY GANGLIA. S.W. Halvorsen and D.K. Berg. Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093.

Chick ciliary ganglion neurons are parasympathetic and have nicotinic acetylcholine receptors (AChRs) that mediate primary synaptic transmission through the ganglion. The ACh response of the neurons in culture can be modulated by a cAMP-dependent process. We report here that AChRs purified from chick ciliary ganglia have a subunit composition similar to brain AChRs and different from muscle AChRs. In addition, one of the receptor subunits can be phosphorylated in the membrane by cAMP-dependent protein kinase.

AChRs were isolated from embryonic ganglia by ion exchange and immunoaffinity chromatography. Analysis of the purified and radioiodinated material by SDS-PAGE revealed two major polypeptides with M_rs of 52 K and 60 K. Both components appear to be constituents of the AChR since they were specifically depleted from ganglion extracts by the immunoaffinity column and were prevented from binding to the column when an excess of anti-AChR antibody was used to compete with the immunoaffinity resin for the receptor. Partial proteolytic digestion of the isolated polypeptides with papain produced distinct peptide maps for the two species, indicating that they represent separate subunits. Both subunits are glycoproteins since their mobilities on SDS-PAGE were markedly increased by treatment with glycopeptidase F.

The ability of the receptor to serve as a substrate for cAMP-dependent protein kinase was examined by incubating a ganglionic membrane fraction with the catalytic subunit of the enzyme and [γ-^{32}P]ATP. AChRs were then immunoaffinity purified and analyzed by SDS-PAGE autoradiography. A 60 K [^{32}P]-labeled protein was identified, corresponding to the 60 K AChR subunit. Additional experiments will be required to determine the significance of the phosphorylation for receptor regulation. (Supported by NS12601 and NS25916)

242.4

EXPRESSION OF NICOTINIC ACETYLCHOLINE RECEPTORS AND RECEPTOR mRNA IN A SUBPOPULATION OF CHICK DORSAL ROOT GANGLION NEURONS *IN VIVO*. R.T. Boyd[1], M.H. Jacob[2], A.E. McEachern[1], and D.K. Berg[1]. [1]Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093 and [2]Worcester Foundation for Exptl. Biology, Shrewsbury, MA 01545.

The dorsal root ganglion (DRG) contains subpopulations of neurons that can be distinguished by various criteria including size, fields of innervation, and neuropeptide content. We report here that a stable proportion of DRG neurons *in vivo* expresses an mRNA encoding the neuronal acetylcholine receptor (AChR) alpha3 subunit, and many DRG neurons have functional nicotinic AChRs.

In situ hybridization on cryostat sections with an alpha3 probe at high stringency revealed alpha3 RNA in chick DRG neurons at all ages examined (embryonic day 8 to posthatch 1 year). Little decline occurred in the size of the neuronal subpopulation with age: 22 ± 2% of the neurons in DRG sections from 1 month old chicks contained detectable hybridizing material as did 18 ± 2% of the neurons in DRG sections from one year old chickens. Northern analysis and RNase protection experiments found no differences in the alpha3 gene product in dorsal root ganglia and that expressed in brain and ciliary ganglia.

The presence of AChRs in DRG neurons was confirmed by immunological and electrophysiological studies. An anti-AChR monoclonal antibody together with fluorescently labeled avidin-biotin antibody complex detected AChRs in 16 ± 3% of neurons in 18 day embryonic DRG sections. Intracellular recording from freshly dissociated neurons of the same age indicated that a quarter of the cells (16 out of 67 tested) had ACh sensitivities that averaged about 30% as high as those observed for ciliary ganglion neurons in culture. Another quarter (16 out of 67) had lower levels of ACh sensitivity while half of the neurons had no detectable response to ACh. d-Tubocurarine (100 µM) reversibly blocked the ACh responses. The results demonstrate that a subpopulation of chicken DRG neurons expresses functional nicotinic AChRs *in vivo*. (Supported by NS12601 and NS21725)

242.5

EFFECTS OF DENERVATION ON CLUSTERS OF ACETYLCHOLINE RECEPTOR-LIKE MOLECULES ON THE SURFACE OF CARDIAC GANGLION CELLS. P.B. Sargent, D. Pang*, and J.M. Lindstrom, Division of Biomedical Sciences, University of California, Riverside, CA 92521, and The Salk Institute for Biological Studies, San Diego, CA 92138.

The effect of denervation on the number, size and distribution of clusters of nicotinic acetylcholine receptor (AChR)-like molecules was examined in the parasympathetic cardiac ganglion of the frog *Rana pipiens*. AChR-like immunoreactivity was measured using immunofluorescence and immunoperoxidase techniques and a monoclonal antibody (mAb 22) made against AChRs from *Electrophorus electricus* which cross-reacts with AChR-like molecules on the cardiac ganglion cell surface. In normally innervated ganglia peroxidase staining is found in small patches occupying about 0.4% of the cell surface. Most (about 80%) of the patches are located at sites of synaptic bouton contact, where the immunoreactivity is found in register with the postsynaptic electron-dense specialization. Immunofluorescence studies indicate that several patches of immunoreactivity are often associated with single boutons, which can have several active zones. Following 2 weeks of denervation there is a 30% decrease in the number of patches of AChR-like immunoreactivity on the cell surface and a 4-fold decrease in their size. Immunoreactive patches on denervated neurons tend to be more dispersed than normal. These results suggest that subsynaptic clusters of AChRs on the ganglion cell surface are not stable following the loss of innervation. Immunoperoxidase studies using mAb 22 in frog skeletal muscle indicate no loss of AChR-like patches nor any change in their size after 2 weeks of denervation. Thus, the myofibers and neurons studied here respond differently to denervation: AChR clusters appear to be stable after short periods of denervation in muscle but not in nerve. (Supported by NIH NS24207).

242.6

CHOLINERGIC LIGAND EFFECTS ON EXPRESSION OF FUNCTIONAL NICOTINIC RECEPTORS BY THE TE671 HUMAN MEDULLOBLASTOMA CLONAL LINE. Ronald J. Lukas, Division of Neurobiology, Barrow Neurological Institute, 350 West Thomas Road, Phoenix, AZ 85013.

Cells of the TE671 cerebellar line are of primitive neuroectodermal origin and express nicotinic acetylcholine receptors (nAChR) that are fully sensitive to functional blockade by alpha-bungarotoxin (Bgt) and otherwise resemble the class of nAChR that also are found at the vertebrate neuromuscular junction. Other studies from this laboratory have shown that chronic treatment (24-48 hr) of TE671 cells with certain nicotinic agonists induces an increase in the expression of cell surface and crude particulate fraction binding sites for radiolabeled Bgt. This report concerns the contrasting effects of chronic drug treatments on the expression by TE671 cells of functional nAChR detected by the use of $^{86}Rb^+$ ion efflux assays.

Loss of functional nAChR activity is seen following chronic exposure to carbamylcholine or nicotine with dose-dependences comparable to those observed for activation of nAChR function by shorter term (1-5 min) exposure to carb or nic, evolves quickly ($T_{1/2}$=5 min) and reverses slowly (est. $T_{1/2}$=24 hr). Effects of chronic agonist treatment are not blocked by coincubation with d-tubocurarine (d-TC) or decamethonium (C_{10}), but rather are mimicked by chronic exposure to d-TC or C_{10} alone with a dose-dependence that is similar to that observed for d-TC- or C_{10}-mediated blockade of shorter-term agonist activation of nAChR function. By contrast, chronic pancuronium or alcuronium (1mM) treatment has no effect alone, but rather protects functional nAChR sites from agonist-induced down-regulation. The full presentation of these data will include discussions of technical considerations and the general implications of these results on the interpretation of data concerning and the mechanisms underlying the regulation of receptor expression.

242.7

CONFORMATIONAL CHANGES DURING FORMATION OF THE COMPLEX BETWEEN α-BUNGAROTOXIN AND SYNTHETIC PEPTIDES THAT MIMIC A BINDING DOMAIN. E. Hawrot and Q.-L. Shi*. Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The region containing residues 173-204 of the α-subunit of the nicotinic acetylcholine receptor is a major determinant of the α-bungarotoxin (BGTX) binding site as shown by a number of studies including those utilizing synthetic peptide mimics (prototopes). Several shorter peptides within this region also bind BGTX but often with reduced affinity as compared to the 32mer. Previous binding studies have relied on solid-phase assays that may underestimate binding affinities. We have now developed soluble binding assays based on spectroscopic techniques which, in addition, provide valuable structural information and reveal a number of conformational changes that occur upon complex formation.

We have used circular dichroism (CD) and proton nuclear magnetic resonance (NMR) to measure BGTX binding to several short synthetic peptides. CD changes upon binding provide a sensitive probe of changes in secondary and tertiary structure in a number of other ligand-protein interactions. We detect large CD changes in both the near and far UV regions upon addition of equimolar amounts (2.5-60 μM) of BGTX and a synthetic 18mer (α-residues 181-198). The far UV CD spectrum is indicative of a large increase in β structure, whereas the near UV CD spectrum strongly suggests a large change in the environment of the side chains, especially the aromatic residues as well as those regions surrounding the disulfide bridges in BGTX. Binding could be detected under acidic conditions (pH 3) as well as at pH 7. Similar CD changes occur upon addition of BGTX to a synthetic 12mer containing α-residues 185-196 and indicate an apparent K_D of 1 μM. NMR investigations confirm the structural changes in aromatic residues of both BGTX and the synthetic peptides. Measurements of the spin-lattice relaxation rates (T_1) for the aromatic protons in the 12mer indicate a decreased mobility of these residues upon binding. In addition, a disulfide bond between the adjacent cysteines (Cys192-Cys193) does not appear to be essential for BGTX binding as monitored in CD assays. An 11mer (α-residues 186-196) with an Ala substituted for Cys192 continues to bind BGTX as measured by changes in CD. Binding does, however, become pH sensitive with this amino acid substitution, being undetectable under acidic conditions.

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242.8

MONOCLONAL ANTIBODIES AGAINST α-BUNGAROTOXIN RECOGNIZE NICOTINIC BUT NOT MUSCARINIC RECEPTOR LIGANDS. M. Quik, S. Geertsen*, R.L. Kenigsberg* and A.C. Cuello (SPON: P. Etienne). Dept. Pharmacol., McGill U., Montreal, Canada.

The aim of this study was to raise monoclonal antibodies (mAb) against α-bungarotoxin (α-BGT) which could recognize an epitope on the toxin molecule that interacted with the receptor. Hybridomas secreting mAbs against α-BGT were produced from the fusions of lymphocytes from hyperimmune animals with myeloma cells. Screening for the presence of anti-α-BGT Abs was done using the ^{125}I-α-BGT binding assay. Several mAbs were identified which interfered with the binding of α-BGT. Their crossreactivity with cholinergic receptor ligands was subsequently investigated. MAb spB57 interacted most potently with nicotinic agonists; it also interacted with d-tubocurarine, but to a lesser degree, and not with other nicotinic antagonists. Muscarinic agents did not crossreact with spB57. Another mAb nsB8 also bound to α-BGT; however, cholinergic ligands did not interact with nsB8.

These results indicate that mAb spB57 raised against α-BGT crossreacts more potently with nicotinic agonists than antagonists. This could suggest that the α-BGT site is more closely linked to the agonist recognition site than the site to which nicotinic antagonists bind. This latter feature may distinguish the nicotinic α-BGT site from other nicotinic receptor populations.

242.9

STIMULATION OF ARACHIDONIC ACID RELEASE IN TRANSFECTED CELLS EXPRESSING CLONED MUSCARINIC RECEPTORS.

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A family of genes encoding four distinct muscarinic receptors (m1-m4) have been cloned (Science 237, 527), and stably expressed in A9 L cells. When the m1 and m3 receptors were stimulated with carbachol there was a rapid rise of liberated arachidonic acid (AA), cAMP, and phosphatidylinositol (PI) turnover, while m2 and m4 receptor stimulation had no effect on these second messengers. Pretreatment with the phorbol ester PMA for 30 minutes caused a marked acceleration and amplification of m1 and m3 receptor mediated AA release. In contrast m1 and m3 mediated PI turnover was inhibited by a 30 minute PMA pretreatment. PMA caused no change in basal AA release or PI turnover. To examine the role of protein kinase C, the cells were preincubated with PMA for 24 hours to desensitize protein kinase C, and the PMA amplification of AA release was abolished. AA release was also unaffected by the cAMP analog, dibutyryl cAMP, or the phosphodiesterase inhibitor IBMX. The phospholipase A_2 (PLA_2) inhibitor mepacrine (100μM) inhibited 79% of the AA release while inhibiting 30% of PI turnover.

Carbachol stimulation of the m1 and m3 receptors also decreased mitogenesis as measured by [3H] thymidine incorporation. The cyclooxygenase inhibitor, indomethacin (10μM) partially blocked (20%) the carbachol induced inhibition of mitogenesis of m1 transfected cells while having no effect on basal mitogenesis.

These experiments demonstrate that AA release by the m1 and m3 receptors is regulated independently of PI turnover and cAMP accumulation. Furthermore the differential effects of PMA and the PLA_2 inhibitor on AA release and PI turnover indicates that the main source of AA release mediated by m1 and m3 muscarinic receptor activation is phospholipase A_2.

242.10

Cloned muscarinic receptors couple to different G-proteins and second messengers. Mark R. Brann*, Bruce R. Conklin*, S. V. Pe nelope Jones*, Nick M. Dean*, Regina M. Collins*, Tom I. Bonner* and Noel J. Buckley*.(SPON: M. Walton) Laboratories of Molecular Biology & Neurophysiology, NINCDS, Laboratory of Cell Biology, NIMH, Laboratory of Biological Chemistry, NCI, Metabolic Diseases Branch, NIDDK, National Institutes of Health, Bethesda MD 20892.

The existence of four distinct muscarinic receptor subtypes (m1-m4) has recently been shown using molecular cloning methods (Science 237, 527). The ability of these receptors to couple to different signal transducing G-proteins and second messenger systems, was examined using A9 L cell lines stably expressing each of the cloned receptors. The interactions of the muscarinic receptors with G-proteins were characterized by measuring the effect of guanine nucleotides, and pertussis and cholera toxins on the affinity of the agonist carbachol for each receptor. Guanine nucleotides decreased the affinity of carbachol for the m1, m2, and m3 receptors, but had no effect on m4 receptors, suggesting that m4 receptors do not couple with a G-protein endogenous to A9 L cells. Pertussis and cholera toxin decreased the affinity of carbachol for m2 but not to m1 and m3 receptors, indicating that m2 receptors couple to a different G-protein. Application of agonist to m1 and m3 receptors increased cAMP levels, phosphatidylinositol hydrolysis, arachidonic acid release and conductance through Ca++ dependent potassium channels. Pertussis and cholera toxin treatments had no effect on these responses, and the m2 and m4 receptors had no detectable effects on these second messengers. These data suggest that muscarinic receptors couple to different G-proteins and second messengers.

242.11

AN M_1 MUSCARINIC RECEPTOR EXPRESSED IN THE TRANSFECTED MURINE FIBROBLAST B82 CELLS IS COUPLED TO THE HYDROLYSIS OF INOSITOL LIPIDS. L. Mei, J. Lai, W.R. Roeske, J.C. Venter and H.I. Yamamura. Depts of Pharmacology & Internal Medicine, Univ. Arizona, Tucson, AZ 85724; Section of Receptor Biochemistry and Molecular Biology, LCMN, MINCDS, NIH, Bethesda, MD 20892.

The radioligand binding properties of the muscarinic receptor expressed in the transfected B82 cells and its possible coupling interactions were studied. [^{3}H](-)QNB binding to the intact transfected cells was saturable and displaceable by atropine. The K_d and B_{max} values of [^{3}H](-)QNB were 12 pM and 17 fmol/10^6 cells, respectively. Inhibition studies of [^{3}H](-)QNB binding to the intact transfected cells suggested that the muscarinic receptor is of an M_1 type since it has high affinity for pirenzepine but low affinity for AF-DX 116. The muscarinic agonist carbachol stimulated [^{3}H]IP_1 accumulation in the transfected B82 cells, which could be inhibited by pretreatment of the cells with pertussis toxin or by muscarinic antagonists. The rank order of muscarinic antagonists in inhibiting carbachol-induced [^{3}H]IP_1 accumulation was atropine > PZ > AF-DX 116, in a good agreement with that from ligand/[^{3}H](-)QNB competition studies. Carbachol at 10 mM had neither stimulatory nor inhibitory effect on the basal or PGE_1-induced cAMP formation in the presence of 5 mM IBMX. These results suggest that the M_1 muscarinic receptor expressed in the transfected B82 cells is coupled to the hydrolysis of PI possibly via a pertussis toxin-sensitive G protein.

242.12

USE OF BACCULOVIRUS FOR THE EXPRESSION OF CLONED DNA ENCODING G-PROTEIN COUPLED RECEPTORS. M. Dennis*, M. Caron* and P. Payette* (SPON: E. MacKenzie) Biotechnology Research Institute, 6100 Royalmount Ave., Montréal, Québec, Canada, H4P 2R2.

Detailed studies of the molecular basis of receptor function require homogeneous preparations of receptor protein in sufficient amounts for biochemical and biophysical analyses. We are investigating the use of bacculovirus for the high-level expression of two G-protein coupled receptors from cloned DNA in insect cells. Genomic clones encoding yeast α-mating factor receptor STE2; a gift from J. Thorner, Univ. of Calif.) and human muscarinic receptor HM1 subtype (generously provided by D. Capon, Genentech, Inc.) were subcloned into the F1 - containing plasmid pTz and single stranded DNA prepared. Synthesis of the second strands was primed with synthetic oligonucleotides (32-42 n) complementary to the 5' and 3' ends of the coding sequences, but containing mismatches specifying Bam H1 restriction sites, and transformants screened by hydridization at high stringency with the ^{32}P-labeled oligonucleotides. Two rounds of mutagenesis yielded plasmids containing the yeast STE2 coding sequence flanked by Bam H1 sites; analogous work is proceeding with the human HM1 sequence. The coding portions for the two receptor genes are to be cloned into the Bam H1 site of the plasmid pAc 373 for homologous recombination into bacculovirus and expression in SF9 insect cells. This work was supported in part by the Medical Research Council.

243.1

SCOTOMA INDUCED BY FOCAL ACTIVATION OF CALCARINE CORTEX WITH THE MAGNETIC COIL. R.Q. Cracco, V.E. Amassian, P.J. Maccabee*, J.B. Cracco*, A. Rudell* and L. Eberle*. Depts. of Neurology and Physiology, SUNY, Health Sci. Ctr. at Brooklyn, New York 11203.

Using ourselves as subjects, the effect of magnetic coil (MC) stimulation on perception of letters randomly permutated in groups of 3 by a computer and displayed horizontally or vertically was studied. Topographical relations were investigated by placing a Cadwell MC tangentially on the scalp such that the distal edge was 2 cm above the inion and then shifting it to the left or right using a visual stimulus - MC pulse interval of 100 ms. Letters were rarely correctly reported with midline placements of the MC but shifting it to the left or right caused more errors in reporting letters at the extreme right or left, respectively, implying a focal cortical effect. Similarly, shifting the MC rostrally caused failure in reporting the bottom letter of a vertical array.

Stimuli delivered by the MC at midline locations were then given 0 to 200 ms after the visual stimulus at 20 ms increments. The letters were nearly always correctly reported at visual stimulus - MC pulse intervals either less than 60-80 ms or greater than 120-140 ms; letters were rarely correctly reported at intermediate intervals of 80-100 ms. Thus by 120-140 ms after the visual stimulus, information related to letter recognition is relayed from human calcarine cortex.

243.2

LOCAL CONNECTIONS OF HUMAN VISUAL CORTEX. A. Burkhalter and K.L. Bernardo*. Department of Neurosurgery & McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

Local connections in the human cortex are largely unknown. We have, therefore, begun to study connections within primary visual cortex (V1) and its reciprocal projections with area V2, using fiber tracing with the fluorescent dye, DiI, (Godement, P., et al., Development, 101:697, 1987) in post-mortem, fixed human visual cortex.

Experiments were performed in 2 adults. After injections into V1, tangential sections through layers 2 and 3 showed a sunburst pattern of labeled terminal clusters (300um in diameter) surrounding the injection site, similar to the projections between blobs, or between interblob regions, in non-human primates. In transverse sections we identified clustered, interlaminar projections from layer 2/3 to 5, layer 5 to 2/3 and 4Cb to 4A. The projections from V1 to V2 terminated in layers 2,3 and 4, avoiding infragranular layers. Large V2 injections revealed projections to V1 terminating in 300um wide clusters in layers 2,3,4A and 5. The center-to-center spacing was 500um. These results show that modern tracing techniques can be used to study connections in the human brain. In addition, they demonstrate that the local connections in V1 in humans and monkey are similar, and that the reciprocal connections between V1 and V2 show the laminar organization typical for forward and feedback projections. (Supported by NIH grant EY05935).

243.3

CYTOCHROME OXIDASE IMMUNOHISTOCHEMISTRY IN THE PRIMATE VISUAL CORTEX. R. F. Hevner and M. Wong-Riley. Department of Anatomy & Cellular Biology, Medical College of Wisconsin, Milwaukee, WI, 53226.

Studies of various brain regions using cytochrome oxidase (C.O.) histochemistry have shown that C.O. activity is not distributed homogeneously between and within these regions. In the primate visual cortex, C.O. activity forms distinct laminar and areal patterns (*e.g.*, puffs). This pattern can change over time in response to altered impulse activity from the retina (Wong-Riley and Carroll, Nature 307, 1984). How then is local C.O. activity regulated in the brain? We hypothesized that the amount of C.O. protein might be an important factor. We chose to test our hypothesis by C.O. immunohistochemistry. We reported last year that we had successfully purified C.O. (from calf brain) for use as an antigen. Since then we have obtained antisera from rabbits immunized with C.O. Antibodies to C.O. were demonstrated by ELISA, indirect immunoprecipitation of C.O. with protein A-Sepharose 4B, and slot blot. The antibodies reacted intensely with C.O. subunit IV by Western blot. We applied the antibodies in immunohistochemical studies of the macaque striate cortex, using the indirect immunoperoxidase and ABC methods. In sections cut tangential or perpendicular to the cortical surface, the immunohistochemical pattern closely resembled that seen by C.O. histochemistry. In particular, puffs of high C.O. immunoreactivity were visible in the supragranular layers in either plane of section. Omission of the primary antibody or substitution of pre-immune serum abolished the pattern.

These results support our hypothesis that the amount of brain C.O. protein is directly related to the activity of C.O. under normal conditions. We can not, however, rule out a role for variable molecular activity (turnover number) of the enzyme. In future studies we will see if the relationship between C.O. activity and amount holds true under experimental conditions.

Macaque brains were gifts of Drs. V. Haughton and H. Canh. (Supported by NIH NS18122 & EY05439 to MWR, and an MCW MSTP Fellowship to RFH.)

243.4

IMMUNOSTAINING REVEALS MORPHOLOGY AND DISTRIBUTION OF MEYNERT CELLS IN PRIMATE VISUAL CORTEX. B.A. McGuire and J.R. Naegele. The Rockefeller University, New York, NY 10021.

Meynert cells have been intriguing due to their large size, sparse distribution and projections to extrastriate areas such as MT. To learn more about their quantitative anatomy, we have stained macaque striate cortex with a monoclonal antibody raised against fixed cat lateral geniculate nucleus. Several neuronal cell types were immunoreactive, however Meynert cells were identified by their location in uppermost layer 6 and by their distinctively large size: the diameter of the labeled somas averaged about 27 μm, and that of the basal dendritic trees measured up to 1.4 mm. The immunoreactivity diffusely filled the cytoplasm and dendritic shafts, but left spines unstained. All layer 6A neurons of comparable size were immunoreactive, suggesting that every Meynert cell was stained. The population was distributed with some regularity, though some cell-free gaps of 300-400 μm in diameter were also present. The average area of the basal dendritic tree reconstructed for 7 cells was 0.412 mm^2, giving an overall coverage of 9.6. It will be important to determine the correspondence between these immunoreactive Meynert cells and both the cytochrome oxidase-rich blob columns, thought to be important in color processing, and the area MT-projecting cells, believed to be an important component of a system for analyzing motion.

243.5

SEGREGATION OF NEUROPEPTIDE Y-CONTAINING NEURONS OUTSIDE CYTOCHROME OXIDASE PUFFS IN THE MONKEY VISUAL CORTEX DEVELOPS IN THE ABSENCE OF VISUAL INPUT. R.O. Kuljis and P. Rakic. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510-8001.

Neuropeptide Y-containing neurons (NPYn) are situated predominantly outside the cytochrome oxidase (CO) puffs in layers II/III of macaque striate cortex (Kuljis & Rakic, Neurosci. Abs. '87). The distribution of NPYn (avidin-biotin-peroxidase) in relation to CO puffs (histochemistry) was analyzed in layers II/III of a normal newborn, and a 10-month old macaque enucleated binocularly six weeks before birth. Findings are expressed as: total No. of NPY neurons/No. neurons inside puffs/No. neurons expected inside puffs if uniformly distributed/% volume of layers II/III occupied by puffs/statistical significance (Chi square). In the neonate: 104/15/23/22%/p<0.46. In the enucleate: 130/1/16/12%/p<0.05. These findings indicate that the segregation of NPYn observed in adult animals begins prenatally and is completed postnatally. Visual experience (absent in the neonate), and in particular, retinal input (absent in the enucleate), appear not essential to trigger or maintain the segregation.

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243.6

ARBORIZATION OF INDIVIDUAL AXONS FROM AREA V2 TO V1 IN THE MACAQUE MONKEY: A STUDY USING PHA-L. K.S. Rockland and A. Virga*. Eye Research Inst., Boston, MA. 02114 and ENRM V.A. Hospital, Bedford, MA. 01730.

In the macaque monkey, area V2 projects back to V1, with terminations (labeled by HRP or 3H-amino acids) mainly in layers 1 and 5. We have injected Phaseolus vulgaris (PHA-L) into area V2 in 4 monkeys, with the goal of clarifying aspects of axon morphology, as described below. Branching and Extent. Two axons (of 18 reconstructed) terminated in a "restricted" pattern: a single cluster (300um or 500um) in layers 1 and 2. More typically (16 profiles), axons ascended (either unbranched or with branches in the deeper gray matter) to L.1, where they had a "widespread" distribution. After branching asymmetrically, they traveled in L.1 for 0.75-0.90mm(6 axons), 1.0-1.8mm (5 axons), 2.2-2.8mm (4 axons), or 3.45mm (1 axon). Thus, individual axons from V2 can traverse 1-3 hypercolumn units in V1. Terminations. Delicate sprays of boutons occurred at intervals along the main trunk in L.1. Spacing, although variable, tended to be 300um or 600um. Frequently, secondary branches, parallel to the main branch in L.1, had scattered boutons along their length, as well as clustered sprays. Both beads and spines were observed. Layers. Supragranular terminations occurred mainly in L.1, but also L.2. There were arborizations in L.5; but these were consistently more restricted (200um-500um wide) than the supragranular ones. (EY07058)

243.7

VISUAL RESPONSES OF NEURONS IN AREA V2 AND IN THE SUPERIOR TEMPORAL SULCUS OF THE MACAQUE MONKEY DURING REVERSIBLE INACTIVATION OF AREA V1. **J. Bullier and P. Girard*. INSERM U 94, 16 av. Doyen Lépine, 69500 BRON, FRANCE.**

We have examined how the reversible inactivation of area V1 by cooling affects the responses of cells in area V2 and in the superior temporal sulcus (STS). We first determined that neural responses in area V1 are completely blocked for temperatures ranging between 6 and 20°C. After having measured the temperature gradients in the cortex, we recorded in a region of V2 sufficiently distant from the cooled region that the neuronal activity could not be directly blocked by the cold. Recording was also restricted to a region of V2 representing the same portion of the visual field as the cooled area in V1.

In these conditions, the overwhelming majority of area V2 neurons (215 out of 218 recorded sites) ceased to respond when V1 was reversibly inactivated.

In contrast, in MT and surrounding areas in the STS, many neurons with receptive fields contained within the region of the visual field represented in the cooled region of V1 remained active when V1 was reversibly blocked.

These results suggest that, for a number of neurons in STS, the visual information is not exclusively channeled through area V1. Area V2, on the other hand, appears to be almost entirely under the control of area V1.

243.8

CORTICAL CONNECTIONS OF MT AND DL IN THE PROSIMIAN GALAGO: EVIDENCE THAT MODULAR SEGREGATION OF PARALLEL PATHWAYS IS A PRIMITIVE FEATURE IN PRIMATES. L. A. Krubitzer and J. H. Kaas. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

In monkeys, parallel streams of visual processing involve neurons that project from distinct modules in area 17 to separate bands of neurons in area 18, which in turn relay either to the middle temporal visual area (MT) and onto the parietal lobe, or to the dorsolateral visual area (DL or V-4) and onto the temporal lobe. A demonstration of such connections in prosimian primates would suggest that these streams developed early in primate evolution, and a segregation of parallel streams is a general feature of primate visual cortex. To investigate this possibility, we injected one retrogradely transported tracer (fast blue) into MT, and another (diamidino yellow) into DL in the same galago (Senegalensis) to determine if inputs from area 18 were nonoverlapping. Cortex was flattened and cut parallel to the surface to favor visualization of bandlike patterns of label in area 18. Alternate sections were stained for cytochrome oxidase (CO). The CO dense bands typically seen in area 18 of monkeys were only weakly and inconsistantly revealed in galagos. Nevertheless, neurons labeled by MT injections were completely segregated from neurons labeled by DL injections. The zones of label from DL and MT while in the same general region of area 18 were offset from each other. Neurons related to both injections formed bandlike arrays, as in monkeys. Thus, separate visual processing streams related to temporal and parietal lobe functions probably differentiated over 50 million years ago, perhaps even before the primate divergence from Toketherian mammals. Supported by EY-02686.

243.9

DISTRIBUTION AND MORPHOLOGY OF NEURONS IN CAT AREA 17 THAT PROJECT TO THE MEDIAL BANK OF THE LATERAL SUPRASYLVIAN SULCUS. G. Einstein and D. Fitzpatrick. Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

We have used retrograde transport tract tracing methods combined with intracellular injections of lucifer yellow in lightly fixed tissue sections (Einstein and Fitzpatrick, Soc. Neurosci. Abstr., 13:676, 1987) to study the distribution and morphology of Area 17 neurons that project to the lateral suprasylvian sulcus (LS). Most of the LS projection originates from a heterogeneous population of pyramidal neurons distributed from the top of layer II to the upper portion of layer IV. These range in size from 100 um^2 to 500 um^2 and exhibit a variety of dendritic branching patterns; some neurons, at the top of layer II, have nearly radial dendritic fields that ramify extensively in layer I while others, in III and IV, have more conventional apical and basal dendritic systems. A population of neurons in the infragranular layers also projects to LS; among these are large pyramidal cells in layer V and small, bitufted neurons in layer VI. The morphological variation observed within and between layers suggests that the projection from Area 17 to LS comprises functionally diverse classes of neurons. Since this projection arises, in part, from the infragranular layers some of these neurons may also project to subcortical targets. Supported by NEI grants EY06661 and EY05546.

243.10

PRESUMED AFFERENTS FROM AREAS 17 AND 18 TO THE CAT'S CLARE-BISHOP AREA: RESPONSE PROPERTIES AND FUNCTIONAL ORGANIZATION. Helen Sherk Dept. of Biological Structure, U. of Washington, Seattle, WA 98195.

Areas 17 and 18 in the cat have two major cortical targets, area 19 and the Clare-Bishop area (CB). A novel technique was used to investigate the responses and functional organization of the of the pathway to CB. Local injections of kainic acid were made in CB to silence neurons; it was then possible to record directly from what appeared to be axon terminals. Their response properties indicated that these were cortical in origin, most likely from areas 17 and 18. Axons from both simple and complex cells could be identified.

A detailed comparison was made between a sample of about 400 afferents and a similar sample from laminae 2+3 of area 17. Response properties were quite similar. A somewhat larger fraction of the afferents were direction-selective. End-stopping, on the other hand, was extremely rare. Afferents' response characteristics were markedly different from those of neurons in CB.

In long tangential penetrations through CB, afferents showed a clear organization according to preferred orientation. Within this framework, afferents were also ordered according to preferred direction.

Supported by EY04847 and the Alfred Sloan Foundation.

243.11

BANDED CONNECTIONS BETWEEN AREAS 17 AND PMLS IN CAT VISUAL CORTEX: POSSIBLE FUNCTIONAL ROLE FOR GLOBAL MOTION PROCESSING. Josef P. Rauschecker and Andreas Kreiter*, Max-Planck-Institut für biologische Kybernetik, Tübingen, FRG

We have reported recently (Rauschecker et al., J. Neurosci. 7: 943-958, 1987) that many neurons in the cat's lateral suprasylvian visual cortex (PMLS) prefer stimulus movement away from the area centralis ("centrifugal motion"). This finding gave a hint as to a possible functional role of PMLS in the evaluation of optic flow and 3-D motion. We are now trying to determine how this global network property is generated from other inputs. It is known that PMLS receives inputs from cortical areas 17, 18 and 19, among others, as well as from parts of the LP-pulvinar complex that show the same kind of centrifugal direction bias as PMLS (Rauschecker, Progr. in Brain Res., 1988). Here, we are especially interested in the organization of cortical input to PMLS and have started investigations using two different experimental approaches.

Physiologically, we have corroborated our original finding with more quantitative and randomized methods. In addition, using global flow field stimuli reveal cooperative effects between different parts of the visual field that may in part be responsible for the global processing properties found. Anatomically, by making small injections of rhodamine labelled latex beads and/or fluorogold into a localized part of PMLS (after identifying the direction preference of the same site by unit recording), we find that one such "column" receives input from distinct clusters of cells in area 17. In serial reconstructions these clusters form parallel bands of labelled cells, often with a periodicity close to 0.5 mm. As a next step we are doing simultaneous recording from PMLS and from area 17 trying to correlate the activity of neurons in both areas with regard to direction preference.

243.12

BACKGROUND DEPENDENCE OF DIRECTION SELECTIVITY IN CORTICAL NEURONS OF AREAS 17, 18, 19, 7 AND PMLS OF THE CAT. **K. Krüger*, H.R.O. Dinse (SPON. K. Behrend), Dept. of Zoology III, Biophys. Sect., University of Mainz, Saarstr. 21, D-65 Mainz, West Germany**

Neurons in visual cortical areas 17, 18, 19, 7 and PMLS were stimulated with a bar superimposed on a large field visual noise (2-dimensional Gaussian white noise covering 50 x 50 degrees of the visual field). The bar was always moved with optimal orientation, length and velocity, the background was kept stationary or moved at different velocities inphase or anti-phase to the bar. An index (DI) of the degree of direction selectivity was determined on the basis of the maximal amplitude in the PSTHs. According to this index, all cells were subdivided in three classes of direction selectivity (non-direction selective, direction sensitive, direction selective). For each class we determined the percentage of cells whose classification of directionality could be influenced by changes of the background condition.

1. The percentage of direction selective cells is very specific in the 5 cortical areas tested. Direction selective cells (DI > 50) were found predominantly in area 18 and PMLS. 2. Direction selectivity of a cell depends on the stimulus (background condition) in 70% of all tested neurons. 3. While in areas 17, 18, 19 and area 7 only 15-30% of all neurons are "direction-permanent" for all conditions tested, i.e. their degree of directionality is not influenced by the background condition, PMLS is a rather special case insofar as more than 50% of all PMLS cells are "direction permanent". We found no difference for the two eccentricity classes tested (10 deg within area centralis, peripheral visual field).

We conclude that PMLS is especially qualified to code absolute directions of motion independently of the stimulus configuration.

244.1

A PARADOXICAL RESULT: INHIBITION *INCREASES* OPTIC NERVE ACTIVITY. R. B. Barlow, Jr., Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

Inhibition has long been known to have a major role in processing information in the visual system. Transmission of inhibitory signals among neighboring elements of the retina can modulate gain and enhance borders and edges in the visual field. These inhibitory actions extend the dynamic range of the retina and enhance contrast sensitivity.

I report here that inhibition in the *Limulus* eye may serve the additional function of preserving the response of the light-adapted retina. Exposing a single retinal receptor (ommatidium) to daytime illumination for >15 min often causes a complete cessation of its optic nerve response.

A normal response can be restored immediately upon illumination of a nearby region of the retina to generate inhibition. Offset of illumination of the neighboring region again leads to a rapid cessation of the optic nerve activity from the recorded unit.

A paradoxical result: onset of inhibition increases optic nerve activity while offset of inhibition decreases it.

Inhibition thus appears to be essential for maintaining the response of the light-adapted retina. Inhibition may achieve this function by *protecting* spike encoders from the effects of high excitatory inputs.

Supported by NIH grant EY-00667 and NSF grant BNS-8709059.

244.2

WHOLE-CELL CURRENTS AND SINGLE-CHANNELS ACTIVATED BY GABA AND GLYCINE IN GANGLION CELLS FROM THE GOLDFISH RETINA. B. Cohen* and G. L. Fain. The Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles CA 90024

GABA and glycine evoked currents in most ganglion cells isolated from the goldfish retina. Whole-cell currents were carried by Cl^- since their reversal potential shifted with E_{Cl} when isethionate was substituted for internal Cl^-. Bicuculline methochloride selectively blocked GABA-induced currents. Bicuculline, strychnine, and picrotoxinin blocked both GABA-induced and glycine-induced currents.

The slope conductance for the most frequent state of the GABA channel in symmetric 140 mM Cl^- was 14 pS. The extrapolated reversal potential was 2 mV. Glycine channels in symmetric 140 mM Cl^- could be separated into 3 groups based on the slope conductance of their most frequent state: 25-26, 29-30, and 40 pS. The reversal potentials for the groups were around 0 mV. The 25 and 30 pS channels had a substate around 14 pS. When isethionate was substituted for internal Cl^-, the reversal potential for the glycine channels shifted negatively. The I-V data were well fit by the Goldman current equation for Cl^-.

Supported by NIH grants EY 05234 and EY01844.

244.3

ALPHA (TYPE I) GANGLION CELLS OF RAT RETINA DIFFER FROM THOSE OF OTHER MAMMALS. L. Peichl* (SPON: J.I. Nelson). Max-Planck-Inst. f.Hirnforschung, D-6000 Frankfurt 71,FRG

Alpha cells, common to mammalian retinae, are large ganglion cells with a distinctive dendritic morphology. They branch in an inner or outer stratum of the IPL and are the presumed correlate of on-centre and off-centre Y cells respectively.

The present study used intracellular injections of Lucifer Yellow and reduced silver staining in retinal whole mounts to quantify rat alpha cells (termed type I cells by other authors). Outer stratifying alpha cells (α_o) show significantly denser dendritic branching compared to inner alpha cells (α_i). Their dendritic field sizes increase differently with eccentricity (central retina: α_o 280-340 µm Ø, α_i 220-280 µm Ø; peripheral retina: α_o350-500µm Ø, α_i 570-780µm Ø). The size difference is complemented by a difference in the density ratio α_i: α_o which is 4:3 in central retina and shifts towards 1:3 in peripheral retina, ensuring a constant dendritic overlap (coverage) of 3-4 fold for either population. The qualitative and quantitative differences between rat inner and outer alpha cells constitute a departure from the general mammalian pattern where inner and outer alpha cells are similar. The larger dendritic fields of inner (on) alpha cells suggest their receptive field centres are larger than those of outer (off) alpha cells. The greater number of outer alpha cells may reflect dominance of the "off" pathway.

244.4

QUANTITATIVE / FRACTAL ANALYSIS OF DENDRITIC TREES OF IDENTIFIED RABBIT RETINAL GANGLION CELLS F. R. Amthor. Dept. of Psychology & Neurobiology Research Center Univ. of Alabama at Birmingham 35294

Physiologically characterized rabbit retinal ganglion cells of virtually all major concentric and complex classes have been intracellularly injected with HRP. Each major physiological class is associated with a unique, identifiable dendritic morphology. In addition to differences in their 3-D structures, differences in the flat mount appearances of these cells have been investigated by the use of quantitative analyses of dendritic coverage such as their Fractal / Hausdorf-Besicovitch dimensions. These were computed using both interior box-counting and expanding area algorithms. On-Off directionally selective ganglion cells, which exhibit apparent dendritic loops, consistently have the highest Fractal dimensions of about 1.85, a value rarely found for any non-self intersecting branching structure. Some sparsely branching cell classes such as sluggish-transient concentric cells exhibit two distinct fractal dimensions at "micro" vs. global size size scales, possibly related to overall (skeletal) versus terminal dendritic branching strategies. Fractal and other quantitative analyses allow concentric classes such as brisk versus sluggish and sustained versus transient concentric cells to be distinguished from On-Off and On directionally selective, orientation selective, local edge detecting and uniformity detecting ganglion cell classes, and may suggest branching strategies during growth. Theoretical limits for some of these have been determined.

Supported by EY05070 and the Sloan Foundation.

244.5

WHAT ARE THE DIRECTIONALLY SELECTIVE SUBUNITS OF RABBIT RETINAL GANGLION CELLS? N. M. Grzywacz & F. R. Amthor[1].
Center for Biological Information Processing, MIT E25-201, Cambridge, MA 02139 & [1]Dept. of Psychology & Neurobiology Research Center, Univ. of Alabama at Birmingham 35294

Subunits have been postulated to account for motion selectivity over small distances within the receptive field (RF) center of On-Off direction-selective rabbit retinal ganglion cells. We investigated the spatial and temporal characteristics of these hypothesized subunits by determining extracellular responses to two-slit apparent motions in the null and preferred directions. For apparent motion in the null direction the first slit inhibited the response to the second regardless of whether the distance was a small or large fraction of the RF center. Within this large inhibitory field there was no temporal dependence on distance between the slits, and very little interaction between On and Off mechanisms. Subunit-like behavior thus cannot due to a restricted extent of the inhibitory region, but appears to result from a large inhibitory field interacting locally with excitation. Our data suggest that this inhibitory interaction is mediated by a shunting, rather than a hyperpolarizing mechanism having a time course resembling a low pass filter with τ about 100 ms. Two slit apparent motion in the preferred direction revealed facilitation over a large region with distance-independent temporal properties. Direction selectivity thus involves both inhibitory and facilitatory mechanisms that are spatially asymmetric, and extend over much of the RF center. A synaptic model we have formulated successfully accounts for these results.

-- Supported by EY05070 and the Sloan Foundation. --

244.6

IDENTITIES OF LGN PROJECTING RABBIT RETINAL GANGLION CELLS M.L. Pu* and F. R. Amthor[1]. (Spon. E. Taub) School of Optometry; [1]Dept. of Psychology & Neurobiology Research Center, Univ. of Alabama at Birmingham 35294

In order to determine what classes of ganglion cells project to the Rabbit LGN, diffuse and focal injections of fluorescent tracers Fast Blue and Rhodamine Latex beads, respectively, were made there. Following survival times of about ten days, the dyes were found to be retrogradely transported to projecting ganglion cell somata within the retina. These cells were injected intracellularly with Lucifer Yellow in living and lightly fixed tissue, so that projecting classes could be identified from knowledge of the dendritic morphologies of physiologically identified cells (Amthor et al., Invest. Ophthal. Vis. Sci. Suppl. 27(3):332). The classes of ganglion cells labeled varied somewhat with the survival time, fluorescent dye used and the injection location. Nearly all injections labeled ganglion cells that had large somata. Following Lucifer Yellow injection, many of these exhibited an Alpha-like morphology known to correspond to that of the brisk-transient concentric cells. However, ganglion cells with large somata but sparse dendritic trees were also found, which correspond to the dendritic morphologies of sluggish concentric cells. Some medium to large somata were those of the bistratified dendritic morphologies of On-Off direction selective ganglion cells. Somewhat less frequently labeled were ganglion cells with small dense arborizations and small somata, which correspond to the brisk-sustained-linear concentric cells.

Supported by EY05070

244.7

ASSOCIATION OF ALPHA CELL DENDRITES WITH CHOLINERGIC AMACRINE PROCESSES. N. Vardi, P. Masarachia* and P. Sterling. Dept. Anat. Univ. Penn., Phila, PA 19104.

The cholinergic amacrine cells in cat form planar arbors in the inner plexiform layer (IPL). Within each arbor the processes from adjacent cells associate intimately with each other over about 70% of their lengths. As the bundles cross each other, "loops" are created of irregular size and shape. Loop diameter averages 15μm in the area centralis and increases with eccentricity. The α cells also form planar arbors at the corresponding levels of the IPL. Their crossing dendrites also create loops comparable in size and spacing to those of the cholinergic network. To investigate a possible association between the two networks we first injected α cells with Lucifer yellow and then immunostained for choline acetyltransferase. When viewed in the same focal plane, there were many instances where α and cholinergic processes ran parallel and close together (within 5μm) over long distances (up to 80μm); also, elements of both networks divided or curved in a similar way. About 25% of the total α cell dendritic arbor was aligned with the cholinergic network. When composites of 4 α cells were placed in random orientations upon the cholinergic network, the degree of alignment was about 9%. Thus, the association between α and cholinergic processes is specific, though it is weaker than the association between the cholinergic processes themselves.

244.8

ANATOMICAL CIRCUIT FOR THE GAUSSIAN CENTER OF THE ON-BETA GANGLION CELL. E. Cohen, and P. Sterling. Dept. Anatomy, U. Penn, Phila, PA 19104-6058.

Sensitivity in the ganglion cell receptive field center was observed by Kuffler to peak at the middle and decline toward the edge. The anatomical basis for this distribution, to which Rodiek fitted Gaussian, is unknown. We reconstructed from electron micrographs of serial sections the connections between arrays of cones, CBb_1 bipolars, and on-beta ganglion cells. Adjacent cones converged in small numbers (4-7) on a CBb_1, and adjacent CBb_1s (6-8) converged on a beta cell. We estimate that the complete circuit at this eccentricity (1^o) connects 34 cones to the beta cell via 9 CBb_1s. Bipolar axons near the beta dendritic field center supply many synapses (25-30), and those near the dendritic field edge supply few (1-5). This arrangment weights a cone's contribution according to the number of synapses that its CBb_1 bipolar provides to the ganglion cell. These weights plotted against distance of each cone from the beta dendritic field center have a dome-shaped distribution. This distribution, when convolved with the linespread function of the cat's eye and the luminance profile of a 0.1^o stimulus, matches closely the distribution of sensitivity measured physiologically for a beta cell at the corresponding eccentricity. Thus, the nonuniform distribution of synapses to an on-beta cell from its CBb_1 axons may explain the receptive field's Gaussian center.

244.9

CONE RECEPTIVE FIELD COMPUTED FROM MICROCIRCUITRY AND RECEPTIVE FIELDS OF X AND Y CELLS. R.G. Smith* and P. Sterling. (SPON: A.C.Rosenquist) Dept. Anat, Univ. of PA, Phila, PA 19104-6058.

The origin of the center-surround receptive field in the cat ganglion cell is unknown. A surround is already present at the level of the CBb_1 bipolar cell, whose array contributes significantly to beta (X) and alpha (Y) ganglion cells, suggesting that a center-surround receptive field might be established first in the cone. We computed the cone receptive field by asking: what cone sensitivity profile, when summed linearly over a cone array of appropriate size, spacing, and spatial weighting, would be required to account for the ganglion cell center and surround? Further, would the same cone receptive field computed in this manner account for both X (beta) and Y (alpha) receptive fields? The cone arrays for beta and alpha cells and the spatial weighting functions were drawn from recent anatomical studies from this laboratory (Cohen and Freed); the center and surround measurements for X and Y receptive fields at the corresponding eccentricity (1 deg.) were drawn from Cleland et al. '79 and Linsenmeier et al. '82.

The computed cone receptive field center is about 60 um, nearly equal to that of an X cell (65 um), and sums to match both X and Y cell centers. The computed cone surround is about the same diameter as the X cell's but the amplitude is reduced by about 1/3. The overall result recalls the broad, center-surround receptive field observed in turtle cones by Baylor et al. '71. Possibly in mammalian retina too contrast sensitivity originates at the cone.

244.10

COLLECTIVE CODING IMPROVES THE SIGNAL/NOISE RATIO OF THE GANGLION CELL. Y. Tsukamoto*, R. G. Smith* and P. Sterling. Dept. Anat., Univ. Penn., Phila., PA 19104.

The dome-like distribution of sensitivity in the receptive field center of a beta ganglion cell in cat area centralis expresses a weighted sum of signals from about 34 cones. We explored computationally the hypothesis that the reason for collecting signals from many cones is to improve the ganglion cell's signal/noise (S/N) ratio. Signals in adjacent cones tend to be strongly correlated because of local correlation in the visual scene and optical blurring. Were the signals identical, to pool them with equal weight would improve the S/N ratio as the square root of the number of cones. However, the correlation declines sharply with distance, so cones at the edge of the receptive field center must contribute less to S/N improvement than those near the center. We computed, according to equations resembling those of Yule-Walker, weights for collecting cone inputs that would maximally improve the S/N ratio. For a ganglion cell center of given diameter and cone density, the optimal weighting function is dome-like. For this weighting function and a given cone density, as ganglion cell center diameter increases, the S/N ratio rises sharply and then asymptotes. For this weighting function and a fixed center diameter, the S/N ratio rises linearly with the square root of cone density. Thus, by collecting many signals with a Gaussian weighting function, the beta ganglion cell may optimally improve its S/N ratio by a factor proportional to the square root of cone density.

244.11

FLICKER SENSITIVITY OF MACAQUE GANGLION CELLS. P.R. Martin*, B.B.Lee and A.Valberg* (SPON:G.Fox) Max-Plank-Inst. Biophys. Chem., 34 Göttingen,F.R.G

As reported previously, the sensitivity of phasic ganglion cells to luminance flicker of different temporal frequencies closely approximates that of a human observer. Sensitivity of tonic cells to chromatic flicker at low temporal frequency (tf) is similar to man's, but even higher at high tfs, showing tonic cell activity is attenuated at higher levels. We have also measured sensitivity to silent substitution flicker, where M- or L- cones are selectively modulated. We found red and green on-centre and phasic cells all to have similar sensitivity to M- or L- cone flicker. This means that tonic cell sensitivity to chromatic flicker is derived equally from center and surround, and that tonic cell insensitivity to luminance flicker is a consequence of cone opponency rather than intrisically low sensitivity.

Sensitivity of tonic cells to chromatic flicker could be well predicted from their sensitivity to M- and L- cone flicker, indicating linear summation of cone signals, but sensitivity of phasic cells to luminance flicker could not be so well predicted. This may be another manifestation of non-linear cone summation in phasic cells.

244.12

PATTERN ERG IN RATS FOLLOWING SECTION OF THE OPTIC NERVE. L. Domenici,* N. Berardi,* A. Gravina* and L. Maffei* (Spon: A. Cangiano). Institute of Neurophysiology of CNR, Via S. Zeno 51, 50100 Pisa, Italy.

Flash and pattern ERG were recorded from urethane anaesthetized hooded rats (Long Evans) by means of silver rings or small silver plates inserted under the eyelids. The signal from the stimulated eye was conventionally amplified, filtered and computer analyzed on-line and off-line. The pattern ERG was evoked by phase alternating gratings of various spatial frequencies and contrast. The pattern ERG amplitude was function of contrast and increased up to 30% contrast. The optimum mean luminance of the gratings was betweeen 15 and 20 cd/m^2. Higher luminances resulted in lower pattern responses. The largest amplitudes of pattern ERG were recorded for spatial frequencies of .1 c/deg while the optimum rate of alternation was around 4 Hz. The highest spatial frequency that elicited a reliable ERG response was .35 c/deg. Following section of the optic nerve the pattern ERG showed a progressive decrease in amplitude and eventually disappeared. The flash ERG remained unaffected.

245.1

THE JOURNEY OF YOUNG MIGRATING NEURONS IN THE ADULT CANARY BRAIN. Arturo Alvarez-Buylla* and Fernando Nottebohm (SPON: D. Griffin). The Rockefeller University, New York, N.Y. 10021

The adult bird brain continues to produce new neurons. These cells are born in the ventricular zone (VZ) sometimes up to 5 mm away from the location where some of these neurons finally mature. To study the intervening steps between birth and neuronal differentation, groups of 3 canaries were sacrificed 1, 3, 6, 15, 20, 30 and 40 days after receiving 2 injections of ^{3}H-thymidine. The position and type of labeled cell was mapped in frontal brain sections at the level of the anterior commissure. Small elongated cells that originated from the VZ lining the lateral walls of the lateral ventricle moved laterally into the telencephalic parenchyma. Before day 15 the labeled elongated cells were concentrated in areas rich in radial glia and were frequently attached to radial glia fibers. During this time the leading front of the elongated cells moved away from the VZ at 28um/h. This rate dropped to 8um/h as cells became scattered throughout the section. The number of labeled migrating cells decreased rapidly after day 20 as labeled neurons started to appear throughout the telencephalon. We conclude that the migrating cells are young neurons.

245.2

RADIAL GLIA DIVIDE AND MAY GIVE BIRTH TO NEURONS IN ADULT AVIAN BRAIN. F. Nottebohm, M. Theelen* and A. Alvarez-Buylla*. The Rockefeller Univ., New York, NY 10021

Neurons are born in the lateral wall of the lateral ventricle of the adult avian forebrain. Neurogenesis is particularly intense within the dorsal and ventral tips of this ventricular zone. Radial glia, with bodies in the ventricular zone, are also particularly abundant within these two regions. Many new neurons migrate from there closely apposed to the long fibers of the radial glia. We now report that these same radial glia, with bodies in the ventricular zone, may give birth to the neurons. This was determined as follows. The forebrain of adult female canaries that had received a single injection of ^{3}H-thymidine was cut transversely into 0.5 mm slices. A thin strip of the lateral wall of the lateral ventricle was then dissected and slightly trypsinized. This tissue was smeared onto a glass slide and stained with our anti-vimentin antibody (40E-C), covered with photographic emulsion and incubated for autoradiography for 4 weeks. Eighty percent of the ^{3}H-thymidine labeled cells were positive to the antibody and of these 68% had single processes of the kind that characterize radial glia. Since proliferation of cells in the ventricular zone of the adult avian brain is closely related to neurogenesis, and since the vast majority of our labeled cells had radial glia morphology, we suggest that neurons are born in the ventricular zone when radial glia undergo mitosis.

245.3

ANALYSIS OF PREFERENTIAL GENE EXPRESSION IN THE SONG CONTROL NUCLEUS HVC OF CANARIES. D. F. Clayton, M. Huecas*, and K. L. Nastiuk. Lab. of Animal Behavior, The Rockefeller Univ., New York, NY 10021.

HVc, a primary song control nucleus in the telencephalon, exhibits profound anatomical and functional plasticity in response to steroid hormones. To provide molecular probes for analyzing gene regulation and anatomical organization in this nucleus, cDNA clones that hybridize to mRNAs more abundant in nucleus HVc than in midbrain plus hindbrain were selected from an HVc library. The anatomical distribution of these mRNAs was determined using in situ hybridization. None of the RNAs yet examined is absolutely specific for the nucleus, although specificity for cell subsets with unique distributions in the brain is evident. These results extend our earlier observations of overlapping patterns of mRNA distribution in brain cell subsets (Clayton et al., Neuron May 1988). Further analysis may identify genes that respond to steroids in HVc, and that may be involved in regulating the neuronal growth in adulthood characteristic of this nucleus.

245.4

BIRTH OF LONG PROJECTION NEURONS IN DEVELOPING AND ADULT AVIAN BRAIN. M. Theelen*, A. Alvarez-Buylla* and F. Nottebohm (SPON: G.Adelman). Rockefeller Univ., New York, NY 10021

The higher vocal center (HVc) of the canary brain has two projections, one to nucleus robustus archistriatalis (RA) and another one to area X of lobus parolfactorius. To determine the birth dates of neurons projecting to these two targets, 38 canaries of both sexes received ^{3}H-thymidine at one of the following ages: E5, E9, E10, E12, and post hatching days 10, 35, 58, 93, 120 and 240. At 13 months all birds received injections of the retrograde tracer fluorogold into RA of one hemisphere and into area X of the other hemisphere, and were killed 4 days later. As many as 76% of the cells backfilled from area X were labeled with ^{3}H-thymidine before hatching, vs. a maximum of 0.7% of RA backfilled cells. The percent labeled per day between hatching and 240 days was 0.2% for X projecting cells and 0.8% for RA projecting cells. In addition 2 birds were treated with ^{3}H-thymidine at 18 months of age. In them 0.1 - 0.3 % of the neurons backfilled from RA were labeled per day of ^{3}H-thymidine treatment. Thus, one type of HVc long projection neuron is produced predominantly during early development, whereas another type is produced throughout development and during adulthood. This is the first report of long projection neurons produced in adult vertebrate brain.

245.5

NGF EFFECTS ON CHOLINERGIC INTERNEURONS OF THE ADULT RAT STRIATUM AND NUCLEUS ACCUMBENS. S. Varon, T. Hagg, H.L. Vahlsing*, M. Manthorpe. Dept. Biology, Univ. of California, San Diego, La Jolla, CA 92093.

Cholinergic neurons of the striatum (STR)(caudate-putamen) respond to NGF with a dose-dependent increase in choline acetyltransferase (ChAT) activity during development, but this response decreases at an early postnatal stage. Adult STR contains low levels of NGF and NGF-mRNA and no (low affinity) NGF receptors can be detected by regular immunocytochemical techniques, although some neurons are able to bind radiolabelled NGF with high affinity. We addressed the question whether adult CNS cholinergic interneurons of the STR and nucleus accumbens (ACC) are still responsive to (exogenous) NGF, as are those in the basal forebrain.

NGF or vehicle was administered by unilateral intraventricular infusion through a small 32 gauge cannula to normal adult rats and to rats that had received a unilateral transection of the fimbria-fornix. Sections were evaluated for immunostaining of ChAT (1E6, P.Salvaterra) and NGF-receptor (192-IgG, E.M. Johnson). The ChAT-reactivity of the neuropil and neuronal cell bodies was increased and the average cross-sectional area of the somata enlarged by 40% in the STR and by 20% in the ACC on the side of the NGF infusion. This response could be elicited on either side of the fimbria-fornix transection, as well as in normal, non-lesioned animals. Unlike the case of the medial septum neurons, the increase in ChAT was not accompanied by an appearance or intensification of immunoreactive receptors for NGF. These results indicate that the apparently undamaged adult cholinergic interneurons of the STR and ACC are still responsive to exogenous NGF and this effect is most likely transduced by the high affinity receptor for NGF. Supported by NIH grant NS 16349

245.6

NGF-REGULATED PLASTICITY IN THE ADULT NERVOUS SYSTEM. J. Diamond, M. Holmes* and B. Visheau*, Dept. Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5.

During our studies that revealed the NGF dependency of collateral sprouting of heat-nociceptive C fibers in adult rat skin (PNAS, 84:6596, 1987) we observed that daily anti-NGF administration not only prevented the expansion of the "heat-fields" into surrounding denervated skin, but actually caused them to shrink, by about 50% in a month. We measure nociceptive fields in the anesthetized rat by a spinal reflex; thus we are examining a possible "central" origin of the shrinkage due, e.g., to reduced substance P (Schwartz et al., 1982, Brain Res. 244:378) causing a reduced synaptic drive. We are however obtaining evidence for a peripheral effect of the treatment. In the EM we see occasional abnormal sensory C axon profiles in normal skin, but significantly more in the skin of anti-NGF treated animals. Moreover, using a new "trophic assay", we find that the time for the C fiber terminals to degenerate following axotomy is almost halved in skin from animals on 4 wks of anti-NGF treatment. We suggest: (i) that there is a normal turnover of sensory C fiber terminals; (ii) that this is regulated by endogenous NGF; (iii) that this NGF is critical to the maintenance of both the function and the structure of these nerve endings.

Generalized, our findings point to a plasticity in the adult nervous system that is regulated by endogenous growth factors.

245.7

AXOTOMY-INDUCED LOSS OF BOTH CHAT AND NGF-RECEPTOR POSITIVE MEDIAL SEPTUM NEURONS IS REVERSED BY DELAYED NGF TREATMENT.

T. Hagg, H.L. Vahlsing*, B. Fass, M. Manthorpe, S. Varon. Dept. Biology, Univ. of Calif., San Diego, La Jolla, CA 92093.

Intraventricular Nerve Growth Factor (NGF) infusion in the adult rat can prevent and, if delayed, reverse most of the disappearance of axotomized medial septum cholinergic (MSC) neurons immunostained for choline acetyltransferase (ChAT). The reversibility indicated that the apparent loss may be largely due to a loss of ChAT enzyme rather than to neuronal death. We have repeated and extended previous findings by immunostaining the MSC neurons for the low affinity NGF-receptor (192-IgG, E.M. Johnson) in addition to the ChAT marker (1E6, P. Salvaterra) and extending the delay time between axotomy and NGF treatment. NGF or vehicle was continuously infused into the ipsilateral ventricle for 14 days beginning immediately, 7 days or 95 days after unilateral fimbria-fornix transection. Additional animals were infused for 1, 3 or 7 days beginning 7 days post-lesion. The number of ChAT-positive MSC neurons dropped to 50% by 7 days post-lesion and to a baseline of 20% by 14 days or longer in vehicle infused rats. All the neurons remained detectable by 14 days when NGF treatment was started immediately post-lesion. A 90% level was rapidly achieved in 3 days when NGF infusion was started after 7 days. Even when delaying the infusion up to 95 days, 60% of the neurons were again detectable. The NGF-receptor immunoreactive MSC neurons dropped to 50% after 7 days post-lesion and to a baseline of 35% by 14 days in vehicle infused rats. Full protection resulted when a 2 week NGF infusion was started immediately after the transection. The receptor positivity was fully restored in 3 days if treatment was started 7 days post-lesion. A 65% level was reached when treatment was delayed for 95 days. Thus, NGF treatment can re-establish both ChAT and NGF-receptor positivity of axotomized MSC neurons. Supported by NIH grant NS 16349 and NS 25011

245.8

SEXUAL MATURITY-DEPENDENT NEURAL PLASTICITY IN THE PRE-PACEMAKER NUCLEUS OF ADULT KNIFEFISH.

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Eigenmannia (Gymnotiformes, Teleostei) can modulate its otherwise very stable electric organ discharge (EOD) by gradual frequency shifts during courtship and the jamming avoidance response, and by abrupt shifts in the context of agonistic and reproductive behavior. The EOD, produced by a specialized electric organ, is under the control of a pacemaker nucleus (PN) in the medulla oblongata. Injection of horseradish peroxidase into the PN only labelled cells within the diencephalic pre-pacemaker nucleus, PPN (Heiligenberg et al., Brain Research, 211:418, 1981). Microstimulation experiments revealed a morphological and functional dichotomy of the PPN showing that both types of EOD modulations can be elicited from two distinct areas within the PPN (Kawasaki et al., J. Comp. Neurol., in press; Rose et al., J. Comp. Physiol., in press).

As shown in this study, in both sexes the normalized length of the dendritic arbors of the PPN is larger in mature animals than in immature specimens. Even more pronounced are differences in the number of varicosities on the dendrites: Fish with well developed gonads show up to 20 times more varicosities than do fish with immature or regressed gonads. These varicosities may represent growing buds, or regions of synaptic contact, or both.

245.9

INCREASED PHRENIC NERVE ACTIVITY OCCURRING WITHIN HOURS OF AN IPSILATERAL C2 SPINAL CORD HEMISECTION IN ADULT RATS.

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Recent studies in our laboratory have demonstrated a rapid morphological alteration of the normal ultrastructure of the phrenic nucleus within 4 hours of an ipsilateral C2 spinal hemisection. We have suggested that these changes may be related to functional recovery of the ipsilateral hemidiaphram which was paralyzed by spinal cord injury. The present study was designed to determine if there is an increase in phrenic nerve activity that temporally coincides with our observed morphological changes. A left C2 spinal hemisection was followed by the immediate application of xylocaine to the right phrenic nerve. Rectified and integrated compound action potentials were then recorded from the left phrenic nerve. Current flow through the nerve was calculated. The animals were allowed to survive and left phrenic nerve activity was again recorded at various intervals up to 5 days post hemisection. Immediate post injury current flow was subtracted from the current flow measured at a later interval for each animal. The results showed a significant increase in phrenic nerve activity occurring within hours after spinal cord injury. By 4 hours post spinal cord injury, left phrenic nerve activity could be recorded without the application of xylocaine to the right phrenic nerve. These results correlate with our previous morphologic studies. Supported by U.S. Public Health Service Grant NS-14705.

245.10

NEURAL PLASTICITY IN AFFERENT AND EFFERENT PATHWAYS TO THE URINARY BLADDER FOLLOWING URETHRAL OBSTRUCTION IN THE RAT.

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Partial urethral ligation in rats produces functional changes consistent with obstruction and detrusor hyperreflexia, as well as enhancement of a spinal micturition reflex. Changes in the neural pathways to the bladder (BLD) were examined by injecting 50 µl of 1% WGA-HRP or 4% fluorogold (FG) into opposite sides of the BLD of obstructed (OBSTR) and control rats. Fast blue dye was injected into the distal colon as an internal control. OBSTR rats showed enlargement of FG labelled BLD postganglionic (PG) cells (553.5±175.2 μ^2,n=4), but not colon cells in the pelvic ganglion compared to controls (298.8±60.7 μ^2,n=4). No difference was noted in size of PG BLD neurons in the lumbosacral chain ganglia in control (544.3±122.7 u^2) vs OBSTR rats (570.2±125.5 μ^2). BLD afferent cell sizes in the L_6 and S_1 dorsal ganglia were also increased in OBSTR rats. A 40% increase of BLD afferent terminals in the L_6 and S_1 spinal cord in the region of the parasympathetic nucleus was found in OBSTR rats. These changes may be produced by increased neuronal activity, increased axonal transport of tracer or sprouting of terminals.

245.11

CHANGES IN THERMAL PLASTICITY OF RAPHE NEURONES AFTER INTERRUPTION OF DIFFERENT PATHWAYS.

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Neurones in the nucleus raphe magnus (NRM) have been shown to belong to the thermoafferent system. In cold-acclimated guinea-pigs (CA, 6-9 weeks in 4°C) average maximum activity and average basic spike rate of warm-responsive NRM units were significantly larger than in normal-acclimated animals (NA, 21°C).

After interruption of the dorsal and rostral afferents and efferents of the NRM by microcuts no significant differences were seen in the average frequency-temperature-characteristic of warm-responsive NRM units in NA guinea-pigs. By contrast, after interruption of the dorsal and rostral pathways in CA animals basic spike rate was significantly reduced (to the basal level in NA animals), whereas the maximum spike rate was not changed. Control lesions of lateral and ventrocaudal pathways did not show any effect in NA guinea-pigs.

It is concluded that the cold-adaptive increase of average peak activity of NRM units after long-term cold acclimation is generated at a lower level, whereas the increase of tonic basal spike rate is mediated by higher pathway loops. In summary, interactions in complex neuronal networks of the lower brainstem may be involved in generating thermal plasticity.

246.1

SHORT AND LONG TERM LEARNING IN THE NEMATODE *C. ELEGANS*

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Although to date relatively little is known about the learning and memory capabilities of *C elegans*, the unique combination of detailed genetic, developmental, and neuroanatomical data available for this animal provides an excellent basis for exploring mechanisms underlying the development and expression of behavioral plasticity.

To begin an analysis of non-associative and associative learning in *C. elegans* we have focussed on a simple form of learning, habituation. In these studies we elicited responses by delivering a vibratory stimulus through the agar medium. Animals responded to this stimulus by reversing direction, moving several body lengths backwards. With 60 presentations of stimuli at a 10 sec ISI reversals became progressively shorter, changing to pauses in forward motion, and eventually responding ceased. A scorer blind to the experimental design rated videotaped responses on a scale of 0-4, (4= reversal >1 body length; 0= no response). We observed significant habituation with a mean initial response of 3.35 +0.95, and a mean habituated response of 0.8+0.28, (N=10, t(9)= 5.314, p=.0005). Significant dishabituation was observed in response to a shock of 60V with an increase above the mean habituated response to 1.9+.35, (N=10, t(9)=2.577, p=.03). We tested for long term habituation by presenting 6 blocks of 20 habituation stimuli over two days, testing on the third day, 15 hours after training. When compared to control animals, trained animals showed significantly greater habituation (Mean Control 1.8+.19, N=7; Mean Experimental 1.0+.18, N=7, t(12)=2.9, p=.01).

Thus, the nematode *C. elegans* is capable of two non-associative learning processes, habituation and dishabituation, and is also capable of long term learning, retaining habituation training for at least 15 hours.

246.2

POSSIBLE LOCI FOR HABITUATION OF THE LEECH LOCAL BENDING REFLEX. S.R. Lockery and W.B. Kristan, Jr. Dept. of Biol., Univ. of Calif., San Diego, La Jolla, CA 92093.

Local bending, a defensive withdrawal reflex, habituates upon repeated mechanical stimulation. To study the mechanism of habituation, we used a semi-intact preparation that reduces the behavioral circuit to one sensory neuron (the P cell), two motor neurons (cells 3 and L), and a number of interneurons. The connection from the P cell to the L cell is monosynaptic, while the connection to cell 3 is polysynaptic. Motorneuron recordings during habituation indicated that the functional plasticity for habituation resides solely in the polysynaptic pathway since the number of stimulus-evoked action potentials in the L cell was constant while the number of cell-3 impulses declined. To further localize the plastic site, we identified seven interneurons that received short latency epsps from the P cell and, when depolarized by current injection, activated cell 3. Three interneurons appeared to make a necessary contribution to cell-3 activity: hyperpolarizing them reduced the number of cell-3 action potentials evoked by P-cell stimulation. For one interneuron, cell 218, hyperpolarization reduced the number of action potentials by up to 50%. Repeated stimulation of the P cell sufficient for habituation decreased the amplitude of epsps recorded from cell 218. Repeatedly depolarizing cell 218 by current injection reduced the number of action potentials recorded from cell 3 in response to depolarization by as much as 100%. Thus the polysynaptic pathway of the local bending reflex contains two possible sites of habituation.

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246.3

SENSITIZATION OF THE TRITONIA ESCAPE SWIM: BEHAVIORAL AND CELLULAR MODIFICATIONS. W.N. Frost, G. Brown* and P.A. Getting. Dept. Physiology & Biophysics, Univ. of Iowa, Iowa City, IA 52242.

It has previously been shown that in the sea slug Tritonia, repeated noxious epithelial stimulation produces habituation of escape swimming. We now report that a single stimulus produces rapid sensitization of multiple aspects of the swim: number of cycles, onset latency and threshold. Cycle number and onset latency were examined by comparing, for each animal, a swim produced by a tail stimulus alone (T) with a swim produced by a tail stimulus coming 5 minutes after a head stimulus (H-T). The response to the tail stimulus of the H-T sequence had a larger number of cycles (6.8 vs 5.4) and a shorter latency (8.2 vs 4.3 seconds) than the response to the tail-alone stimulus ($p<.05$, N=10). A control group receiving T-H on day 1 and T on day 2 showed no difference in cycle number or latency. In a second experiment the T intensity was first adjusted to be subthreshold for producing a swim. In 10 of 14 animals this tail stimulus became suprathreshold in the H-T paradigm ($p<.01$), indicating that the head stimulus produced sensitization (a reduction) of the threshold for the subsequent tail stimulus.

In isolated brain preparations, brief electrical stimulation of pedal nerves triggers the neural program for the escape swim. Following such a stimulus, the interneuron C2 displayed: 1) a prolonged increase in excitability as measured by the number of spikes elicited by a 5 second constant current pulse (avg pre = 7, avg post = 23) and, 2) a decrease in the amplitude of the spike undershoot (avg pre = 5.3mV, avg post = 3.0mV), both measured from the same resting potential ($p<.05$, N=5).

Using a computer reconstruction of the known swim circuitry we have begun an evaluation of the relationship between the multiple behavioral changes and the circuit modifications underlying sensitization. Our preliminary findings suggest that the change in C2 excitability may contribute to the increase in cycle number.

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246.4

ASSOCIATIVE LEARNING IN HERMISSENDA II. ROLE OF FOOT CONTRACTION IN CS-MEDIATED REDUCTION OF PHOTOKINESIS. L.D. Matzel*, B.G. Schreurs*, I.I. Lederhendler*, and D.L. Alkon (SPON: R. Desimone). Lab. of Molecular and Cellular Neurobiology, NIH, Bethesda, MD 20892.

We have recently reported that pairings of light with orbital rotation result in a prolonged associative reduction of photokinesis in Hermissenda. Here, we examined the contribution of foot contraction to that response. The degree of unconditioned foot contraction was found to be directly proportional to the speed of the rotation. Contraction was evident for up to 30 sec after the offset of rotation and was concomitant with a reduced rate of locomotion during that period. This graded unconditioned response to rotation was directly related to the strength of the conditioned response that rotation supported as indexed by the reduction of photokinesis following light(CS)-rotation(US) pairings. Moreover, foot contraction was found to be a short latency conditioned response to light following light-rotation pairings and persisted long after the onset of the light. In total, these results indicate that associative reduction of photokinesis is mediated by a long-lasting contraction of the Hermissenda's foot. The observation of conditioned contraction is consistent with a previous report (Lederhendler, Gart, & Alkon, 1986) demonstrating a transfer of properties from the US to the CS, and may be mediated by direct excitatory pathways from the photoreceptors to the motoneurons of the foot.

246.5

ASSOCIATIVE LEARNING IN HERMISSENDA: I. PROLONGED CS-MEDIATED REDUCTION OF PHOTOKINESIS. B.G. Schreurs*, L.D. Matzel*, I.I. Lederhendler* and D.L. Alkon (SPON: J.S. McIntosh) LMCN, NIH-NINCDS, Bethesda, MD 50892.

Pairings of a light and high-speed rotation result in a reduction of phototactic behavior in Hermissenda crassicornis. High-speed rotation prevents precise specification of stimulus onset due to the gradual onset characteristics (approx. 1-2s). To overcome these onset characteristics, previous studies have employed rotation of long duration (30s) to ensure maximum stimulus intensity and have yielded conditioning using "simultaneous" pairings. We report three experiments employing a shaker device to provide intense, short latency, orbital rotation of brief duration (2s). Experiment 1 revealed that pairings of light (3s) and orbital rotation (2s) reduced posttest movement around a circular track relative to unpaired light and rotation. Experiment 2 showed marked reduction of movement at interstimulus intervals of 1 and 2s but not at 10s. Experiment 3 examined the role of stimulus onset and offset and revealed that forward overlapping stimulus presentations were superior to sequential or simultaneous presentations.

246.6

OPERANT CONDITIONING OF IDENTIFIED NECK MUSCLES AND INDIVIDUAL MOTOR NEURONS IN *APLYSIA* D.G. Cook & T.J. Carew, Dept. Psychology, Yale University, New Haven, CT 06520.

Aplysia can be operantly trained to change their head-waving behavior to avoid presentation of an aversive bright light (Cook & Carew, 1986). In the present study we asked whether the neck muscles involved in head-waving (Lateral Columellar Muscles [LCMs]), as well as individual neck motor neurons, are capable of operant conditioning.

EMG activity in the LCMs was recorded using implanted cuff electrodes in freely moving animals. Differential (left vs. right) EMG output of a CONTINGENT group (N=15) was operantly trained with light, while a YOKED CONTROL group (N=15) received non-contingent reinforcement. Following training, CONTINGENT animals showed a significant change in differential EMG activity compared both their own baseline ($p<.025$) and to YOKED CONTROLS ($p<.025$). YOKED CONTROLS showed no learning.

Next, using a split-foot preparation, intracellular recordings were made from single neck motor cells in the pedal ganglion. CONTINGENT cells (N=8 preparations) received either uptraining (light whenever firing rate fell below baseline); or downtraining (light whenever firing exceeded baseline). YOKED CONTROL cells (N=8) received non-contingent reinforcement. Following training CONTINGENT trained cells showed a significant alteration in firing rate, ($\bar{x}$ change = 93 A.P.s, $p<.05$), largely due to successful downtraining (4 of 4 in predicted direction, compared to 2 of 4 uptrained cells). YOKED CONTROLS showed no significant change from baseline.

Our results show that identical training procedures can contingently modify intact behavior, muscle activity, and the excitability of individual motor cells. It will now be important to determine the neuronal sites which produce the learning we observe at all three levels of analysis.

246.7

ANALYSIS OF DIRECTED HEAD MOVEMENTS IN RESPONSE TO FOOD STIMULI IN *APLYSIA*

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Aplysia, when oriented in their characteristic feeding posture, respond with head and neck movements directed toward food stimuli that are applied briefly to various loci on their tentacles or rhinophores. Video analysis showed that the velocity and the final turning angle of the head correlates with the eccentricity of the stimulus position. In all cases, the turning angle of the animal greatly exceeds the stimulus position, i.e., the animal markedly over-shoots. In contrast to a brief stimulus, a maintained stimulus leads to centering of the food, followed by one or several bites. During a biting response, stimuli are completely ineffective in eliciting a turning response, although bites per se do not suppress ongoing turning movements. This inhibition is not present during head turning movements. Having established some of the behavioral characteristics of the head orienting response, we were in a position to examine the properties of central neurons that might mediate the response. As a first approach we focussed on neurons in the cerebral B cluster, since available data (e.g., Fredman & Jahan-Parwar 1980; Bablanian et al. 1987) made them candidate motoneurons for head orientation. These cells respond to tactile and chemical stimulation on the tentacles, and firing the cells leads to contraction of muscles in the neck and body wall. Dye fillings and intracellular recordings from neurons in the B cluster in isolated ganglia and semi-intact preparations revealed two distinct types of neurons, which differ in their morphology, their electrophysiological properties, and their response to tentacle stimulation. However, the properties of neither type of B cluster neurons matched the criteria for involvement in head orientation, as established by the behavioral studies. Chronic recordings of units in the cerebro-pleural connective (most likely spikes from contralateral B neurons) showed activity which was correlated with stimulation of both the ipsi- and the contralateral side of the head. The peak of the neuronal activity occurred immediately after the stimulus but did not persist while the animal was turning its head and did not generally occur during 'spontaneous' head movements. We are currently investigating other neurons that might be involved in head orientation to food.

246.8

ACTIVITY OF CBI-2 OF *APLYSIA* ELICITS BITING-LIKE RESPONSES.

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Biting is a consummatory feeding behavior involving coordinated movements of the buccal mass (biting apparatus) with a characteristic radula protraction-retraction sequence. Rhythmic biting is elicited when a food stimulus is continuously applied to the inner lips (perioral zone). An interganglionic cerebral-buccal interneuron (CBI-2) was previously identified which evokes a robust rhythmic buccal motor program (BMP). We have found that the BMP involves phasic activation of various identified buccal neurons, including ARC motoneurons B15 and B16, interneurons B4 and B5, and the putative central pattern generating neurons B31 and B32. B15 and B16 fire in a pattern that is seen during normal biting in intact animals (see Cropper et al., these abstracts), but is not observed in the same cells during swallowing, rejection, or withdrawal. CBI-2 also drives cerebral neurons, including the serotonergic, metacerebral cells. The other cerebral neurons, however, may not be necessary for activation of the BMP since the efficacy of CBI-2 driving of the BMP is little affected by blockade of synaptic transmission in the cerebral ganglion with 10 mM Co++. In dissected preparations consisting of the cerebral and buccal ganglia, and the buccal mass, firing of CBI-2 reliably produces rhythmic buccal mass movements which are similar in topography and timing to biting movements seen in intact, food-aroused animals. Video image analysis was used to show that the rhythmic buccal movements elicited by CBI-2 exhibit a build-up in speed and strength, similar to that seen in normal biting. Our data thus indicate that CBI-2 may play a role in the initiation and maintenance of biting. The identification of a neuron that drives a BMP and a specific class of consummatory behavior in a dissected preparation, opens the way for detailed analyses of central and peripheral mechanisms of neuromodulation underlying food-induced arousal and satiation of feeding in *Aplysia*, and will permit comparison with similar neurons that have been described in other molluscan species.

246.9

GUT STIMULATION EVOKES A SLOW IPSP IN HISTAMINERGIC NEURON C2, MEDIATED BY CCK-GASTRIN LIKE IMMUNOREACTIVE BUCCAL NEURON, B18.

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The identified mechanoafferent neuron C2 makes extensive synaptic contact with neurons controlling feeding in Aplysia, and is believed to shape aspects of the feeding response as well as contribute to the maintenance of food-induced arousal. The synaptic output of C2 is powerfully gated by inhibitory input to the cell, including a slow inhibition evoked by esophageal nerve stimulation. We show here that this slow IPSP is caused by B18. Esophageal nerve stimulation with a 1 second, 10Hz train of pulses results in a burst of fast IPSPs in C2 followed by a long lasting (several seconds) hyperpolarization, and a simultaneous burst of spikes in B18. Firing B18 by injecting depolarizing current has similar effects on C2, and hyperpolarization of B18 to prevent its firing during esophageal nerve stimulation abolishes C2's response. When the amplitude of B18's IPSP in C2 is augmented by bathing the cerebral ganglion with high Ca++ containing saline, single B18 spikes induce in C2 a fast IPSP followed by a slow, long lasting (relative to the membrane time constant) IPSP. These IPSPs are likely monosynaptic, as they follow B18 spikes one for one when B18 is made to fire at high frequency. In double labeling experiments B18 neurons iontophoretically marked with Lucifer Yellow showed immunoreactivity with a CCK/gastrin antiserum.

The nature of the esophageal nerve inputs to B18 were studied in preparations with the esophagus and gut left connected to the buccal ganglion. Mechanical stimulations mimicking dilation of the esophagus/anterior gut caused B18 depolarization and firing similar to those observed with esophageal nerve stimulation, and our electrical stimulations are therefore likely activating esophageal/anterior gut mechanosensory inputs to B18. This pathway thus allows sensory input from the esophagus/anterior gut access to neurons involved in the control of the buccal mass and food induced arousal, and may effect modifications in feeding behavior as a function of the state (e.g., dilation) of the esophagus/anterior gut.

246.10

GILL ARITHMETIC: INTERACTIONS BETWEEN IDENTIFIED MOTOR NEURONS AND THEIR EFFECT ON EVOKED BEHAVIORS OF APLYSIA GILL.

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The movements evoked by depolarization of individual gill motor neurons, although variable in amplitude, are qualitatively consistent. Two cells, RDG and L7, produce a very similar movement involving principally Shorten and Antiflare. The movement produced by L9 is a weak Lift; LDG1 combines Shorten, Antiflare, and Roll; and LDG2 produces a movement combining Flare, Curl, and Roll. Simultaneous depolarization of qualitatively congruent cells such as two LDG2s or L7 and RDG produces a movement whose amplitude is greater than the sum of the individual movements. Also L9 enhances the effect of L7, while making no obvious change in the movements of other cells. L7 and LDG2 add in amplitude as reported previously (Kupfermann et al. 1974). Their qualitative interaction will be discussed. The effect of hyperpolarization or depolarization of major motor neurons on the GWR may depend on behavioral state. In suppressed preparations hyperpolarization of L7 and/or RDG may have no effect, qualitative or quantitative, on the GWR.

246.11

A FIRST ATTEMPT TO MINIMIZE THE NUMBER OF NEURONS THAT PARTICIPATE IN THE APLYSIA GILL-WITHDRAWAL REFLEX.

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Previously published results from optical recordings suggested that as many as 300 neurons in the abdominal ganglion might be involved in the gill-withdrawal reflex. We began efforts to determine if the reflex could be elicited in conditions where a smaller number of neurons might be involved. We compared the number of active neurons in two situations, the control situation with sea water bathing the siphon, ganglion, and gill, and the experimental situation where both the siphon and gill were bathed in a low-calcium high-magnesium sea water to block contraction and possible recurrent sensory feedback to the ganglion. Surprisingly, the number of active neurons in the experimental situation was slightly larger in three preparations; 107% $\pm$ 1% (mean and standard error of the mean) of the control value. When the time courses of the increased spike activity following the water jet to the siphon were compared in the two situations, they were found to be similar. Recurrent activity from siphon and gill contractions did not have large effects on the neuron activity in the ganglion.

246.12

SENSITIZATION AND SENSORY SIGNALS IN APLYSIA I. NEUROMODULATOR EFFECTS ON PERIPHERAL EXCITABILITY.

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Previous work (and next abstract) suggests coordinate central modulation of single sensory neurons at multiple cellular loci during sensitization. Recently we found that noxious stimulation causes long-term (10-15d) enhancement of peripheral sensory excitability. Here we describe acute effects of peripherally applied neuromodulators on tail mechanosensory thresholds of individual VC neurons. Threshold was determined (blind) before, during, and after injection of either seawater or an endogenous neuromodulator (each 10^{-6}M except ACh, 10^{-5}M) into the tail field. The test sequence was then repeated with the other solution. Comparisons to seawater within animals showed significant threshold depression by SCP_B, and significant elevation by dopamine, FMRFamide, and ACh. 5HT depressed threshold during later pretests of cells in the same animal, obscuring within-animal 5HT effects. Comparisons between animals, however, showed significant depression of threshold by 5HT for at least 10-15 min after washout and equally persistent elevation by FMRFamide. Similarities to central neuromodulatory patterns in these cells suggest that bidirectional modulation of sensory signalling by noxious stimuli may be produced centrally and peripherally by the same set of neuromodulators.

246.13

SENSITIZATION AND SENSORY SIGNALS IN APLYSIA II. MODULATION OF CENTRAL BURSTING AND SPIKE CONDUCTION. A.L.Clatworthy*and E.T. Walters. Dept. Physiol. & Cell Biol., U.Texas Med.Sch, Houston, TX 77225.

Regenerative bursts triggered by soma stimulation after noxious training indicate sensitization can alter sensory spike patterns at central loci (Walters, 1987). We now report that nerve stimulation causing afferent spike trains like those evoked by cutaneous injury elicits long-latency regenerative spikes centrally, which add to the afferent volley. 49 of 66 VC sensory neurons (74%) showed regenerative bursts (1-15 spikes). Regenerative bursts required more intense nerve stimuli (2.5 x VC axon threshold), indicating a heterosynaptic component. In 13 of 20 cells soma hyperpolarization reversibly blocked regenerative bursts ($p<.001$), indicating a central locus. Centrally applied $CoCl_2$ (100mM) blocked regenerative bursts in 9 cells, altered the bursts in 3 cells, and had no effect in 3 cells. Regenerative bursts appeared to originate outside the soma. Short-latency spikes evoked one-for-one by nerve stimulation could also be modified in the CNS: afferent volleys from 1 nerve were sometimes completely or partially blocked, or enhanced, by stimulating a second nerve. Noxious stimuli can thus modulate several aspects of sensory spike signalling, in addition to synaptic and perhaps transducing functions (preceding abstract).

SEROTONIN RECEPTORS II

247.1

5-HT2 RECEPTOR REGULATION IN RAT FRONTAL CORTEX: PUTATIVE SELECTIVE AGONIST AND ANTAGONIST STUDIES. M. R. Pranzatelli. Department of Neurology, Columbia University, NY, NY 10032.

DOI [1-(2,5-dimethoxy-4-iodo-phenyl aminopropane)-2] has recently been suggested as a selective 5-HT2 receptor agonist. Because of the potential importance of such a tool for investigations of 5-HT2 receptor regulation, receptor binding studies were performed in rats after chronic daily treatment with DOI, the selective 5-HT2 antagonist ketanserin, or with vehicle. In naive frontal cortex, competition experiments revealed shallow curves with significantly different IC50s (nM) for (±)DOI of 2.3 (^{3}H-DOB), 44.4 (^{3}H-ketanserin), and 2216 (^{3}H-5-HT). Yet chronic daily treatment with DOI (3-9 mg/kg) down-regulated 5-HT2 sites in frontal cortex identified with either ^{3}H-ketanserin (-60%) or with ^{3}H-DOB (-75%), without affecting 5-HT1 sites. Kds were not altered. Chronic treatment with ketanserin (10 mg/kg) also down-regulated both ^{3}H-ketanserin (-37%) and ^{3}H-DOB (-55%) sites in frontal cortex, without changes in 5-HT1 sites. These data indicate that in chronic treatment, DOI, like ketanserin, is highly selective for 5-HT2 vs 5-HT1 sites at behaviorally useful doses. However, the similar effects of a representative putative 5-HT2 selective agonist and antagonist on proposed high- and low-affinity 5-HT2 sites suggests that the regulation of 5-HT2 sites, as so defined, is unusual and unlike that of 5-HT1 sites. Supported by NIH grant 1-KO8-NS01158 and the Myoclonus Research Fund.

247.2

[^{125}I]-2-(2,5-DIMETHOXY-4-IODOPHENYL)AMINOETHANE (2C-I) LABELS THE GTP-SENSITIVE STATE OF THE 5-HT$_2$ RECEPTOR. M. P. Johnson*, D. E. Nichols, and C. A. Mathis*. (SPON: G.K.W. Yim). Depts. of Med. Chem. & Pharmacog. and Pharmacol. & Toxicol., Purdue Univ., Sch. of Pharmacy, W. Lafayette, IN 47907.

Recent studies of 5-HT$_2$ receptor binding have focused on the agonist state of the receptor using racemic [^{3}H]-DOB (Lyon et al., Mol. Pharmacol. 31:194, 1987) or the isomers of [^{125}I]-DOI (Johnson et al., Neuropharmacol. 26:194, 1987) as radioligands. We present here evidence that a new achiral ligand, [^{125}I]-2C-I labels the same site. Saturable specific binding (70%) was found in rat frontal cortex. Scatchard analysis yielded a single site model with K_D=1.52±0.10 nM and B_{max}=87±3.8 fmol/mg. Addition of 1 mM GTP inhibited specific binding to less than 10%.

Displacement results showed a low affinity for the 5-HT$_{1A}$/5-HT$_{1B}$ antagonist pindolol, as well as for DA, NE, histamine, and 8-OH-DPAT. The purported 5-HT$_{1B}$ agonist TFMPP had high affinity for this site. Stereoselectivity was demonstrated for the more active isomers of the hallucinogens DOI, 5-MeO-AMT and DMCPA. It was found that 4-OH or 5-MeO substitution on the DMT nucleus to give psilocin and 5-MeO-DMT, respectively, yielded a 3-4 fold increase in affinity. Therefore, [^{125}I]-2C-I can be used to label the 5-HT$_2$ receptor, with the advantages of high specific activity and an achiral molecule, avoiding the need to use pure isomers of DOB or DOI as ligands.

247.3

R(-)-4-^{77}BROMO-2,5-DIMETHOXYAMPHETAMINE [^{77}Br-R(-)DOB] LABELS A NOVEL 5-HYDROXYTRYPTAMINE BINDING SITE IN BRAIN MEMBRANES. S.J. Peroutka, A. Hamik, M.A. Harrington, C.A. Mathis*, P.A. Pierce and S.S.H. Wang. Department of Neurology, Stanford University, Stanford, CA 94305.

R(-)-4-^{77}Bromo-2,5-dimethoxyamphetamine (^{77}Br-R(-)DOB) was synthesized to a high specific activity (7500 ± 200 Ci/mmol) and used to label membrane-associated recognition sites in rat brain. ^{77}Br-R(-)DOB displayed high affinity (K_D = 0.15 ± 0.02 nM) for a relatively low density of binding sites (B_{max} = 0.31 ± 0.04 pmole/g tissue) in rat cortical membranes as compared to ^{3}H-ketanserin (K_D = 0.65 ± 0.1 nM; B_{max} = 6.2 ± 0.6 pmole/g tissue). Guanine, but not adenine, nucleotides were found to inhibit specific ^{77}Br-R(-)DOB binding. 10^{-4} M GTP did not eliminate specific ^{77}Br-R(-)DOB binding but caused a competitive inhibition of the radioligand. A significant correlation ($p < 0.01$) exists between drug potencies for ^{77}Br-R(-)DOB labeled sites and both 5-HT$_2$ binding sites ($r = 0.64$) and 5-HT$_{1C}$ binding sites ($r^2 = 0.68$). However, the sites do not appear to be identical. We conclude that ^{77}Br-R(-)DOB labels a unique 5-HT recognition site in rat brain which does not coincide with previously described 5-HT binding site subtypes. The ^{77}Br-R(-)DOB site does not appear to be a high affinity "state" of the 5-HT$_2$ receptor but may play a role in the pharmacological effects of hallucinogenic drugs.

247.4

5HT-2 RECEPTOR PET QUANTIFICATION USING UNLABELLED ANTAGONISTS. D.F. Wong*, A. Gjedde*x, C. Ross*, R. Frank*+, R.F. Dannals*, P. Thyrum*+, D. U'Prichard, H. N. Wagner, Jr* (SPON: W. Jankel). Johns Hopkins Med. Inst., xMontreal Neuro Inst. and +ICI Pharmaceuticals Group, Wilmington, DE.

We have developed a model for reversible neuroreceptor radioligands competing with inhibiting drugs. For measuring human 5HT-2 receptors parameters in vivo we used the putative 5HT-2 antagonist, ICI 169,369. Six subjects received 30 milligram BID of the drug or placebo in a double blind crossover study for 1 wk. each. Subsequently each subject received C-11 NMSP IV, frontal cortex (FC) binding was measured by PET. Input function was determined by plasma sampling throughout the study with simultaneous drug samples. Rate constants K1-k4 and volumes of distribution at equilibrium were determined. The percentage change from control to drug PET scan for the parameters k3/k4 declined during drug 5-40%; maximum FC/cerebellum declined 3-20%. Using partition coefficient assumptions, and in vitro affinity ratios, preliminary Bmax & KD estimates averaged 93 pmoles/g, 18nM, resp. This is analogous to using competition with irreversible binding (JCBFM 6:137, 1986). Although Bmax can be measured by varying specific activity (J Nuc Med 28:611, 1987) this protocol allows screening new drugs with C-11 NMSP & even more specific PET ligands.

247.5

INDOLEALKYLAMINES AND PHENALKYLAMINES HAVE OPPOSITE EFFECTS ON PHOSPHATIDYLINOSITOL HYDROLYSIS IN RAT CORTEX. P.A. Pierce and S.J. Peroutka. Department of Neurology, Stanford University School of Medicine, Stanford, CA 94305.

5-Hydroxytryptamine (5-HT) stimulates phosphatidylinositol hydrolysis at micromolar concentrations via the 5-HT_2 receptor in rat cortex (Conn,P.J. and Sanders-Bush, E.,Neuropharmacol.,23:993,1984). This response is blocked by the 5-HT_2 antagonist ketanserin. The phenalkylamines 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB) are partial agonists, producing a lower PI stimulation relative to the 5-HT PI response. This phenalkylamine PI response is also blocked by ketanserin. In contrast, the indolealkylamines d-lysergic acid diethylamide (d-LSD) and N,N-dimethyltryptamine (DMT) only slightly stimulate PI hydrolysis at high concentrations (greater than 10 uM). This response is not blocked by ketanserin. At high concentrations, this ketanserin-insensitive PI stimulation has been reported for 5-HT_2 antagonists (Conn, P.J. and Sanders-Bush, E.,J. Pharmacol. Exp. Ther.,242:552,1987). At lower concentrations, d-LSD (10 nM) and DMT (1 uM) significantly inhibit the PI response to 10 uM 5-HT. These data indicate that phenalkylamines (DOI, DOB) are partial agonists at the 5-HT_2 receptor in rat cortex while indolealkylamines (d-LSD, DMT) are antagonists at the 5-HT_2 receptor yet stimulate PI hydrolysis through another interaction at high concentrations.

247.6

ACUTE AND CHRONIC EFFECTS OF HP 370 ON D_2 AND 5HT_2 RECEPTORS AND DA METABOLISM. C.A.Wilmot, A.M.Szczepanik*, P.G. Conway, L.R.Brougham*, J.C.Wilker* and D.B. Ellis*. Department of Biological Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville NJ 08876.

HP 370 4,9-dibromo-6-(4-methyl-1-piperazinyl)benzo[b]pyrrolo [3,2,1-jk][1,4]benzodiazepine, is a potential antipsychotic which resembles clozapine (CLZ) in behavioral and electrophysiological tests predictive of an atypical agent but, unlike CLZ, HP 370 is inactive at cholinergic receptors. Receptor autoradiography was used to examine site-specific interactions of CLZ and HP 370 with D_2 and 5HT_2 receptors and compared to the typical neuroleptic haloperidol (HAL). CLZ and HP 370 produced 50-75% inhibition of [^{3}H]-spiperone binding to 5HT_2 receptors after acute treatment with 10-40 mg/kg, ip, which persisted for at least 24 hours with HP 370. Chronic administration of CLZ (20 mg/kg, ip) or HP 370 (20-40 mg/kg, ip) decreased the B_{max} for cortical 5HT_2 receptors by 55% and >80%, respectively, with no significant changes in the B_{max} for D_2 receptors in the striatum or n. accumbens. Neither acute nor chronic treatments with HAL affected binding to 5HT_2 receptors. HP 370 increased the ratios of DOPAC/DA and HVA/DA to 175-250% of control following a single dose (20-40 mg/kg, po) but not following chronic dosing. Similar to CLZ, the effects of HP 370 on DA metabolism were not potentiated by amfonelic acid. Acutely, HP 370 (10-40 mg/kg, ip) also significantly antagonized HAL-induced catalepsy. These studies suggest that strong interactions with 5HT_2 receptors may be a significant component of the profile of an atypical neuroleptic.

247.7

1-(2,5-DIMETHOXY-4-IODOPHENYL)-2-AMINOPROPANE (DOI) ACTS AT 5-HT2 RECEPTORS TO CONTRACT THE PORTAL VEIN IN VITRO AND INHIBIT FEEDING IN VIVO. L.E. Schechter, P.M. Consigny* and K.J. Simansky.Depts. Pharmacol. & Physiol., Med. Col. PA, Phila., PA 19129.

Previously we have reported that DOI produces an anorexic action in rats that is blocked by the 5-HT2 antagonists LY53857, ketanserin and 1-(1-naphthyl)-piperazine but not by the peripherally-acting 5-HT2 antagonist xylamidine (Psychopharmacol. 94(3):342, 1988). This contrasted sharply with the ability of xylamidine to antagonize the anorexic effect of peripherally administered serotonin. Thus, either DOI does not act as a 5-HT2 agonist at peripheral 5-HT2 sites or xylamidine does not block the anorexic effect because this action is mediated in the brain. Accordingly, DOI was tested in the rat portal vein which is a model for peripheral 5-HT2 receptor stimulation. Using noncumulative additions of agonists, DOI contracted the portal vein in a dose-related fashion [4 nM-40 uM; ED50 = 38 (7-201) nM] with full agonist activity compared to that of serotonin [max contraction (g) = .82 ± .08 vs. .90 ± .03, respectively] . In contrast, adding DOI in a cumulative fashion reduced the maximal response. Thus depending upon the experimental conditions DOI can appear to be a full or partial agonist. Notably, xylamidine produced a dose-related antagonism (1-100 nM) of the contractile effect of DOI (80 nM) with an ID50 of 6 (2-16) nM. The contractile activity of DOI (80 nM) was also blocked by spiperone (100 nM) and ketanserin (100 nM) but not by pindolol (20,000 nM). In vivo, spiperone (0.17 umol/kg) antagonized the anorexic effect of DOI (6 umol/kg) in rats. Taken together, the results demonstrate that DOI is a 5-HT2 agonist that can stimulate peripheral 5-HT2 receptors in the portal vein. Furthermore, this action is blocked by xylamidine. In view of our previous findings, this suggests the anorexic action of DOI is mediated at central 5-HT2 receptors. Supported by NIMH 41987 to KJS.

247.8

SEROTONIN EXCITATION OF FACIAL MOTONEURONS: MEDIATION THROUGH A 5-HT-2 RECEPTOR. Kurt Rasmussen and George K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06508

Serotonin (5-HT) and norepinephrine (NE) increase the excitability of facial motoneurons by producing a slow depolarization. We have examined this response with ligands selective for various 5-HT receptor subtypes in an effort to determine the subtype(s) of 5-HT receptor(s) involved in this response. In chloral hydrate anesthetized male rats, DOM, a putative 5-HT-2 agonist, applied iontophoretically (20 nA) or systemically (100 µg/kg) acts as an agonist while 8-OH-DPAT, a 5-HT-1A agonist, applied iontophoretically has little or no effect. The action of DOM differs from that of 5-HT in that it was much longer in duration (e.g., > 30 min) following a single iontophoretic application. Similar results were obtained for these compounds when applied iontophoretically to facial motor neurons recorded in the in vitro brain slice preparation. Intracellular recordings in the brain slice preparation show that DOM, like 5-HT, causes a slow depolarization of facial motoneurons, but its action is much more prolonged than that of 5-HT. The non-selective 5-HT antagonist methysergide was effective in blocking the action of 5-HT, but not NE, when administered systemically in vivo (0.1-0.2 mg/kg) and iontophoretically in vitro (10 nA). The selective 5-HT-2 antagonist ritanserin was also effective in blocking the action of 5-HT, but not NE, when administered systemically in vivo (0.2-0.4 mg/kg) and when added to the bath in vitro (20 µM). However, another 5-HT-2 antagonist, LY 53857, was not able to block the action of 5-HT and may display some agonist activity when applied systemically in vivo or iontophoretically in vitro. Thus, the action of 5-HT on facial motor neurons seems to be due primarily to activity at 5-HT-2 receptors, however this 5-HT-2 receptor may have different properties than 5-HT-2 receptors in other brain regions.

247.9

^{3}H-QUIPAZINE LABELS 5-HYDROXYTRYPTAMINE$_3$ RECEPTORS IN RAT CORTICAL MEMBRANES. C.M. Milburn, A. Hamik and S.J. Peroutka. Department of Neurology, Stanford University, Stanford, CA 94305.

5-Hydroxytryptamine$_3$ (5-HT_3) receptors have been extensively characterized in the peripheral nervous system and have recently been identified in the central nervous system. Since quipazine displays potent antagonist effects at peripheral 5-HT_3 receptors, ^{3}H-quipazine was used to radiolabel putative 5-HT_3 receptors in rat cortical membranes. ^{3}H-quipazine labels a single population of binding sites with an affinity of 1.3 ± 0.1 nM. Specific binding was defined as the excess over blanks taken in the presence of 100 nM ICS 205-930. Quipazine, ICS 205-930, BRL 43694A and Lilly 278584 had affinities of 1 nM or less for this site. The affinities of 5-HT, bufotenine, MDL 72222, mCPP, metoclopramide and RU 24969 were between 10 and 500 nM, and that of cocaine was 2880 nM. Mesulergine, ketanserin, methysergide and d-LSD were inactive (i.e., ≥ 50,000 nM). Nucleotides did not significantly inhibit binding to these sites. The pharmacology of this site suggests that ^{3}H-quipazine labels 5-HT_3 receptors in rat cortex.

247.10

SPECIES VARIATIONS OF [^{3}H]QUIPAZINE BINDING TO 5-HT_3 RECEPTORS. A. Hamik* and S.J. Peroutka (SPON: E. Smith). Department of Neurology, Stanford University Medical Center, Stanford, CA 94305.

[^{3}H]Quipazine has been shown to label 5-HT_3 receptors in rat brain. (Peroutka and Hamik, Euro. J. Pharm., 148:297, 1988) Specific binding was defined as the excess over blanks in the presence of 100 nM ICS 205-930. Eight species were examined for specific [^{3}H]quipazine binding to 5-HT_3 receptors. In rat cortex, the sites were labelled with a K_D of 1.3 ± 0.1 nM and a B_{max} of 2.9 ± 0.5 pmol/g-tissue. Pig cortex showed a K_D of 2.1 ± 0.2 nM and a B_{max} of 2.6 ± 0.2 pmol/g-tissue. By contrast, no significant specific binding was shown in human, cow, rabbit, guinea pig, chicken, or turtle brain. For example, in the presence of 0.6 nM [^{3}H]quipazine, rat cortex shows a total binding value of 1,270 ± 30 cpm, and a non-specific binding value of 540 ± 10 cpm. In human cortex, total [^{3}H]quipazine binding equals 610 ± 40 cpm, and non-specific binding equals 550 ± 20 cpm. Therefore, species variations in either the density or presence of 5-HT_3 receptors appear to exist.

247.11

5-HT_3 RECEPTORS CONTROL DOPAMINE RELEASE IN THE LIMBIC SYSTEM OF FREELY-MOVING RATS. A.Imperato* and L.Angelucci* Farmacologia 2a, Università di Roma "La Sapienza", P.le A.Moro 2, 00185 Roma, Italia. (SPON: M. Karobath)

Classication of 5-hydroxytryptamine (5-HT) receptors into 5-HT_1 , 5-HT_2 and 5-HT_3 subtypes, has been recently proposed on functional and pharmacological grounds. Contrary to 5-HT_1 and 5-HT_2 receptors found in various tissues, including neurons of the CNS, until now 5-HT_3 receptors have only been demonstrated in PNS.Here we show, by the use of microdialysis, that 5-HT_3 receptors, located on neurons in the ventral tegmental area (VTA), regulate dopamine release in the nucleus accumbens of conscious rats. ICS 205-930, a potent and selective 5-HT_3 receptor antagonist, either systemically (50 ug/Kg s.c.) or into the VTA (0.5 ug), prevented the increase in dopamine release by s.c. morphine, nicotine, ethanol. This is the first direct evidence of the existence of functional 5-HT_3 receptors in the brain. Since the mesolimbic DAergic system is thought to mediate the rewarding properties of abuse drugs,these findings point to a possible therapeutic usefulness of ICS 205-930.

247.12

CHARACTERIZATION OF [^{125}I] DOI BINDING SITES IN RAT BRAIN. D.J. McKenna[1], C.A. Mathis*[2] and S.J. Peroutka[1]. 1. Department of Neurology, Stanford University Medical Center, Stanford, CA 94305. 2. Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

DOI is a hallucinogenic phenylalkylamine which interacts stereospecifically with serotonergic receptors. Autoradiographic investigations have demonstrated [^{125}I]DOI specific binding in regions known to contain high densities of 5-HT_2 receptors. Scatchard analysis of [^{125}I]DOI binding in rat cortex indicates that [^{125}I]DOI labels an intermediate density of sites compared to [^{77}Br]-R-DOB and [^{3}H]ketanserin (0.31, 4.9 and 6.2 pmol/g tissue, respectively). The observation that DOI labels only 70 to 80% of the number of sites labeled by [^{3}H]ketanserin suggests it may label a unique 5-HT_2 receptor subtype in the rat central nervous system. Nucleotides did not effect either the K_D or B_{max} values. The results of competition studies using various subtype-selective 5-HT ligands at [^{125}I]DOI-labeled sites will be presented, and the relevance of the "DOI site" to other 5-HT receptor subtypes will be discussed.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM I

248.1

DIFFERENTIAL ROLES OF GABA AND GLYCINE AS INHIBITORY NEUROTRANSMITTERS IN CAT EXTRAOCULAR MOTOR NUCLEI. R.F. Spencer, R.J. Wenthold, and R. Baker. Dept. Anat., Med. Col. of Virginia, Richmond, VA 23298; Lab. Neuro-otolaryngol., N.I.N.C.D.S., N.I.H., Bethesda, MD 20892; and Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

The immunohistochemical localization of GABA and glycine in the cat oculomotor, trochlear, and abducens nuclei has been examined by light and electron microscopy. In the oculomotor nucleus, GABA-immunoreactive synaptic endings were distributed within all motoneurone subdivisions except the medial rectus, in which there was a near absence of labelled profiles. The trochlear nucleus exhibited the highest density of GABA-immunoreactive axo-somatic and axo-dendritic synaptic endings that contained numerous pleiomorphic synaptic vesicles. By contrast, the abducens nucleus was characterized by a paucity of GABA-immunoreactive synaptic endings.

The immunohistochemical localization of glycine demonstrated a high density of immunoreactive synaptic endings in the abducens nucleus that presumably are associated with vestibular, reticular, and prepositus hypoglossi inhibitory inputs. Glycine-immunoreactive synaptic endings in the abducens nucleus also contained pleiomorphic synaptic vesicles and exhibited a widespread soma-dendritic distribution. By contrast, the oculomotor and trochlear nuclei exhibited sparse glycine-immunoreactive staining. These differences appear to be related to differences in immunoreactive staining of axons in the ascending (GABA) and descending (glycine) MLF. The findings are consistent with the differential roles of GABA and glycine as inhibitory neurotransmitters in the vertical and horizontal eye movement systems, respectively.

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248.2

THE CEREBELLOTECTAL PATHWAYS: ORIGINS AND RELATIONSHIP TO THE CROSSED TECTORETICULAR NEURONS IN A PRIMATE. P.J. May and J.D. Porter. Depts. of Anatomy and Ophthalmology, Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

The anatomical relationship of the superior colliculus, an important premotor saccade center, and the cerebellum, which is believed to play a critical role in the feedback regulation of saccades, has not been investigated in primates, the order having the most extensive saccadic repertoire. Our previous retrograde results demonstrated two populations of cerebellotectal neurons: a contralateral one in the posterior interpositus and adjacent dentate nuclei and a bilateral one in the fastigial nucleus. Injection of WGA-HRP into the posterior interpositus and dentate nuclei of the Macaca fascicularis resulted in a moderately dense band of anterogradely labelled terminals in the ventral half of the intermediate grey layer, throughout the rostrocaudal extent of the superior colliculus. The deep grey layer was more lightly labelled. Following injection of WGA-HRP into the fastigial nucleus, labelled terminals were distributed in the same laminar pattern, but were present bilaterally and were restricted to the rostral pole of the colliculus. In both cases, a few retrogradely labelled neurons were observed in the collicular deep layers. To determine the relationship of the cerebellotectal termination pattern to the sources of descending tectal pathways, WGA-HRP was placed unilaterally in the rostral pontine reticular formation. The retrogradely labelled neurons in the contralateral superior colliculus were restricted to the ventral half of the intermediate grey layer, throughout the colliculus. This band of labelled cells co-localized with an acetylcholinesterase rich band in the intermediate grey layer. Labelled cells in the ipsilateral colliculus were located dorsal and ventral to this lamina. The precise laminar correspondence between the distribution pattern of cerebellotectal terminals and the location of the cells of origin of the predorsal bundle (crossed) pathway, indicates that the cerebellum plays a direct role in modifying the activity of saccadic premotor neurons in the superior colliculus. Supported by NEI grants EY07166 (PJM) and EY05464 (JDP).

248.3

OCULOMOTOR-RELATED CONNECTIONS OF THE CEREBELLAR FASTIGIAL NUCLEUS IN THE MONKEY, AND THEIR RELATIONSHIP TO FRONTAL EYE FIELD EFFERENTS. G.R. Leichnetz and A. Gonzalo-Ruiz*, Dept. of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Lesions of the fastigial nucleus (FN) have a profound effect on the production of saccadic eye movements. While the cerebellum is not necessary for the generation of eye movements, it is essential for the coordination of features of the movements (eg. size, velocity) in response to changing motor commands. The frontal eye field (FEF), on the other hand, participates in the initiation of voluntary purposeful saccades in trained monkeys. We have been interested in the interaction of cortical and cerebellar influences in the control of eye movement (see JCN 268: 508-526, 1988).

Both the FN and FEF project to the superior colliculus, supraoculomotor periaqueductal gray, paramedian pontine reticular formation (including raphe), and the nucleus prepositus hypoglossi. The FEF projects to the medial nucleus reticularis tegmenti pontis, and the dorsomedial basilar pontine nuclei (DMPN), and to a lesser extent the dorsolateral basilar pontine nuclei (DLPN). The FN appears to receive input from both DMPN and DLPN. While the FEF projects to preolivary "paraoculomotor" cell groups, including the nucleus of Darkschewitsch, medial accessory nucleus of Bechterew, and dorsomedial parvicellular red nucleus, none of these appear to receive from or project to the FN. The medial accessory olivary nucleus and vestibular complex, which project to the FN, do not receive FEF input. We are now attempting to understand the importance of their points of convergent vs. distinctive connectivity in terms of their contrasting roles in ocular motility.

248.4

PROJECTIONS FROM THE ROSTRAL MESENCEPHALIC RETICULAR FORMATION TO THE SPINAL CORD. An HRP and autoradiographic tracing study in the cat. G. Holstege[1] and R.J.Cowie[2],[1] Dept. Anatomy, Univ. California San Francisco CA 94143 [2] Dept. Anatomy Howard University Washington DC 20059.

Eye and head movements are strongly interconnected, because they play an important role in determining the direction of the visual field. The caudal Field H of Forel, including the rostral interstitial nucleus of the MLF (riMLF) and the interstitial nucleus of Cajal with adjacent reticular formation (INC-G) contain neurons controlling vertical eye and head movements. Injections of ^{3}H-leucine in these areas and HRP spinal cord injections revealed that:

1: Unlike the neurons projecting to the extra-ocular muscle motoneurons, the major portion of the spinally projecting neurons are not located in the riMLF or INC proper but in adjacent areas, i.e. in the ventral and lateral parts of the caudal third of the Field H of Forel and in the INC-G.

2: Neurons in caudal Field H of Forel project, via the ventral part of the ventral funiculus, to the lateral part of the upper cervical ventral horn. This area includes motoneuronal cell groups, innervating the cleidomastoid, clavotrapezius and splenius muscles. At lower cervical levels labeled fibers are distributed to the medial part of the ventral horn.

3: Neurons in the INC-G project, via the dorsal part of the ventral funiculus, bilaterally to the medial part of the upper cervical ventral horn. This area includes motoneurons innervating prevertebral flexor muscles. Further caudally labeled fibers are distributed to the medial part of the ventral horn (laminae VIII and adjoining VII) throughout the length of the spinal cord.

It is proposed that neurons in the caudal Field H of Forel, projecting to the spinal cord, might be especially involved in the control of fast vertical head movements and the INC-G-spinal neurons in slower, smaller movements controlling the position of the head in the vertical plane. Supported by NASA Grant 2-484903-26308

248.5

GAZE CONTROL IN HEAD-FREE CATS: TASK DEPENDENT TRAJECTORIES AND EVIDENCE FOR A COMMON MOTOR DRIVE TO THE EYES AND HEAD. D. Guitton and D.P. Munoz. Neurology and Neurosurgery Dept. McGill University, Montreal, Canada, H3A 2B4

Orienting movements, consisting of combined eye and head displacements, are routinely used to direct the visual axis to the source of a sensory stimulus. Tectoreticulospinal and reticulospinal neurons in the cat are important pathways that participate in the control of eye and head movements. The afferents to these neurons as well as the nature of their movement related discharges suggest that the degree of coupling between eye and head movements can be quite variable. We examined eye-head coupling when cats performed two tasks: 1)a "collicular" task in which the cat oriented to a target that appeared unexpectedly in the periphery (VT task); 2)a more cognitive, "cortical" task in which the cat oriented to a location where it predicted the target would appear (PT task). For a gaze shift of given amplitude both the eyes and head, and consequently gaze, movements were slower in PT compared to VT. The onset times of the eyes and head always covaried. When the target appeared just prior to a gaze shift initiated by predictive cues, the eyes accelerated in mid-flight and so too the head, 20-30 ms later. Thus in all cases the eyes and head appeared to share a common motor drive. The most striking evidence for this mechanism was that the head acceleration and eye velocity profiles when superimposed, could hardly be distinguished from one another.

248.6

SMOOTH PURSUIT INITIATION IN HUMANS WITH CEREBRAL HEMISPHERIC LESIONS. M.J. Morrow* and J.A. Sharpe. Division of Neurology, Playfair Neuroscience Unit, The Toronto Hospital, University of Toronto, Toronto, Ont. M5T 2S8

We studied horizontal smooth pursuit maintenance (SPM) and initiation (SPI) in 18 patients with unilateral cerebral lesions, using magnetic search coil oculography. SPM was recorded in response to nine groups of predictable target sinusoids differing in frequency and amplitude. Responses to unpredictable foveopetal and foveofugal step-ramps measured SPI and allowed distinction between retinotopic and directional pursuit deficits.

Six patients had significant SPM asymmetry, all with lower ipsilateral gains. Two of these did not have directional SPI asymmetry. In contrast, 3 patients without SPM asymmetry had directional SPI deficits with reduced initial eye acceleration and increased latency for ipsilateral step-ramps. Seven patients had retinotopic SPI deficits; only four of these had hemianopic field loss involving the area of SPI loss. Four patients with complete homonymous hemianopia could generate low velocity SPI to targets moving in their blind fields.

These data indicate that directional deficits of SPM and SPI can be disparate. Retinotopic pursuit loss in humans does not specify damage to optic radiations or striate cortex.

Supported by MRC of Canada Grant MT5404 and NIH Grant EY-06040.

248.7

THE USE OF TARGET SPEED IN PLANNING CORRECTIVE SACCADES IN SMOOTH PURSUIT EYE MOVEMENTS IN MONKEY. E.L. Keller and S.D. Johnsen*. Smith-Kettlewell Eye Research Institute and Dept. of Electrical Engineering and Computer Sciences, University of California, Berkeley, CA 94115.

In response to a sudden onset of smooth visual target motion, primates are able to initiate a smooth tracking eye movement that rapidly matches eye speed to target speed. Because of the latency of this response and the possible initial offset in the position of the eyes with respect to the target, this smooth tracking response is generally coupled with a longer latency corrective saccade which reduces the position error between eye and target. Since these corrective saccades actually move the eye very close to the moving target's position, it has been assumed that information about target speed is utilized in the planning of these saccades. However, in the only quantitative study providing a test of this notion (Heywood and Churcher, Vis. Res. 21, 479, 1980), it was found in human subjects that accurate information about target speed was not incorporated into the programming of saccades. We reinvestigated this subject in the monkey, and found that information about target speed is used in this species in the production of corrective saccades. Multiple linear regression analysis supported the hypothesis that the position error and the retinal slip velocity that exist at about 100 msec prior to saccadic onset are both required to provide an accurate statistical prediction of saccade size.

248.8

VERGENCE-RELATED CELLS NEAR THE ABDUCENS NUCLEI J.W. Gnadt, P.D.R. Gamlin, L.E. Mays and Y. Zhang. Dept. of Physiological Optics, School of Optometry, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Twenty-five vergence-related neurons were recorded near the abducens nuclei in 8 macaques. Twenty-three of these cells increased their activity for convergence: mean = 8.2 (spikes/sec)/deg of vergence (range: 2.2 to 20.9). The activity of the cells preceded the vergence movements by a mean of 25 ms, though this varied considerably. Many cells had a smaller, but significant, sensitivity to vergence velocity: mean = 1.1 (spikes/deg)/(deg/sec). Seven cells also increased their firing rate for ipsilateral horizontal conjugate eye movements, and 3 of these showed increases for downward eye position. The mean ratio of vergence to conjugate gains was 5.7. Many of the cells were located near medium-lead burst or omniburst neurons.

In one monkey, recording sites of 5 cells were recovered histologically. Three were in the reticular formation lateral and caudal to abducens, one was at the border of the medial vestibular nucleus and the last was in the lateral vestibular nucleus.

These data have shown that a heterogenous population of vergence-related cells exist in the posterior brainstem and document the prenuclear mixing of vergence and conjugate signals. The purely vergence-related signals would be appropriate as inhibitory input to abducens neurons. (Supported by Grant EY03463 and P30 EY03039).

248.9

LIDOCAINE-INDUCED UNILATERAL INTERNUCLEAR OPHTHALMOPLEGIA: EFFECTS ON ACCOMMODATIVE VERGENCE. P. D. R. Gamlin, J. W. Gnadt and L. E. Mays. Department of Physiological Optics, University of Alabama at Birmingham, Birmingham, AL 35294.

Abducens internuclear neurons decrease their firing rate during symmetrical convergence (Gamlin *et al.*, Invest. Ophth. Vis. Sci. (Suppl) 29:345, 1988). This should result in an inappropriate vergence signal being sent to medial rectus motoneurons during vergence movements. Unilateral lesions of the medial longitudinal fasciculus (MLF) would block this incorrect signal, thereby facilitating convergence.

Reversible unilateral blockade of the MLF was produced by injections of 100 nl of 10% lidocaine HCl in two Rhesus monkeys. Both horizontal conjugate gain and the accommodative convergence/accommodation ratio (AC/A) were measured before and during the blockade. As expected, conjugate adduction was severely affected on the side of the blockade. When the effects of decreased conjugate gain are considered, the net AC/A ratio was increased by MLF blockade. These results confirm that that the MLF is indeed carrying an inappropriate vergence signal, which is cancelled by another, more potent vergence input to medial rectus motoneurons. (Supported by NIH Grant EY03463 and P30 EY03039).

248.10

OCULAR REALIGNMENT DURING AND AFTER SACCADES USING PREPROGRAMMED VERGENCE. L. Levi*, D.S. Zee, T.C. Hain, W.A. Fletcher* and M.L. Rosenberg*. Dept. of Neurology, The Johns Hopkins Hospital, Baltimore, MD, 21205.

The difference in power between anisometropic spectacle lenses induces a prismatic effect that varies with gaze direction. To fuse rapidly, the anisometropic subject needs to change the relative alignment of the visual axes during a saccade. We predicted that this capability would be under adaptive control.

Spectacle-corrected anisometropia was simulated in two normal myopic subjects (-8 and -10 diopters, respectively) by having them wear a contact lens on the right eye and a spectacle lens in front of the left eye for several days. Horizontal and vertical saccades were recorded with binocular coils prior to wearing the combination and after resuming normal corrections. Viewing was both monocular and binocular. In one subject saccades were also measured on first wearing the combination, and immediately prior to resuming his usual correction. The vergence change was derived by subtracting the left and right eye positions.

Both subjects, upon resuming their usual corrections, still demonstrated an intra- and post-saccadic vergence change appropriate to the induced prismatic effect of the spectacle-contact lens combination (e.g. up to 5.3° intra-saccadic and 1° post-saccadic divergence for 20° right saccades, and up to 4.4° intra-saccadic and 0.9° post-saccadic right hyperdeviation for 20° upward saccades). These movements occurred with both binocular and monocular viewing. Whereas this adaptation occurred with both horizontal and vertical saccades, on initially exchanging the spectacle-contact lens combination and the normal correction, an appropriate vergence response to the acute prismatic effect was seen only with horizontal saccades.

These results suggest that the CNS learns to preprogram intra- and post-saccadic vergence movements, which, once learned, occur independently of disparity cues.

248.11

VISUALLY-INDUCED CROSS-AXIS PLASTICITY OF POST-SACCADIC EYE DRIFT. Z. Kapoula, D.A. Robinson and L.M. Optican*. Lab. of Sensorimotor Res., NEI, Bethesda, MD 20892.

Post-saccadic eye drift can be induced optically in monkeys and humans by post-saccadic motion of a visual pattern (Optican & Miles, J. Neurophysiol., 54: 940, 1985; Kapoula et al., Soc. Neurosci. Abstr., 13: 392, 1987). We examined the ability of humans to adapt to horizontal post-saccadic pattern motion coupled with vertical saccades.

Five subjects scanned a random-dot pattern projected onto a 2 m hemisphere. A computer detected the end of each saccade and drifted the pattern exponentially, by 25% of vertical saccade size, leftward after up saccades, right after down saccades. At first subjects followed the pattern motion after a delay of 30-93 ms. After 3 hrs of training, vertical saccades were followed by zero-latency horizontal drift, with no horizontal saccade, in the direction of pattern motion. It had an exponential waveform and mean amplitude of 2.1°. For all subjects, the horizontal drift even persisted after purely vertical saccades in the dark (0.6°).

The drift appeared due to a step in horizontal innervation, presumably from the neural integrator which integrates a neural pulse, but how could that occur with no horizontal pulse? Perhaps the coupling from the output of the integrator is changed or a part of the vertical step is coupled to the horizontal system.

248.12

LONG TERM SACCADIC ADAPTATION DOES NOT AFFECT THE SACCADES EVOKED BY ELECTRICAL STIMULATION OF THE SUPERIOR COLLICULUS.

Edmond J. FitzGibbon and Michael E. Goldberg Laboratory of Sensorimotor Research, National Eye Institute, N.I.H., Bethesda, MD 20852.

Stimulation of the superior colliculus (SC) of a monkey whose saccadic system has adapted to visual targets yields saccades identical to those evoked in the unadapted state. To see if this failure to evoke adapted saccades by electrical stimulation were a property unique to the paradigm of short term adaptation we stimulated SC before and after chronic saccadic adaptation.

Horizontal rectus muscles of one eye of two rhesus monkeys were weakened by injection of botulinum toxin, and a microelectrode implanted in the intermediate layers of the superior colliculus. The normal eye was occluded, and the monkeys viewed the world through the weakened eye. After a week the monkeys made more accurate saccades with the weakened eye and hypermetric saccades with the occluded eye. Saccadic gains were increased by over a factor of 2. Electrical stimulation of SC through the fixed microelectrode evoked saccades similar to those evoked before the start of the adaptation process.

These data suggest that both long- and short-term saccadic adaptation occur in neural centers not excited by electrical stimulation of the SC, either in a region before the SC in the processing stream or a region which acts directly on the saccadic centers in the pons and midbrain reticular formation yet is not affected by electrical activation of the SC.

FEEDING AND DRINKING IV

249.1

EFFECT OF HOUSING AND PRE-TEST HANDLING ON THE HYPERPHAGIC AND HYPOTHERMIC EFFECTS OF 8-OH-DPAT. D.V. Coscina and L. Dixon*. Sect. of Biopsychol., Clarke Inst. of Psychiatry University of Toronto, Toronto, ONT, Canada, M5T 1R8.

Considerable research has shown that drugs which stimulate post-synaptic serotonin (5-HT) receptors can reduce food intake. More recent work suggests that agents which stimulate pre-synaptic 5-HT receptors can enhance feeding by suppressing the release of endogenous 5-HT. Work from this lab with one of these agents, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), has revealed inconsistency in reliably enhancing feeding in sated rats. One difference between our work and that of others who report consistent feeding enhancement is the testing conditions used. We conduct all testing in single wire-mesh homecages while others use plastic tubs for housing, then move rats to new tubs just before and during testing. To determine if such conditions contribute to different findings, we conducted 3 separate studies with adult female rats fed Purina Chow ad lib that were housed and handled both ways, then received 60 ug 8-OH-DPAT vs. saline s.c. In 2 of these studies, 1 hr feeding was enhanced only when rats were housed in tubs. These same rats became hypothermic after 8-OH-DPAT while rats housed in wire-mesh cages became hyperthermic. These findings show that 8-OH-DPAT's ability to modify food intake and body temperature can vary with test conditions. This fact should be considered when attempting to interpret the mechanism of action for this putative 5-HT autoreceptor agonist.

249.2

EFFECT OF DIETARY PROTEIN ON THE ANORECTIC POTENCY OF FLUOXETINE (FLX). E.T.S. Li and S.Q. Luo*. Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, M5S 1A8, CANADA.

Low doses of FLX, a 5-HT uptake inhibitor, selectively suppress carbohydrate (CHO) but not protein (PRO) intake in rats given choices. To investigate further the effect of protein content of the diet on FLX, rats were fed single diets containing 10% PRO- 73.5% CHO, 25% PRO- 58.5% CHO or 40% PRO- 43.5% CHO. The rats received FLX or saline 15 min prior to food access. Compared to saline, FLX decreased 1 hr food intake (mean difference in g given in table,

Casein	FLX (mg/kg, ip)			
%	1	2	2.5	3
10	1.4	6.0*	6.2*	5.9*
25	1.1	5.0*	2.6	5.1*
40	0.2	2.7	3.4*	4.6*

paired t test, *p<0.05). ANOVA followed by Duncan's Multiple Range Test indicate an effect of FLX at 1 mg/kg being weaker than at the other doses (p<0.05). Diet composition was a significant factor (F = 3.47, p<0.037) with the 10% PRO- 73.5% CHO diet different from the 40% PRO- 43.5% CHO diet (p<0.05). We conclude that the anorectic potency of FLX is dependent on the relative proportion of PRO and CHO in the diet.

(Supported by NSERC of Canada)

249.3

THE IMPACT OF SEROTONERGIC AGONISTS ON NOCTURNAL PATTERNS OF MACRONUTRIENT SELECTION. G.F. Weiss* and S.F. Leibowitz. The Rockefeller University, New York, N.Y. 10021.

Evidence suggests that brain serotonin (5-HT) plays an important role in the inhibitory control of appetite for specific foods. We have examined the impact of centrally injected 5-HT, d-norfenfluramine (DNF), fluoxetine (FLUOX) and peripherally injected dexfenfluramine (DF), on the natural patterns of macronutrient intake in freely feeding rats.

The results of these studies demonstrate: 1) Injection of 5-HT (0.5-4 µg) directly into the paraventricular nucleus (PVN) selectively suppresses carbohydrate intake. 2) This effect is most potent in freely-feeding animals and occurs during the first hour of the nocturnal feeding cycle. This is precisely when an increase in PVN content of the 5-HT metabolite 5-HIAA is detected using microdialysis procedures (B.G. Stanley et al., 1987). 3) PVN injection of DNF (0.4-12 µg) and FLUOX (1-20 µg), as well as peripheral injection of DF (0.06-0.25 mg/kg), also selectively suppress carbohydrate intake. 4) Cannula mapping studies indicate that this serotonergic effect is anatomically specific, localized to the medial hypothalamus (PVN and ventromedial nucleus), as opposed the lateral hypothalamus or other extra-hypothalamic areas examined.

Additional results show that medial hypothalamic electrolytic lesions produce carbohydrate hyperphagia and obesity, while blocking the feeding suppressive effect of peripherally injected DF. These results strongly support the hypothesis that 5-HT neurons innervating the medial hypothalamus are physiologically active in regulating appetite for carbohydrate-rich foods, particularly at dark onset, and also in mediating the influence of peripherally injected serotonergic drugs on patterns of eating behavior.

249.4

MAIN SEROTONINERGIC EFFECTS OF FENFLURAMINE ON FOOD INTAKE MAY NOT INVOLVE CENTRAL APPETITE PATHWAYS. D. A. Booth and B. J. Baker*. Neuroscience Labs., Psychology Dep't, Univ. Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

In frequently feeding rats and people, most of the suppression of food intake caused by 5HT actions of the anti-obesity drug dl-fenfluramine (F), including reduced intake of conventional snackfoods between meals, is likely to arise from the retardation of gastric emptying and a consequent prolongation of the appetite-suppressant effect of energy being absorbed from the last meal: peripheral 5HT antagonist xylamidine (3 mg/kg) counters F's effects at 2.5 mg/kg both on gastric emptying rate and on the ratio of meal size to time to the next meal.

In the rapidly eaten first meal by food-deprived rats (the common test for "anorexia") and in a choice between diets differing in texture (as carbohydrates and proteins do), much of the observed suppression of intake or shift in choice can be attributed to F's action on brainstem 5HT synapses within the sensorimotor pattern generators for manipulation, mastication and/or deglutition, and sedation at high doses. F (2.5 mg/kg) alters relative intakes of chow crumbs differing in size (2.5 - 6.5 mm). F's effect on this innate texture preference is modulated by the texture of the nutrient preparation with which the crumbs are mixed (45% casein or calcium caseinate, dextrin or maltodextrin). Furthermore, the previously observed lack of effect of F on the learned protein- or carbohydrate-specific dietary selection cued by odor (Soc. Neurosci. Abstr., 15: 593, 1986) also extends to learned crumb-size cues. We conclude that previous observations attributed to nutrient regulation arose from sensorimotor disruption.

It remains to be shown that any forebrain 5HT action of peripherally administered F affects intake or dietary choice other than via these two sorts of effect on ingestive behavior, i.e. feeding-specific modulation of peripheral satiety signals and non-specific inhibition of central reflexes.

249.5

SERTRALINE AND OTHER SEROTONIN (5-HT) UPTAKE INHIBITORS POTENTLY REDUCE NOCTURNAL FREE-FEEDING IN RATS. K. J. Simansky, J. Lemieux* and F.C. Sisk*, Dept. Pharmacol., Med. Coll. PA, Philadelphia, PA 19129.

5-HT is thought to mediate inhibitory control of feeding. Nonetheless, acutely administered, 5-HT uptake blockers have proven equivocal in producing anorexia. We determined the effects of sertraline (SERT), a potent and highly selective inhibitor of 5-HT uptake, on the amount of dry food eaten by male rats under various conditions. When given to nondeprived rats 30 min prior to lights off, SERT (3.3, 10 and 30 umol/kg, IP) reduced feeding during the first h of the dark period by 32, 66 and 93%. SERT also produced anorexia (-15, -46, -70%) during the ensuing 3 h. Tested at night after 24h deprivation, SERT was less effective (+3, -45, -50%) during the first h and ineffective thereafter. Comparing 20 umol/kg SERT in separate groups, the degree of anorexia was: nondeprived rats in the dark > deprived rats in dark > deprived rats eating during the light period. Moreover, ad lib rats never compensated for the initial anorexia whereas deprived rats did. The 5-HT uptake inhibitors fluoxetine (FLU) and zimeldine (ZIM) (20 umol/kg) also reduced intake in ad lib rats during the dark with SERT=FLU>ZIM. A much smaller dose (5 umol/kg) of SERT, FLU and paroxetine decreased feeding from 0-1, not 1-4 h. The anorexic effect of this dose of SERT was completely antagonized by 0.25 umol/kg methiothepin but not by 0.3 umol/kg spiperone. Thus, normal feeding most sensitively detects the anorexic effect of acutely enhancing synaptic 5-HT in rats. Supported by NIMH 41987 to KJS.

249.6

MICROSTRUCTURAL ANALYSIS OF FEEDING SUPPRESSION AND OTHER BEHAVIORAL CHANGES INDUCED BY N-0437, A SELECTIVE DOPAMINE D2 AGONIST. I.N. Rusk and S.J. Cooper. Department of Psychology, University of Birmingham, B15 2TT, U.K.

N-0437 (5,6,7,8-tetrahydro-6-propyl[2(2-thienyl)ethyl] amino]-1-naphthalenol)hydrochloride) reduces palatable food consumption in nondeprived rats and mice (Rusk, I.N. and Cooper, S.J., Brain Res. Bull., in press). The present experiment undertook a microstructural analysis of N-0437's effects on feeding behavior, and on associated behavioral categories. Adult male rats were observed after administration of 0.3 - 3.0 mg/kg of N-0437, or vehicle, using a counterbalanced within-subjects design (n=8). The rats were trained to consume a palatable sweet mash in a 30 min test. Responses were recorded on-line using a microcomputer according to 7 categories (feeding, oral activity, sniffing, grooming, locomotion, rearing and immobility). Within each category, total duration, bout frequency and bout duration could be calculated. Food intake and local eating rates were also calculated. N-0437 (0.3 mg/kg) stimulated DA autoreceptors, since it induced immobility and yawning, but had no effect on food intake. However, at 1.0 and 3.0 mg/kg, N-0437 significantly reduced food intake, due to a reduction in eating rate with no change in eating duration. Since d-amphetamine and cocaine reduced food intake through a reduction in eating duration (unpub.observations), N-0437 is quite different in its feeding suppressant effect.

249.7

CENTRALLY ADMINISTERED D-1 AND D-2 SELECTIVE RECEPTOR ANTAGONISTS REDUCE SWEET TASTE REWARD IN THE RAT. LH Schneider, CA Watson*, and GP Smith. Dept. Psychiatry, NY Hosp-Cornell Med Ctr, White Plains, NY 10605.

We have previously shown that peripheral (i.p.) administration of selective antagonists of the dopamine (DA) receptor subtypes inhibits the positive reinforcing effects of sham-fed sucrose in rats, i.e. sweet taste reward. Here, we compared the effects of equimolar treatment with the D-1 antagonist SCH 23390 and the D-2 antagonist raclopride when given centrally (i.c.v.) or i.p. on 30-min sham intakes of a 10% sucrose solution. Male Sprague-Dawley rats (n=6) were chronically implanted with a lateral ventricular cannula and a gastric fistula. Rats were tested after a 4.75 h food deprivation and 15-min after an i.p. injection (2 $ml\text{-}kg^{-1}$) and i.c.v. infusion (10 $ul\text{-}kg^{-1}$). Baseline intakes were 36 $\pm$ 1 ml/30 min. We found that both receptor antagonists were significantly more potent when given i.c.v. than i.p. For D-1 receptors, doses of SCH 23390 which produced complete and partial significant suppressions when infused i.c.v. had no effect when injected i.p. For D-2 receptors, raclopride was clearly more potent i.c.v. than i.p.; its ID_{50} i.c.v. was subthreshold i.p. These data provide further evidence that central D-1 and D-2 receptor mechanisms mediate sweet taste reward. The neuroanatomical loci of the critical D-1 and D-2 sites remain to be determined.

Supported by NS24781 (LHS).

249.8

SUCROSE RESPONSIVENESS PREDICTS THE ONSET OF OBESITY: RELATION TO HYPOTHALAMIC α-NORADRENERGIC SYSTEM. J. A. Grinker, M. Jhanwar-Uniyal, W. D. Block*, M. Darwish* and S. F. Leibowitz. Univ. MI., Ann Arbor, MI 48109 and Rockefeller Univ., NY., NY. 10021.

Chronic administration of norepinephrine into the paraventricular (PVN) and ventromedial (VMN) nuclei increases daily carbohydrate ingestion and body weight gain, while decreasing energy expenditure. The present study aimed to: 1) Categorize rats on the basis of their natural sensory responsiveness to sucrose and determine its relationship to the development of dietary induced obesity; and 2) Relate this sucrose responsiveness and dietary induced obesity to α-noradrenergic receptors in discrete hypothalamic areas.

Male Sprague-Dawley rats on lab chow were tested for sensory responses during acute presentations of sucrose solutions. High and low sensory responsiveness was defined on the basis of slope of regression of solute consumed to concentration. In a 2nd experiment rats received either lab chow or high fat diet (lab chow + 30% wt/wt crisco) for 6 weeks. Six hypothalamic sites, PVN, medial preoptic area, dorsomedial nucleus, VMN, perifornical lateral hypothalamus and arcuate nucleus and median eminence, were micropunched. Standard radioligand binding technique, using the α_2-noradrenergic receptor agonist [3H] p-aminoclonidine (2 nM) and α_1-noradrenergic receptor antagonist [3H] prazosin (0.5 nM) were employed. Results demonstrate: 1) High sucrose responders became obese on high fat diet. 2) High sucrose responsiveness is associated with a small decrease in α_1- and α_2-receptors in the PVN. 3) Density of PVN α_2-receptors is higher in dietary induced obese rats. These results suggests that PVN α-noradrenergic receptors may be involved in development of obesity.

249.9

GALANIN INCREASES EXTRACELLULAR NOREPINEPHRINE IN THE PARAVENTRICULAR HYPOTHALAMUS AS MEASURED BY MICRODIALYSIS. S.E. Kyrkouli, B.G. Stanley and S.F. Leibowitz, The Rockefeller Univ., NY.

Galanin (GAL), a neuropeptide which coexists with norepinephrine (NE), specifically stimulates food intake when injected into the paraventricular hypothalamus (PVN) of satiated rats. Evidence suggests that GAL may act by modulating the release of endogenous NE in the PVN.

To test this, GAL (1.0 μg) was injected into the PVN of satiated rats. Using a small, removable microdialysis probe, consecutive samples of dialysate (40 μl/20 min) were collected for 80 min before and 120 min postinjection. Extracellular levels of NE and of the DA and 5-HT metabolites, DOPAC and 5-HIAA, were measured by HPLC-EC. Galanin reliably increased extracellular NE from 3.0 to 7.2 pg ($p<0.01$, by ANOVA). This effect occured within 20 min after the injection and lasted for about 80 min. There was no change in the other metabolites measured. Preliminary experiments with NPY revealed no increase in extracellular NE. These effects, consistent with previous findings, support the view that GAL specifically releases NE in the PVN and suggest that this peptide may stimulate feeding by acting through endogenous NE release.

249.10

DEFENSIVE BURYING OF FLAVORS PAIRED WITH LITHIUM BUT NOT AMPHETAMINE. L.A. Parker. Dept. of Psychology, Sam Houston State University, Huntsville, TX 77341.

Although rats demonstrated avoidance of both lithium- and amphetamine-paired flavored solutions, only lithium-paired flavors elicited a defensive burying response. On each of three conditioning trials, rats were presented with milk solution which was immediately followed by an injection of 127.2 mg/kg LiCl, 3 mg/kg d-amphetamine or 9 mg/kg d-amphetamine in the CS+ groups or by an injection of physiological saline in the CSc groups. One week after the final conditioning trial, the rats were tested for both conditioned avoidance of the flavored solution (CTA) and for defensive burying of the spout of the bottle containing the flavored solution. The amount consumed of milk and water and the change in the height of the bedding material at the point at which the spout entered the chamber were measured at hourly intervals over a seven hr test period. The results support the contention based upon our prior work that lithium-based, but not amphetamine-based, CTAs are mediated by a palatability shift.

249.11

HEAD FIXATION DURING ROTATION INHIBITS FORMATION OF MOTION-INDUCED CONDITIONED FOOD AVERSION IN SQUIRREL MONKEYS. C.R. Wilpizeski and L.D. Lowry*. Dept. of Otolaryngology, Jefferson Med. Col., Philadelphia, PA 19107.

Head fixation is known to inhibit motion-induced vomiting. Because conditioned food aversion (CFA) appears to be another facet of motion sickness, it is hypothesized that immobilization also prevents development of CFA. Squirrel monkeys were exposed for 2 hours per day for 5 consecutive days to each of the following conditions: (1) head fixation without horizontal rotation, (2) fixation during 30 rpm rotation, (3) rotation while nonrestrained. Banana intake was quantified immediately before each daily session. When permitted free movement during continuous rotation, subjects exhibited frequent emesis and a significant daily decrease in banana intake (CFA). On the other hand, when fixed, with or without rotation, neither emesis nor CFA occurred. If the development of CFA is presumed to signify the presence of motion-induced nausea or malaise, then head fixation prevents subjective symptoms as well as objective signs of motion sickness.

249.12

TASTE AVERSION OR HYPERRESPONSIVENESS TO PALATABILITY FOLLOWING AREA POSTREMA LESIONS? Naomi Tomoyasu and Nancy J. Kenney. Dept. of Psychology, Univ. of Washington Seattle, WA 98195.

Area-postrema-lesioned (APX) rats overingest novel, palatable foods. To assess whether the novelty or the palatability of foods underlies such overingestion, 6 APX and 7 sham-lesioned (CONT) rats were fed a palatable AIN diet for the first 8 days after ablation Six APX and 7 CONT rats were fed pelleted chow during this period. For the next 2 days, all rats were offered both AIN and milk adulterated with .05% quinine (choice days).

On the 1st choice day, pellet-fed APX and CONT rats and AIN-fed CONT rats did not differ in food choice consuming 60-86 Kcal AIN and 7-13 Kcal quinine-milk. APX rats which were fed AIN after ablation took only 15 ± 10 Kcal AIN but ingested 37 ± 14 Kcal quinine-milk. On the 2nd choice day, quinine-milk intake of the AIN-fed APX rats decreased to 29 ± 9 Kcal and those of the remaining groups increased to 18-25 Kcal. AIN intakes of AIN-fed APX rats remained low (21 ± 10 Kcal) compared with those of the other groups (61-80 Kcal).

If the novelty of foods is equal, palatability has similar effects on food choice of APX and CONT rats. Ingestion of a food following APX results in an aversion to that food such that APX rats will ingest an unpalatable novel food more readily than a palatable food available since ablation.

CARDIOVASCULAR REGULATION V

250.1

CHOLINERGIC, GLUTAMATERGIC, GABAERGIC AND NORADRENERGIC TRANSMISSION IN THE ROSTRAL VENTROLATERAL MEDULLA (RVL) OF NORMOTENSIVE WISTAR KYOTO RATS (WKY) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR). W.H. Cline*, A.M. May*, L.L. Stephenson*, C.R. Kelley* and S.P. Arneric (Sponsor: T.J-F. Lee), Dept. Pharmacol., Southern IL Univ. Sch. Med., Springfield, IL

We sought to determine whether alterations in the pattern of neurotransmission within the RVL, the presumed tonic vasomotor center, may contribute to the initiation of hypertension in SHR. Male WKY and SHR were studied at 4-6 weeks when systolic blood pressure did not differ between the two groups (WKY: 142±2; SHR: 149±3 mmHg; p>0.05, N=15). In micropunches of the RVL (1.5 mm dia. x 0.5 mm thick), at the level of the C_1 adrenaline cell group, release of endogenous glutamate (Glu), gamma-aminobutyric acid (GABA) and ^{3}H-acetylcholine (^{3}H-ACh) were measured in vitro; while turnover of norepinephrine (NE) following metryosine (250 mg/kg, i.p.) was measured in vivo. Spontaneous and K^+ (55mM)-evoked release of Glu, GABA and ^{3}H-ACh did not differ between WKY and SHR (p>0.05, N=4); nor did NE content (WKY: 11.8±2.9; SHR: 14.4±10.6 ng/mg prot.) or NE turnover (WKY: 1.2±0.9; SHR: 1.2±1.3 ng/mg prot./hr). CONCLUSION: Initiation of hypertension in SHR does not appear to be the result of derangements in neurotransmission within the RVL related to the sympathoexcitatory transmitters Glu and ACh or the sympathoinhibitory transmitters GABA and NE. (Support: American Heart Assn., IL Affiliate, to S.P.A and W.H.C).

250.2

BLUNTED PRESSOR RESPONSIVENESS TO QUINPIROLE, A DA D2 AGONIST, IN CONSCIOUS DOCA/NaCl HYPERTENSIVE RATS IS RELATED TO ANP RELEASE. Y.F. Chen, H. Jin*, R. Paul*, S. Nagahama* and S. Oparil. Hypertension Program, University of Alabama at Birmingham, AL 35294.

The selective dopamine (DA) D2 agonist quinpirole (QUI) causes an acute centrally mediated pressor response in conscious normotensive rats that is blunted in the DOCA/NaCl model. To examine the hypothesis that the blunted pressor response to QUI in DOCA/NaCl rats is due to enhanced atrial natriuretic peptide (ANP) release, plasma ANP and max pressor response (ΔMAP) to QUI were measured in 4 wk DOCA/NaCl (MAP=165±3 mmHg) and control rats (MAP=110±1 mmHg) after QUI (1 mg/kg, i.v.) administration with or without pretreatment of domperidone (DOM, 2.5 mg/kg, i.v.), a peripherally acting DA D2 antagonist. Results (mean ±SEM):

	ANP (pg/ml)			ΔMAP (mmHg)	
	Basal	QUI	QUI+DOM	QUI	QUI+DOM
Control	117±16	260±59*	103±14	22±2	32±3
DOCA/NaCl	260±32Δ	501±52*Δ	273±47Δ	10±2Δ	33±5

Δp<0.05 compared to control; *p<0.05 compared to basal, n=8-10. In addition, replacement with exogenous ANP (1 μg/kg, i.v.) attenuated the pressor response to QUI in DOM pretreated DOCA/NaCl and control rats. These data indicate that peripheral DA D2 receptors modulate ANP secretion in the rat and support the hypothesis that the blunted pressor response to QUI in DOCA/NaCl rats is related to enhanced ANP release.

250.3

BETA-2 ADRENOCEPTOR MEDIATED VASODILATION IN THE HYPERTENSIVE RAT. R.F. Kirby, C.H. Woodworth, & A.K. Johnson. Depts. of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa.

Activation of peripheral beta-adrenoceptors by circulating epinephrine produces vasodilation in the skeletal muscle vasculature of the rat. We have previously found that blockade of beta-2 adrenoceptors during an acute stressor leads to enhanced pressor responses in the borderline hypertensive rat (BHR) but is without effect in the normotensive Wistar-Kyoto (WKY). The enhanced pressor response of the BHR appears to be a trait inherited from the spontaneously hypertensive rat (SHR) which also exhibits exaggerated pressor responses to acute stress following beta-2 adrenoceptor antagonism. These results suggested that the vasculature of BHR and SHR may be more sensitive to the vasodilatory effects of circulating epinephrine. However, in the present study SKY, BHR, and SHR showed equivalent depressor responses to direct beta-2 adrenoceptor activation with graded doses of salbutamol. The results indicate that differential release of adrenomedullary epinephrine during acute stress may occur among the three strains producing greater activation of beta-2 adrenoceptors that mediate vasodilation.

Supported by research grant HL33447.

250.4

DEPRESSOR EFFECT OF ANGIOTENSIN II IN THE BRAINSTEM OF RENAL HYPERTENSIVE RATS. R, Mosqueda-Garcia, C.J. Tseng*, M. Appalsamy*, D. Robertson*. Div. Clinical Pharmacology, Vanderbilt University, Nashville, TN 37232.

Angiotensin II (AII) is a potent vasoconstrictor. Central or peripheral administration of AII increases BP, more pronounced in spontaneously hypertensive (SHR) than in normotensive rats. In this study we investigated the sensitivity of renal hypertensive rats to AII administration into the area postrema (AP) and in the nucleus of the solitary tract (NTS).

Two kidneys one clip renal hypertensive Sprague-Dawley rats (renal-HTN), sham-operated normotensive (NT) and (SHR) were anesthetized with urethane and placed in a stereotaxic frame. The dorsal surface of the medulla was exposed for intra-AP and NTS administration of AII (5, 20 and 200 ng) and adenosine (615 ng). Administration of 200 ng of AII into the AP increased BP and HR in NT rats (+13±5 mmHg; +10±4 b/m) and in SHR (+18±2 mmHg; +20±2 b/m). In contrast, in renal-HTN rats the same dose of AII decreased BP and HR (-59±5 mmHg, -73±9 b/m). The smaller doses of AII also decreased cardiovascular parameters in this group of rats, whereas pressor responses were observed in the other groups of rats. Similarly, in the NTS, AII was depressor in the renal hypertensive rats and pressor in the other two groups. In contrast, intra-AP or NTS adenosine elicited similar qualitative depressor effects in the 3 groups of rats, with the renal-HTN rats the most sensitive and the SHR the least sensitive. These results indicate that in the development of renal hypertension central structures change not only the sensitivity but also the type of final cardiovascular response to AII.

250.5

GABAergic NEURONS AND OPIATE RECEPTORS ARE INVOLVED IN INITIATION OF GENETIC HYPERTENSION. Bang H. Hwang. Terre Haute Ctr. for Medical Education, Indiana Univ. Sch. of Med., Terre Haute, IN 47809.

Spontaneously hypertensive (SHR) rats and Wistar-Kyoto (WKY) rats were used. The μ and δ oplate receptor binding, as well as GABAergic terminal density in the brainstem were evaluated. Blood pressure was determined by tail-cuff method. The μ and δ oplate receptor binding was studied by using ^{125}I-FK33824, and ^{125}I-enkephalin plus morphiceptin respectively. GABAergic terminals were revealed by immunocytochemistry using antibody to glutamic acid decarboxylase (GAD). This study showed that μ receptors were increased in the nucleus tractus solitarius (NTS), dorsal motor nucleus of the vagus, and spinal trigeminal nucleus at the pre-hypertensive stage (3 weeks of age) of SHR rats, but not at the well-established hypertensive stage (11 weeks of age). In contrast, δ opiate receptors were decreased at the pre-hypertensive stage. GAD-positive GABAergic terminals were reduced in the NTS of SHR rats at the pre-hypertensive stage, but not at the hypertensive stage. Collectively, this study provides evidence to support that GABAergic neurons and opiate receptors may play important roles in initiating hypertension seen in SHR rats, but are not closely associated with the maintenance of hypertension. (supported by NIH grant NS25087).

250.6

CONTRIBUTION OF PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVH) TO THE MAINTENANCE OF TWO-KIDNEY ONE-CLIP (2K1C) RENAL HYPERTENSION. T.X. Zhang* and J. Ciriello (SPON: J.P. Girvin). Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

We have previously shown that PVH is involved in the hypertensive process in several different experimental models of hypertension. The present study was done to investigate the contribution of PVH neurons to the maintenance of the elevated arterial pressure (AP) in the 2K1C model of renal hypertension. Rats were randomly assigned to two groups: in one group a 0.2 mm diameter clip was placed on the left renal artery and in the other group the artery was exposed but not clipped. Thirty days later the hypertensive 2K1C and the normotensive sham-clipped animals were divided into two additional groups: one group received bilateral electrolytic lesions of PVH and the other sham-PVH lesions. In the sham-clipped rats PVH lesions did not alter AP. However, AP in the 2K1C-PVH lesioned rats (120 $\pm$ 7 mmHg) was significantly reduced, compared to 2K1C-sham-PVH lesioned animals (167 $\pm$ 8 mmHg), to a level not different from sham-clipped rats that received PVH lesions (118 $\pm$ 3 mmHg) or sham-PVH lesions (124 $\pm$ 5 mmHg). AP remained approximately 52 mmHg lower in the 2K1C-PVH lesioned group then in the 2K1C sham-PVH lesioned group throughout the study. These data suggest that renal hypertension is dependent on the integrity of PVH neurons. (Supported by HSFO).

250.7

AFFERENT RENAL NERVE (ARN) STIMULATION ALTERS PLASMA LEVELS OF VASOPRESSIN AND OXYTOCIN IN THE AWAKE RAT. J.K. Simon*, J. Ciriello and N.W. Kasting (SPON: T.E. Feasby). Dept. of Physiology, Univ. of Western Ontario, London, Canada and Dept. of Physiology, Univ. of British Columbia, Vancouver, Canada.

We have recently shown that stimulation of ARN in the cat increases plasma levels of arginine vasopressin (AVP) and in the rat excites putative neurosecretory AVP cells in the hypothalamus. The present study was done to obtain direct evidence for changes in plasma levels of AVP and oxytocin (OXY) in the conscious rat during electrical stimulation (5 min, 10s pulse train, 40 Hz, 5 ms pulse duration, 500 μA) of the left ARN using bipolar cuff electrodes. Rats were instrumented with arterial and venous cannulae for the recording of pressure and removal of blood samples for assay of AVP and OXY by radioimmunoassay, respectively. Blood samples were taken 1 hr prior to and at the end of the 5 min ARN stimulation period. ARN stimulation elicited a pressor response (8$\pm$2mmHg) and significantly elevated plasma levels of AVP from 0.65$\pm$0.28 pg/ml to 2.00$\pm$0.48 pg/ml and of OXY from 6.36$\pm$1.34 pg/ml to 12.88$\pm$3.24 pg/ml. These data demonstrate that in the conscious rat afferent inputs from the kidney contribute to reflex pathways that alter the release of the neurohypophyseal peptides AVP and OXY to influence circulatory and body fluid homeostasis. (Supported by HSFO).

250.8

EFFERENT CONNECTIONS OF THE MEDIAN PREOPTIC NUCLEUS (MnPO) IN THE RAT. S. Roder* and J. Ciriello (SPON: J.D. Cook). Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

The MnPO has been implicated in cardiovascular and body fluid regulation. However, the efferent connections of the MnPO have not been adequately mapped. This study was done to investigate the distribution of fibers originating in MnPO using the anterograde transport of the tracer Phaseolus vulgaris leucoagglutinin (PHA-L). PHA-L was iontophoresed into MnPO and after a survival period of 7-12 days, transverse sections of the forebrain and brainstem were processed immunohistochemically for the demonstration of PHA-L containing fibers and terminals. Cells within the injection site containing PHA-L were observed in the dorsal portion of MnPO. In the forebrain these cells were observed to give rise to axons projecting to the medial and lateral septum, vertical limb of the n. of the diagonal band of Broca, medial preoptic n., supraoptic n., subfornical organ, paraventricular n., lateral and posterior hypothalamic n., and medial habenular n. In the brainstem, fibers and terminals were observed in the central grey, dorsal tegmental n. and in the dorsal, median and pontis raphe n. These data have provided evidence for the anatomical substrate by which MnPO can influence circulatory and body fluid regulatory mechanisms. (Supported by HSFO).

250.9

NUCLEUS MEDIANUS (NM) LESIONS ALTER THE DRINKING RESPONSE TO ANGIOTENSIN II (ANG II) INJECTED INTO THE SUBFORNICAL ORGAN (SFO). M.B. Gutman*, D.L. Jones and J. Ciriello (SPON: G.G. Ferguson). Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

It is known that ANG II acts at SFO to elicit increases in arterial pressure (AP), drinking and vasopressin secretion and that SFO projects directly to NM. To investigate the role of NM in the AP and drinking responses to ANG II injected into SFO or intravenously studies were done in awake unrestrained rats. Microinjection of ANG II into SFO elicited drinking (7.9 $\pm$ 0.8 ml in 15 min) and pressor (15 $\pm$ 1 mmHg) responses. Intravenous infusion of ANG II elicited a pressor response (36 $\pm$ 3 mmHg) but not a drinking response. Lesion of cells in the dorsal NM (dNM) with kainic acid resulted in a significant attenuation of the drinking but not the pressor response to ANG II injected into SFO (8.3 $\pm$ 0.8 ml in 15 min pre-lesion and, 1.9 $\pm$ 1.4 ml in 15 min post-lesion). Injection of the vehicle into dNM and kainate lesions of structures adjacent to dNM had no effect on the pressor or drinking responses. Similarly there was no effect of these lesions on the pressor response to ANG II injected intravenously. These data indicate that cells in dNM are part of a forebrain neural circuit that mediate the drinking but not the pressor response to ANG II acting at SFO. (Supported by HSFO).

250.10

EFFERENT PATHWAYS FOR SYMPATHETIC RESPONSES FROM INSULAR CORTEX. D.F.Cechetto, V.C.Hachinski, S.J.Chen *. Robarts Research Inst./Dept.of Physiology, U.of Western Ontario, London, Ont. N6A 5K8.

Insular cortex (IC) stimulation elicits a number of autonomic responses. The IC projects directly to subcortical sites, including the lateral hypothalamic area (LHA), the parabrachial nucleus (PB) and the nucleus of the solitary tract (NTS), which in turn project to sympathetic preganglionic areas. To determine which sites mediate the sympathetic responses originating from the IC, sympathetic nerve discharge (SND) responses resulting from IC stimulation were recorded before and after synaptic block with cobalt (COB) injections in the LHA, PB or NTS. Blood pressure, heart rate and multi-unit SND from the renal nerve were continuously monitored in 31 rats anesthetized with a-chloralose or urethane. Single pulse IC stimulation (200 uA, 1.0 ms) elicited an early increase and two later decreases in SND in chloralose-anesthesized animals, while urethane-anesthesized animals did not exhibit the initial increase. Ipsilateral COB injections (500 nl) into LHA significantly attenuated all SND responses elicited by IC stimulation. COB into the ipsilateral PB did not affect the SND responses. Ipsilateral COB injections into the NTS (500 nl) significantly enhanced the initial increase in SND. Bilateral COB injections into the LHA or NTS did not significantly attenuate or enhance the SND changes compared to ipsilateral injections only. These results suggest that the LHA is a necessary component of the descending pathway for sympathetic responses originating in the IC, while synaptic blockade in the NTS enhances sympathetic excitation elicited from IC.

(Supported by the Heart and Stroke Foundation of Ontario).

250.11

FEAR-INDUCED CHANGES IN PLASMA CATECHOLAMINES AND CORONARY HEMODYNAMIC FUNCTION. Richard L. Verrier and Fernando A. Moya-Huff. Cardiovascular Laboratories, Harvard School of Public Health, Boston, MA 02115.

We have demonstrated that provocation of an angerlike state in dogs produces significant elevations in plasma norepinephrine (NE) levels with only moderate alterations in epinephrine (E) concentration. These changes are accompanied by increases in heart rate (HR), systemic blood pressure (BP), and coronary blood flow (CBF) (Circulation 75:249, 1987). The goal of this study was to model the fear state and to characterize the attendant neurohumoral and hemodynamic responses. Five dogs were instrumented for determining ECG, BP, and left circumflex CBF. Fear was induced by having an aggressive animal challenge the test subject's access to food. This paradigm elicited intimidation behavior as evidenced by cowering, ear retraction and somatic tremor. (Means ± SEM; *p<0.05).

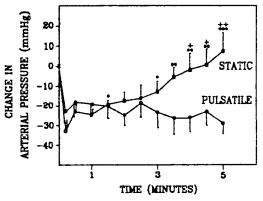
	Control	Fear	% Change
HR (bpm)	96±4	132±5*	38
BP (mmHg)	96±4	119±8*	24
CBF (ml/min)	27±6	40±11*	48
NE (pg/ml)	245±24	378±53*	54
E (pg/ml)	187±33	328±48*	75

Thus, the fearlike state elicited significant hemodynamic changes and a prevalent increase in plasma E over NE. The neurohumoral response is distinct from that produced by anger, wherein NE is dominant.

250.12

RAPID RESETTING OF THE REFLEX RESPONSES TO STATIC AND PULSATILE PRESSURES AT THE ARTERIAL BARORECEPTORS IN CONSCIOUS DOGS. D. Mendelowitz* and A.M. Scher* (SPON:F.R. Robinson, Jr.). Univ. of Washington, Seattle, WA. 98195

Rapid resetting of the baroreceptors occurs within 5 minutes. The reflex from these receptors, (the baroreflex) has been shown to reset in some studies, but not in others. In many studies in which resetting was observed the baroreceptors were exposed to static pressure. In all studies in which resetting did not occur pressure remained pulsatile. This study tests the hypothesis that static pressures elicit transient responses and pulsatile pressures evoke sustained baroreflex responses. One baroreceptor region, the right carotid sinus, was surgically prepared for reversible isolation in 6 dogs with all other arterial baroreceptors denervated. Sinus pressure was changed from control levels to either an a) elevated static or b) elevated pulsatile pressure for five minutes. The elevated static pressure caused an initial decrease in arterial pressure which decayed rapidly, -32.7±5.5 mmHg initial, +7.6±8.9 mmHg at five minutes, p<.005. In contrast with elevated pulsatile pressure the responses were sustained throughout the sinus pressure change, -23.2±5.3 mmHg initial, -29.0±4.8 mmHg at five minutes, p>.4. Thus pulsatile pressures may prevent the resetting that occurs with static pressures at the baroreceptor and/or within the central nervous system.



BLOOD/BRAIN/NERVE BARRIER I

251.1

HOW SIGNIFICANT IS THE BLOOD-BRAIN BARRIER? M. Tangoren*, R. Broadwell, E. Moriyama*, C. Oliver*, and A. Wolf* (SPON: M. Salcman). Div. Neurosurgery, Univ. MD Sch. Med., Balto., MD 21201 and NIDR, NIH, Bethesda, MD

We reported previously that labeling of CNS pericytes with blood-borne tracer protein in the mouse was attributed to the absence of a cellular barrier at the level of the meninges (J. Comp. Neurol. 251: 260, 1986). Blood-borne HRP escapes fenestrated vessels in the dura mater, presumably passes extracellularly through the arachnoid mater, reaches the pial surface for entry to the perivascular clefts, and eventually is endocytosed by pericytes throughout the CNS. Conversely, blood borne lectin-HRP fails to enter the CNS of mouse and rat by this extracellular route, due to lectin-binding occluding open junctions in the arachnoid mater, but does undergo adsorptive transcytosis through the BBB (PNAS 85: 632,1988). We now report that the meninges of the rat are not a barrier to blood-borne HRP and perhaps peptides. A series of rats was injected intravenously or into the carotid artery with 0.5-1ml of 40-250mg of native HRP/saline or with 0.67-1ml of transferrin-HRP (1-7mg/ml); post-injection times were 10mins-2hrs. At 10mins. the macromolecules were endocytosed by cerebral endothelia with no evidence for transcytosis through the BBB; however, cerebral pericytes were labeled with HRP reaction product at 1hr. Reaction product of native HRP was evident on the pial surface and within the Virchow-Robbins spaces at 10mins. Perfusion-fixation of the brain at 10mins but not after 30mins resulted in extravasations of HRP throughout the forebrain. The results in the rat suggest that, as in the mouse, blood-borne proteins, peptides (i.e., transferrin, insulin) and other molecules may circumvent the BBB by way of the meninges for entry to the CNS and that perfusion-fixation of the CNS may rupture tight junctional complexes between endothelial cells. Similar studies in the primate are in progress. Supported by NINCDS grant 18030.

251.2

HOMING TO THE CNS OF ^{14}C-LABELED MYELIN BASIC PROTEIN (MBP) REACTIVE LYMPHOCYTES MEDIATING ADOPTIVELY-TRANSFERRED EAE. A.H. Cross*, B. Cannella* and C.S. Raine, Dept. of Pathology, Albert Einstein Coll. of Med., Bronx, NY 10461.

Experimental allergic encephalomyelitis (EAE) in SJL/J mice is well-characterized pathologically in its acute and chronic forms and is known to be mediated by T cells of helper phenotype. The present study analyzed the CNS homing, distribution and ultimate fate of labeled effector cells in EAE induced by adoptive transfer. LNC blasts from syngeneic SJL/J donors immunized 10 d previously with MBP were labeled with ^{14}C-thymidine after 3 d stimulation in vitro with MBP. Recipients developed an illness indistinguishable from that induced by unlabeled cells. At day 8, ^{14}C cells were noted in perivascular (pv) cuffs in the spinal cord and cerebrum. During chronic phases, ^{14}C cells were still present in pv cuffs, representing <5% of cuff cells. During relapses, ^{14}C cells were again limited to pv infiltrations in CNS areas which correlated with new signs. In comparison to acute illness, ^{14}C cells at relapse were fewer. Despite extensive inflammatory infiltration into CNS parenchyma, ^{14}C, MBP-specific cells were confined to the immediate pv cuff.

In view of very small numbers of specific cells being apparently sufficient for extensive disease and their restriction to pv areas, we speculate that the ^{14}C, MBP-immune lymphocytes orchestrate the influx of other cells that mediate demyelination. [Supported by NMSS FG-749-A-1 and NS 08952]

251.3

ANTIBODY INFLUX FROM BLOOD TO CSF IN CSF-IMMUNIZED RATS. H.F.Cserr, M.J. DePasquale*, and P. Knopf*. Physiology and Biophysics, Brown University, Providence, RI 02912.

We have previously demonstrated that the ratio of antibody titers, CSF/serum, to human serum albumin (HSA) is elevated following HSA injection into a lateral cerebral ventricle, as compared to intramuscular injection in Sprague-Dawley rats. We have now investigated the possibility that antibody transport from blood to CSF contributes to the elevated CSF antibody titers in CSF-immunized animals. Rat anti-HSA serum was infused i.v. for 180 minutes, using a schedule which yielded a step-increase in serum anti-HSA titer. CSF was withdrawn from the cisterna magna, without a change in pressure, at a rate of 2 µl/min. Experiments were conducted in non-immunized, control rats and in rats previously immunized via CSF with up to 3 doses of 100 µg HSA. CSF albumin, CSF total protein, and changes in CSF and serum anti-HSA titers were measured. CSF albumin (25+4, n=17) and total protein (79+5, n=26) remained unchanged and in the normal range in all animals, indicating that brain barrier function was normal. In the control group, Δ CSF/Δ serum titer ratios for anti-HSA ranged from 1/7600 to <1/26,000 and, in 15 of 21 of the CSF-immunized rats, from 1/5,700 to <1/38,000. In contrast, 6 of the CSF-immunized rats showed elevated titer ratios, ranging from 1/350 to 1/3,000. Results are consistent with facilitated antibody transport from blood to CSF in 30% of the rats immunized by HSA injection into CSF. Supported by PHS NS11050.

251.4

TRACER STUDIES OF PERIVASCULAR SPACES SURROUNDING BLOOD VESSELS ON THE SURFACE OF THE RAT BRAIN. T. Ichimura* and H.F. Cserr. (SPON: K. Chapman). Physiology & Biophysics, Brown U., Providence, RI 02912.

Recent studies suggest that blood vessels on the brain surface are surrounded by a perivascular space (PVS), which is continuous with the perivascular (or Virchow Robin) space within the parenchyma (e.g. Am. J. Physiol., 246:F835 1984). We have examined relationships of the surface PVS to the parenchymal PVS and general subarachnoid space (SAS) in Sprague Dawley rats. Anatomical tracers (India ink, or 1% Evans blue and 5% albumin in saline) were injected over 15-30 min using a glass micropipet through the intact dura into a PVS, the overlying SAS, brain tissue (1 µl), or cisterna magna (30 µl). Tracer distribution was followed for up to 90 min, by direct observation and in unfixed, lyophilized or fixed sections. After 30 min, tracers injected into the PVS of surface vessels distributed preferentially within surface and parenchymal PVSs, but also leaked into SAS. After 60 min, tracer injected into any of the four injection sites distributed widely, appearing in and around PVSs of the circle of Willis (CW) and in the SAS along the olfactory tract. This indicates that PVSs around parenchymal and surface vessels, including the CW, are continuous and in open communication, but that they are not entirely separated from the general SAS. Tracer movement within the PVS is not unidirectional, possibly reflecting the mixing action of the vascular pulse. Supported by NIH grant NS-11050.

251.5

ELECTROLYTE MOVEMENT IN RAT RETINA STUDIED BY ION MICROSCROPY M.S. Burns* (SPON: R. Joy). Dept. Ophthal. Univ. California, Davis, CA 95616

The retinal pigment epithelium (RPE)/choriocapillaris (CC) complex forms the outer blood-retinal barrier (BRB) and the retinal vessel endothelial cells form the inner BRB. The relative movement of electrolytes across these barriers was studied using secondary ion mass spectrometry equipped with a digital image analysis system. Long-Evans rats were perfused for 5 minutes prior to sacrifice with normal Ringer's solution containing Na and K, or with Ringer's in which Li was substituted for Na and Rb for K. Freeze-drying techniques were used for sample preparation to preserve microlocalization of diffusible ions. Selected areas of retina were analyzed quantitatively.

There was no difference in electrolyte content between the central and peripheral retina and there was no difference in Na and K content with either normal or Li/Rb perfusion, indicating that Li and Rb were acting as tracers. No barrier to Na (Li) movement was apparent in the choroid to sclera pathway, but Li did not leave the inner retinal vessels, despite a high local intravascular Li concentration. Li moved from the CC into the retina and formed a gradient across the retina. High endogenous content of Rb was found in these retinae. In regions with selective loss of the RPE cells, Li and Rb freely entered the neural retina, but Na and K levels were unchanged. These data show the importance of the RPE in controlling retinal electrolyte content, but also indicate that inner retinal homeostatic mechanisms maintain Na and K levels in the absence of RPE.

251.6

C-FRAGMENT OF TETANUS TOXIN: A NONTOXIC CARRIER FOR DELIVERY OF PROTEINS INTO THE CENTRAL NERVOUS SYSTEM. P.S. Fishman*, J.M. Savitt*, and D.A. Farrand* (SPON: N. Bass). VA Res.Labs, Dept. of Neurology, Univ. of Maryland, Sch. of Med.,Baltimore, MD 21201.

Tetanus toxin and its atoxic carboxyl terminus fragment (C-fragment) bind avidly to receptors concentrated at synapses, and are readily internalized by neurons through endocytosis. Previous investigators have been successful in using C-fragment as a vector to enhance internalization of other proteins by neurons in tissue culture, and by neurons innervating a local injection site. We have previously demonstrated the systemically administered C-fragment is internalized by all neuronal types with processes outside the blood-brain barrier (particularly motorneurons). We now have shown that proteins coupled to C-fragment are internalized from a systemic source in a similar distribution. Horseradish peroxidase (HRP) was covalently linked to C-fragment using the periodate activiation procedure of Nakane and Kawoi. Mice were injected by intraperitoneal, intravenous,and intramuscular routes with O.2 - 2 mgm of C-fragment-HRP conjugate. Animals were sacrificed at 1-5 days after injection and nervous sytem tissue analyzed with HRP histochemistry. Intense labeling of motorneurons was seen in all animals. Peroxidase activity was also observed in sensory ganglion cells, preganglionic autonomic neurons and neurons of several hypothalamic nuclei. Minimal labeling of neurons in a similar distribution was seen after injection of free HRP in excess of 50 times the injected HRP content of C-fragment-HRP conjugates. All animals remained well until the time of sacrifice. C-fragment-HRP conjugates are polymeric with molecular weights in excess of 500 kD. Conjugates containing C-fragment; HRP; and a third protein have also been synthesized using the same procedure. We are currently examining these conjugates as a means of enhancing the delivery of proteins of choice into the CNS. (Supported by the Veterans Administration.)

251.7

BLOOD-TO-BRAIN TRANSPORT OF MPTP. N.J. Riachi*, J.C. LaManna, and S.I. Harik Dept. of Neurology, CWRU, Cleveland, Ohio 44106

Systemic MPTP causes acute Parkinsonism in man and other primates. With intracarotid administration, even very small amounts of MPTP produce neurotoxicity. To investigate the blood-brain barrier transport characteristics of MPTP, we used the indicator-fractionation method with a bolus injection of [^{3}H]MPTP and [^{14}C]butanol into the right atrium of anesthetized adult male Wistar rats. The animals were decapitated at intervals ranging from 20 sec to 30 min after the bolus injection. Regional brain samples and the anterior tongue were obtained immediately after decapitation for dual isotope counting. Like butanol, MPTP was almost completely extracted by the brain on the first pass. However, while butanol was rapidly washed out as a function of time, MPTP and/or its products were trapped in the brain. The relative ^{3}H to ^{14}C extraction ratio thus increased from ~ 1 at 20 sec to ~ 12 by 30 min. The tongue failed to concentrate MPTP or its metabolites like the brain, with the ratio of ^{3}H to ^{14}C not exceeding 2 by 30 min. Metabolism of MPTP into polar compounds, less likely to cross biological membranes, may explain its retention by neural tissue. The exact site at which MPTP is trapped within the brain remains unknown. Supported by USPHS grant HL-35617.

251.8

CEREBRAL IRON UPTAKE IN THE YOUNG RAT. A.J. Dwork*, M. Durkin*, M. Osman*, N.J. Willson* and A.I. Barkai* (SPON: R.R. Goodman). NYS Psychiatric Inst., NY, NY 10032.

In the rat, iron acquired by the brain early in postnatal life undergoes little subsequent exchange with peripheral stores, and a deficiency of dietary iron at this time leads to an irreparable deficit in cerebral iron. The current study examines the extent of cerebral uptake and the neuroanatomic distribution of iron absorbed at various ages. Two-, 4-, or 6-week-old rats were injected i.p. with a trace dose of $^{59}FeCl_3$ and were sacrificed after various intervals (up to age 8 weeks) by cardiac perfusion. At the time of sacrifice, radioactivity was determined in blood, serum, and the left half of the brain; the right half was sectioned for autoradiography. When the injection was made at 2 weeks of age, brain radioactivity remained constant from the second postinjection day. However, the distribution of radioactivity became gradually more discrete to resemble that of ferric iron in the brain of the adult rat. The ratio of brain/blood radioactivity rose gradually, due to a loss of blood radioactivity, and showed the same 1.6-fold increase between 2 and 4 weeks postinjection for rats injected at age 2 or 4 weeks. The brain/blood ratio 2 weeks postinjection for rats injected at 4 or 6 weeks of age was 25% that for rats injected at age 2 weeks, indicating an active but diminished uptake by the brain at the later ages. Serum radioactivity was 4% that of blood 1 day postinjection and low enough thereafter to be explained by microscopic contamination with hemoglobin, suggesting that intracerebral rearrangement, rather than exchange between brain and blood, accounts for the changes in distribution.

251.9

ENDONEURIAL CAPILLARY PA TO ALBUMIN IN SCIATIC NERVES OF RATS MAINTAINED ON A LEAD DIET. A. Weerasuriya*, G. L. Curran*, and J. F. Poduslo. (SPON: J. W. McLaren) Membrane Biochem. Lab., Depts. Neuro. & Biochem./Molec. Biol., Mayo Fdn., Rochester, MN 55905

The permeability coefficient-surface area product (PA) of endoneurial capillaries in rat sciatic nerve to radiolabeled rat serum albumin (RSA) was examined in rats maintained on 4% lead carbonate and their corresponding pair-fed controls. Circulation time for the i.v. injected ^{125}I-RSA bolus was 60 min and 2 min for ^{131}I-RSA (residual endoneurial vascular volume indicator). After 5 weeks of the lead diet, there was no significant difference between the PA of lead fed rats and that of their pair-fed controls, even though their blood lead levels were in excess of 1.8 µg/ml. At 6 weeks, the PA in lead fed rats was 50% more than that of the pair-fed controls and by 8 and 10 weeks the PA of lead fed rats was more than double of that in the pair-fed controls. The above results are in accordance with previously observed increases of blood nerve barrier permeability in the initial stages of lead neuropathy. Furthermore, these results show that the i.v. bolus injection technique can be used to assess small changes in endoneurial capillary PA to large protein molecules in other models of experimental neuropathy. (Supported by NS14304-P4)

251.10

GALACTOSEMIA AND ALDOSE REDUCTASE INHIBITOR: EFFECT ON BLOOD-NERVE BARRIER PERMEABILITY AND WATER CONTENT OF THE RAT PERIPHERAL NERVE. K.C.Wadhwani, L.E. Caspers-Velu*, V.A. Murphy, Q.R. Smith, P. Kador*, and S.I. Rapoport. Lab of Neurosciences, NIA, NIH, Bethesda, MD 20892.

Galactosemia causes peripheral neuropathy such as nerve edema. It is not known, however, whether the permeability of the blood-nerve barrier (BNB) is altered in galactosemic animals. We, therefore, determined and compared the permeability-surface area product (PA) for sucrose at the BNB, using an i.v. bolus injection technique (Ohno et al, Am J Physiol, 235:H299, 1978), and the water content of the sciatic nerve in rats fed a normal diet, a 50% galactose diet or a diet containing both galactose and aldose reductase inhibitors (Statil, 0.04% or Alcon 1576, 0.0008%), for a period of 9-11 months. The PA at the blood-brain barrier (BBB) was also determined for comparison. Mean (±SEM) PA's ($\times 10^{-5}$ ml/s.g wet wt, n=7) at the BNB and BBB in control rats were 1.8±0.2 and 1.1±0.1, respectively. Values in a galactose-fed or galactose+aldose reductase inhibitor-fed animals did not differ from control animals ($p > 0.05$). Water content (% wet wt, n=6-8) in the nerve, however, increased significantly in galactosemic rats compared to control rats (76±1 vs 66±1) ($p < 0.01$). Aldose reductase inhibitors reduced water content to control values (Statil: 69±1; Alcon 1576:67±1). Our results show that galactosemia does not alter BNB permeability but leads to nerve edema, which can be prevented by aldose reductase inhibitors.

251.11

NOVEL INTERCELLULAR JUNCTIONS IN INVERTEBRATE CNS. N.J. Lane (SPON: P.D. Evans). AFRC Research Unit, Dept of Zoology, Downing Street, Cambridge, CB2 3EJ, UK.

The peripheral glial cells that surround the CNS in certain groups of primitive arthropods are characterised by unusual junctions. In centipedes and millipedes, (Myriapoda), these glial cells are associated by filamentous "linkers" which produce a reduction, but not an occlusion, of the intercellular cleft. Exogenous tracers, such as HRP or La^{+++}, are unable to penetrate beyond these glial clefts, however. This suggests the existence of an extracellular matrix "barrier" in conjunction with the linkers. These linker junctions are interposed between conventional gap junctions. In freeze-fracture replicas, loosely aggregated gap junctional connexon plaques fracture onto the E face, together with linear alignments of intramembranous particles and furrows; P face ridges complementary to the EF furrows also occur, but it is not clear how the linkers connect into the intramembranous structures, if at all. These structural modifications, or linker junctions, are quite distinct from the conventional tight or septate junctions found between the outer glial cells in more highly evolved arthropods such as the insects and arachnids, and appear to represent a new category of intercellular junction.

SYNAPTIC STRUCTURE AND FUNCTION II

252.1

A LINK BETWEEN ACIDIFICATION AND MEMBRANE CYCLING IN THE TERMINALS OF FROG RETINAL PHOTORECEPTORS. D. Sulzer* and E.Holtzman. Dept. of Biol.Sci., Columbia U., NY 10027

The presynaptic terminals of photoreceptors in Rana pipiens retinas maintained in oxygenated Ringers accumulate the weak-base vital dyes, Neutral Red and Acridine Orange. Dye accumulation is prevented by inhibitors of glycolysis (IAA) and of respiratory metabolism (KCN, DNP). By analogy to observations on non-neuronal cells, we infer that the terminals, or compartments within them, maintain a pH that is low relative to the extracellular milieu. Other "weak bases", including ammonium chloride, benzylamine and tributylamine, can block the uptake of the dyes; these bases are expected to raise the pH within acidified structures. Photoreceptor terminals in retinas exposed to ammonium chloride exhibit alterations in the exocytic-endocytic cycling of synaptic vesicles: Membrane retrieved by endocytosis accumulates in large vacuoles which subsequently can regenerate synaptic vesicles when the ammonium chloride is removed. Vacuolation is made more pronounced by depolarizing the cells with a high potassium medium. The vacuoles appear to be recycling compartments which become enlarged as an osmotic consequence of the low pH-dependent accumulation of ammonium ions within them. Thus, these observations suggest that membrane cycling in the photoreceptor terminals can proceed by way of intermediate compartments with low pH, perhaps comparable to "endosomes". Supported by NEI grant EY03168.

252.2

BRAIN SYNAPTIC VESICLES CONTAIN A SPECIFIC LIPID KINASE ACTIVITY. S.M. Bajjaleh*, T.F.J. Martin* and E. Floor. (SPON: A. Clark). Neurosciences Training Progam and the Departments of Zoology and Anatomy, University of Wisconsin, Madison, WI 53706.

Using highly purified brain synaptic vesicles (Floor et al, J. Neurochem. 50: 1588, 1988), we have identified a lipid kinase activity that is apparently specific to synaptic vesicles.

Membrane fractions at each stage of purification were incubated with [32]P-ATP, extracted under neutral conditions and analyzed by thin layer chromatography (TLC). Two lipid kinase activities were seen. One is a diacylglycerol kinase activity which is found in all membrane fractions. The other activity, specific to synaptic vesicles, produces a phosphorylated lipid that migrates with brain ceramide phosphates in three different TLC systems. Calcium, magnesium and the cationic antibiotic neomycin stimulate this activity.

A mixed micelle assay was used to determine the identity and distribution of the substrate for the synaptic vesicle- specific lipid kinase. Results from these experiments indicate that: 1) brain ceramides are phosphorylated by synaptic, but not by non-synaptic vesicle microsomes, supporting the notion that the synaptic vesicle-specific kinase activity is a ceramide kinase; 2) lipids extracted from non-synaptic vesicle fractions contain the kinase substrate, suggesting that it is the kinase activity, and not the substrate, that is specific to synaptic vesicles.

We are currently investigating the identity and regulation of this kinase, its substrate(s) and substrate producing enzymes.

Supported by NIH grants DK 25861 and NS 24890.

252.3

APLYSIA SYNAPTIC VESICLES CONTAIN SYNAPTOPHYSIN AND ARE ENRICHED IN G PROTEIN. G.J. Chin, S.S. Vogel, A.M. Elste*, and J.H. Schwartz. Howard Hughes Medical Inst., Columbia University, New York, NY 10032.

Synaptophysin, an integral membrane glycoprotein (M_r 38,000), is a major component of mammalian synaptic vesicles. Western blot analysis using an anti-synaptophysin monoclonal antibody (SY38) revealed that a single immunoreactive polypeptide (M_r 29,000) is present in *Aplysia* nervous tissue and greatly enriched in synaptosomes. Immunoreactivity was localized mainly to varicosities and neurites in cryostat sections of paraformaldehyde-fixed ganglia. Fractions containing synaptic vesicles and synaptic plasma membrane were prepared by hypotonic lysis and differential centrifugation. Electron microscopy revealed that the 200,000 x g pellet (SV fraction) consists of lucent and dense-cored vesicles with 50-150 nm diameter; numerous elongated, empty sacs were found in the 16,000 x g pellet (SPM fraction). The enrichments of synaptophysin relative to the synaptosome fraction were 10-fold in the SV fraction and 0.43-fold in SPM. For 5'-nucleotidase, a plasma membrane marker enzyme, the enrichments were 0.74-fold in SV and 2.0-fold in SPM. We conclude that synaptophysin is specifically associated with *Aplysia* synaptic vesicles. Surprisingly, as determined using an affinity-purified polyclonal antiserum (A569AP, kindly donated by S.M. Mumby, U. of Texas) directed against the α subunit of bovine brain G proteins, *Aplysia* Goα (M_r 41,000) was enriched in both the SV and SPM fractions as compared to the synaptosome fraction. Association of Go with intracellular vesicles is supported by the observations that the *Aplysia* pertussis-toxin substrate (M_r 41,000) is also enriched in the SV fraction and that squid axoplasm contains a membrane-bound pertussis-toxin substrate (M_r 41,000; Vogel *et al.*, These Abstracts). We speculate that G protein, like synaptophysin, is specifically associated with synaptic vesicles and may regulate fusion of synaptic vesicles with plasma membrane.

252.4

Immunolocalization of Different Molecular Forms of Acetylcholinesterase by Light and Electron Microscopy. S.N. Abramson, T. Deerinck*, P. Taylor*, and M. Ellisman. Depts. of Pharmacology and Neurosciences, UCSD, La Jolla, CA 92093.

Acetylcholinesterase (AChE) occurs in several structural forms, including a hydrophobic dimer (5.6 S) and an asymmetric oligomer (17 S). These species are thought to be differentially distributed, however, antibodies selective to each species have been difficult to obtain. Knowledge of the amino acid sequences of these forms in *Torpedo californica* has allowed us to generate polyclonal antibodies that are selective for a unique sequence in the catalytic subunits of the 17 S form, and high resolution immunolabeling of cryosections of electric organ has allowed us to determine the differential distribution of AChE forms with respect to synaptic specializations. Several antibody probes were used, including: 1) rabbit polyclonal antibodies that selectively recognize the catalytic subunits of the 17 S form; 2) a mouse monoclonal antibody that selectively recognizes a structural component of the 17 S form; 3) rabbit polyclonal antibodies that recognize the catalytic subunits of both forms; and 4) a mouse monoclonal antibody that recognizes the catalytic subunits of both forms. The sensitivity and specificity of these and other antibodies were determined by western blot, ELISA, and immunoprecipitation assays. Initial immunofluorescent localization revealed that the 17 S form was concentrated near the synaptic cleft, while the 5.6 S form was more evenly distributed over the innervated face of the electrocyte. This was confirmed by a combination of cryoultramicrotomy and immunogold labeling, with conventional and high-voltage electron microscopy. The 5.6 S form was associated with the nerve terminal and the innervated membrane of the electrocyte, while the 17 S form was found mainly in patches at the synaptic appositions.

252.5

RELATIONSHIP BETWEEN SYNAPTIC NOISE AND TEMPERATURE IN FORMAL NEURONS. H. Korn and Y. Burnod*. Lab. Neurobiologie Cellulaire, INSERM, Institut Pasteur, Paris.

The possible influence of synaptic noise on the output of a real neuron, i.e. the Mauthner cell, was investigated and compared with the effect postulated for temperature which is the maximum slope of the sigmoidal input-output function in formal neurons. Probability distributions of quanta released by a population of 42 inhibitory cells, with known binomial release parameters, were reconstructed under different firing rates of the network. The uncertainty of output resulting from this noise was calculated for given excitatory input signals on the basis of the cumulative functions of inhibitory events overcoming these signals. The performance of a system subjected to such fluctuations follows a sigmoidal shape, with a "temperature" which is proportional to the mean number of inhibitory quanta constituting noise. When excitatory and inhibitory quanta occur simultaneously, temperature decreases with the presynaptic firing rate (as does the probability of release). However, during more common situations of asynchrony, the overlap of postsynaptic responses produces a net increase of temperature as the number of active cells and their frequency of discharge increase. Thus, temperature is controlled by presynaptic activity, a notion which could be included in formal models.

252.6

COMPARISIONS BETWEEN COMPUTATIONAL OPERATIONS GENERATED BY ACTIVE RESPONSES IN DENDRITIC BRANCHES AND SPINES, USING SABER. G.M. Shepherd, N.T. Carnevale+, and T.B. Woolf. Sect. Neuroanat., Yale Univ. Sch. of Med., New Haven, CT 06510 and +Depts of Neurol. and Neurobio. and Behvr., SUNY, Stony Brook, NY 11794.

Previous studies of the computational abilities of a dendritic branch demonstrated that active properties in spine heads can lead to the generation of logic operations. We have extended those results by comparing the relative effectiveness of different distributions of active membrane within the branch.

We have used a new analog circuit simulator (Flach et al. Soc. Neurosci. Abs. (1987) p.159) called SABER (Analogy, Inc). Its advantages include: entering nonlinear equations directly; creating hierarchial components so that users can directly input anatomical and physiological details; exploring compartmental values or conductance changes using batch files; and a plotting package supporting output comparisons from different runs.

A prototypical stretch of a spiny dendritic tree was modeled. The four spine heads were 1um by 1um with 0.1um by 1um spine necks. The trunk was 700 um in total length with the spines distributed at 250um (2), 300um (1) and 350um (1) from the most apical end. In previous work an AND gate was simulated by 1 nS excitatory synaptic conductance changes in the two most apical spine heads. When active membrane of the same amount and density was placed at the base of these voltage-gated spines, 2 nS was necessary for the AND operation. As the same number of channels was distributed over more dendritic membrane (lowering the density) the efficacy fell further, at first gradually, and then rapidly, to a point where logic operations were no longer possible. The results further indicated that action potentials generated at either branch or spine locations could appear similar when recorded nearer the soma.

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252.7

MODELS OF LOCAL ELECTRICAL INTERACTIONS WITHIN SPINY DENDRITES OF GRANULE CELLS IN MOUSE OLFACTORY BULB. T.B. Woolf, G.M. Shepherd, C.A. Greer. Sections of Neuroanatomy and Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT 06510

Olfactory bulb granule cells participate in reciprocal dendrodendritic synapses with mitral and tufted cells. The granule cell components of these synapses are restricted to heads of dendritic spines. We wished to test the postulate that local spread of current within granule cell dendritic trees is capable of activating synaptic output from these spines.

We have made detailed reconstructions of spiny dendrites using camera lucida and through focus light photomicrographs (mag=5000X) of Golgi-Kopsch impregnated mouse granule cells. Measurements consisted of the length and diameter of all dendritic processes, spine necks, and spine heads. Measurements were made to within 0.2um, although below about 1 um light diffraction limited this accuracy. We then used an analog circuit simulator (SABER; Analogy, Inc.) to model the effects of transient conductance increases in spine heads and the subsequent passive spread of depolarization in the remainder of the dendritic tree.

The influence of a conductance change of 1 nS in a particular spine head tended to be restricted to a limited subset of the total available spines. The size and organization of these subsets were determined not only by the input conductance but also by the complex interaction of position, closeness of other spines, and spine dimensions. Consequently, markedly different morphologies can correspond to a similar functional subset. Preliminary data were also obtained on the effects of non-linear and temporal interactions between spines. Collectively, the results support the notion that, in the absence of active properties, subsets of granule cell spines may act as spatially restricted processing units.

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TRAUMA I

253.1

THE COINCIDENCE OF HEAD AND CERVICAL SPINE INJURY. D.B.Michael, D.R.Guyot*, and W.R.Darmody*. Depts. of Neurosurgery and Radiology, Wayne St. Univ. Sch. of Medicine, Detroit, MI 48201.

The association of head and cervical spine injury has long been recognized. Reports of the coincidence of these injuries in the literature range from 1.2-19%. This study was undertaken to determine the coincidence and examine the mechanisms of head and cervical spine injuries at a major trauma hospital.

All cases of cervical spine injury (CSI) and head injury (HI) admitted to The Detroit Receiving Hospital during 1987 were identified using the hospital computer data bank and spine unit log book. There were 359 admissions for HI including concussion, skull fracture, and intracranial hemorrhage. There were 92 admissions for CSI including complete or incomplete neurologic syndromes with or without fractures.There were 22 patients with both HI & CSI (both). Thus the coincidence of primary CSI with HI (both/CSI) is 24% while the coincidence of primary HI with CSI (both/HI) is 6%.Mechanism of injury will be demonstrated with appropriate imaging studies.

Our study supports the view that all seriously head injured patients be treated as though a concomitant cervical spine injury is present until proven otherwise. Further, it shows nearly 1/4 of patients with cervical spine injury have also suffered a head injury. This coincidence may be an important consideration in the rehabilitation of such injured patients.

253.2

GFAP-LIKE IMMUNOREACTIVITY IN DAMAGED NEURONS AFTER SPINAL CORD INJURY. D.J. Gower, C.R. Hollman* and M. Tytell. Neurosurg. Section, Univ. Oklahoma, Oklahoma City, OK and Anatomy Dept, Wake Forest Univ. Medical Center, Winston-Salem, NC.

Glial fibrillary acidic protein (GFAP), a cytoskeletal component of the astrocyte, increases dramatically in amount concommitant with astrocyte hypertrophy after CNS injury. This study examined changes in localization of GFAP immunoreactivity after spinal cord injury.

Pentobarbital-anesthetized adult male rats underwent a single-level thoracic laminectomy. A 50 gm/cm concussive spinal cord injury was induced and the cords collected 0, 24, 48, 72, 96, 120, and 144 hrs later. The tissue was fixed by microwave irradiation in Carnoy's solution, paraffin embedded and sectioned at 7 µm. The GFAP was detected using a mouse anti-GFAP monoclonal antibody (1:500 dilution, Chemicon), followed by a rat-absorbed anti-mouse antibody (1:50) and the Vector Co. Elite ABC kit for HRP.

Within 24 hrs of injury, glia in the penumbra around the injury demonstrated typical thickening and lengthening of processes which precedes formation of a glial scar. Concurrently, a small percentage of the retraction balls of damaged axons in the white matter were highly positive for GFAP immunoreactivity. At later times more of these became immunoreactive, as well as portions of axons at a distance from the injury and occasional neurons.

These observations may reflect either the endocytosis by damaged axons of GFAP released from damaged astrocytes or an injury-induced alteration of a neuronal cytoskeletal protein producing an epitope similar to GFAP.

253.3

DIMETHYL SULFOXIDE AND MECLOFENOXANE MAY ATTENUATE NEUROLOGICAL DYSFUNCTION AFTER CLOSED IMPACT INJURY TO THE SPINAL CORD. L.Bunegin,* M.S.Albin and J.Martinez*. Dept. of Anesthesiology, Univ. Tx. Hlth. Sci. Cntr., San Antonio, TX 78284 and Dept. Pathology (Neuropath). Univ. Pgh. Sch. Med., Pittsburgh, PA.

In this study we evaluate the effects of dimethyl sulfoxide (DMSO), a free radical scavenger, and meclofenoxane (MEFOX) an antioxidant-enzyme sitmulator, on acute closed impact injury to the spinal cord. Fifteen (15) barrier raised anesthetized cats were assigned to either the control group (Group 1, placebo five animals), DMSO (Group 2, five animals, 2.5 grams daily), and MEFOX (Group 3, five animals, 50 mg daily). Spinal cord injury was produced by firing a missile impacting on an impounder resting within the confines of an electronic collar seated on the T_9-T_{10} interspace using a force at or greater than 0.8 x 10^7 dynes/kg. Previous data from our laboratory has indicated that 0.8 x 10^7 dynes/kg is the threshold force to produce irreversible paraplegia. Functional evaluation was carried out daily during the subsequent 4 weeks using a Recovery Index Score (RIS) in which the ability to run, move and climb were tested. A score of 16 indicates normal function and a score of 3 indicated paraplegia. The neurobehavioral changes found in each group were analyzed for statistical significance using paired t test with alpha splitting. All control animals were at 7.0 or lower in the RIS (mean 4.6±0.9 SEM). All animals in the DMSO group showed a good level of functional recovery (mean 12.4±1.2 SEM), while the MEFOX group demonstrated paraplegia in 2 and excellent scores in 3 for a mean of 10.6±2.8 SEM. There was no significant statistical differences between the impact forces used in all 3 groups. On the other hand, there was statistical significance in the RIS Scores ($p<0.05$) between DMSO against control and MEFOX against control. We noted no statistical differences between DMSO and MEFOX in the Recovery Index. It appears that significant functional recovery occurred after acute closed impact injury of the spinal cord using DMSO and MEFOX. Supported by a grant from the Moody Foundation, Galveston, Texas.

253.4

NMDA ANTAGONISTS ATTENUATE TRAUMATIC NEURONAL INJURY IN AN IN VITRO MODEL. E.S. Tecoma, M.P. Goldberg, H. Monyer, D.W. Choi. Stanford Univ. Med. Sch., Stanford, CA, 94305.

Recent evidence implicates excitatory amino acid neurotoxicity in the pathogenesis of traumatic CNS injury (Faden AI and Simon RP, Soc. Neurosci. Abstr. 13: 1031). We tested the hypothesis that NMDA antagonists might reduce neuronal loss in a novel cell culture model of traumatic injury.

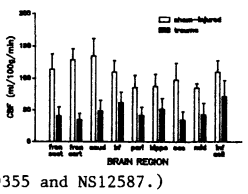
Dissociated cortical cell cultures were prepared from E14 - E17 fetal mice, and maintained in 15 mm wells for 2 - 3 wks in vitro; neurons formed a network on top of a confluent glia monolayer. After transfer into a defined bathing medium, the cell layer was mechanically disrupted by applying a linear scratch with a sterile pipette tip, producing a 2 mm wide tear down to bare plastic. Within 1 - 2 mm of the margin of this tear, there was widespread acute swelling of neuronal cell bodies, followed by neuronal degeneration over the following day. In contrast, in cultures to which the NMDA antagonist dextrorphan (DX) (100 μM) was added prior to this traumatic insult, there was a marked reduction of neuronal swelling and subsequent death in this marginal area (mean severely injured area, defined by dense trypan blue staining of neuronal debris, in 5 consecutive microscopic fields: control 4.21 +/- 0.46 mm^2 (SEM, n = 4), DX treated 0.44 +/- 0.19 mm^2 (n = 4) , $p < 0.001$).

253.5

AUTORADIOGRAPHIC AND MICROSPHERE STUDIES OF CEREBRAL BLOOD FLOW AFTER TRAUMATIC BRAIN INJURY. D.S. DeWitt,* X-Q Yuan,* R.L. Hayes, B.G. Lyeth, L.W. Jenkins, D.S. Prough* (SPON: J. Butterworth), Dept. of Anesthesia, Bowman Gray School of Medicine, Winston-Salem, NC 27103; Division of Neurosurgery, Medical College of VA., Richmond, VA 23298.

Cerebral blood flow (CBF) was studied in rats after fluid percussion injury using the autoradiographic iodo-antipyrine (IAP) or the radioactive microsphere techniques. Rats were prepared for trauma under barbiturate anesthesia (Dixon, et al, J Neurosurg 67:110-119, 1987). Twenty-four hours later, rats were anesthetized with halothane (1%) and surgically prepared for CBF measurements. Anesthesia was decreased to 0.5% in 70% N_2O in both groups and, in one group, CBF was measured 25 minutes after injury (2.3 atm., n=6) or sham-injury (n=6) using IAP. In the other group, microspheres were injected prior to and at two of the following four times (T): 5, 15, 30, 60 minutes after injury (2.5 atm., n=17) or sham-injury (n=20).

After trauma, CBF decreased in all regions studied using IAP, including frontal, parietal and occipital cortices, caudate, basal forebrain, hippocampus, midbrain, and inf. colliculus (figure). In the sham-injured microsphere rats, CBF increased at all time points, probably due to halothane anesthesia. After trauma, MS hemispheric CBF was 14%, 26%, 37% and 45% of pre-trauma values at T5, T15, T30, and T60, respectively. These studies demonstrated that traumatic brain injury resulted in significant decreases in CBF in rats.



(Supported by NIH grants NS19355 and NS12587.)

253.6

PERCUSSIVE INJURY TO THE RAT SPINAL CORD: A MODEL OF AXONAL DAMAGE. D.D. Rigamonti, H. Mena*, J.B. Long, A. Martinez-Arizala, R. A. King*, J. W. Holaday. Walter Reed Army Institute of Research and Armed Forces Institute of Pathology, Washington, D.C. 20307-5100

In an attempt to reproduce the conditions associated with human spinal cord trauma, we developed a novel technique to induce a closed spinal cord injury. Anesthesized, unoperated male Sprague-Dawley rats were secured and suspended from a stereotaxic head holder of a percussion instrument. The impacting device was aligned with the T1 vertebral spinous process and a pneumatically driven metal plunger contacted the animal without fracturing the vertebrae. Animals exhibited hindlimb paralysis after injury and neurologic, hemodynamic and morphologic changes at varied post-injury intervals were evaluated. Anesthetized rats were perfused through the heart with formalin, the spinal cords removed and sections stained with H&E, Nissl, Kluver-Barrera, and Bielschowsky techniques. The lesion, confined to the site of impact, involved the white matter in a diffused manner primarily in the ventral and ventrolateral funiculi with axonal swelling and loss, vacuolation and minimal cellular reaction. There was minimal central gray matter necrosis and cavitation although some neurons exhibited central chromatolysis. Small hemorrhages with hemosiderin pigment deposition and mild gliosis were found in the dorsal horns.

253.7

REGIONAL CEREBRAL BLOOD FLOW (rCBF) RESPONSIVENESS TO HYPOXIA AND HYPERCAPNIA BEFORE AND AFTER BRAIN MISSILE WOUNDING (BMW) IN ANESTHETIZED, PARALYZED CATS. D. Torbati, M.E. Carey*, J.F. Davidson* and J.B. Farrell*. Dept. of Neurosurg. Louisiana State University Medical Center, 1542 Tulane Ave. New Orleans, LA 70112.

The reactivity of rCBF to hypoxia and hypercapnia was measured in 16 brain structures by radioactive microspheres. The rCBF was measured during normoxia and hypoxia (n=7; P_aO_2=55 torr) and during normocapnia and hypercapnia (n=7; $P_a^2CO_2$=60 torr) both before and after BMW. Right cerebral hemisphere was wounded by a 2mm, 31 mg steel sphere penetrating an intact cranium (280 m/s; 1.4 J.). Before BMW, total CBF increased by 75% during hypoxia and by 99% during hypercapnia. After BMW, while total CBF was reduced by 30%, hypoxia and hypercapnia produced no increase in blood flow. Moreover, rCBF reactivity to hypoxia and hypercapnia was reversed in 5/16 structures. During hypoxia the wounded gray and white matter showed the highest decrease in blood flow (-19 and -27% respectively). This inverse reactivity was greater during post-BMW hypercapnia, reaching -37 and -40% in wounded gray and white matter. These data strongly suggest that CBF autoregulatory mechanisms responding to hypoxia and hypercapnia were impaired after BMW. Thus, BMW-induced hypoventilation and/or transient apnea leading to hypoxemia and hypercapnia are detrimental to post-BMW recovery. Supported by contract No. DAMD17-86-C-6098, LAIR, USMRDC.

253.8

THE EFFECT OF SERUM HYPERGLYCEMIA ON THE PHENOMENON OF INCREASED POST-TRAUMATIC ISCHEMIC VULNERABILITY. L.W. Jenkins, K. Moszynski*, B.G. Lyeth, L.P. Miller, W. Lewelt*, D.S. DeWitt*, A. Allen*, T.J. Majewski*, G.L. Clifton*, H.F. Young*, R.L. Hayes, Richard Roland Reynolds Neurosurgical Research Laboratories, Med. Col. of Virginia, Richmond, VA 23298.

Previous studies in fasted rats subjected to a sublethal mechanical injury followed 1 or 24 hours later by a normally sublethal ischemic insult have documented an increased post-traumatic ischemic vulnerability. This is evidenced by bilateral hippocampal CA1 pyramidal death which can be attenuated by combined muscarinic and NMDA receptor antagonists. The present study examines the effect of serum hyperglycemia on this phenomenon. Overt neuronal death was evaluated after identical combined injury and various post-injury survival in rats with pre-injury serum glucose levels of above 400 mg%. Hyperglycemic rats developed status epilepticus and died within 24 hours post-injury. Compared to fasted rats given the same combined insult the pattern of neuronal death was expanded in hyperglycemic rats to additional brain regions demonstrating high glutamate and cholinergic receptor binding. Based upon such spatial relationships, receptor mediated pathology may also be involved in the hyperglycemic exacerbation of neuronal injury following trauma and secondary ischemia. Supported by NIH Grant NS 19950.

253.9

THE EFFECT OF AN APPLIED DIRECT CURRENT FIELD ON FUNCTIONAL AND ANATOMICAL RECOVERY AFTER ACUTE EXPERIMENTAL SPINAL CORD INJURY M.G. Fehlings C.H. Tator and R.D. Linden Div. of Neurosurgery and Playfair Neuroscience Unit, University of Toronto

Recent evidence indicates that direct current (DC) stimulation promotes recovery after acute spinal cord injury in mammals. In this study, the effect of DC field polarity was studied in 30 adult rats. After 53 g clip compression injury of the cord at T1, the rats were randomly and blindly allocated to one of three groups (n=10): one group underwent DC stimulation (14 uA) with the cathode distal to the injury site (normal polarity); a second underwent DC stimulation with the cathode proximal to the lesion (reversed polarity); a third group received sham (0 uA) stimulators. Clinical neurological function was assessed weekly by the inclined plane technique. At 8 weeks after injury, motor and somatosensory evoked potentials (MEP and SSEP) were recorded and horseradish peroxidase (HRP) was introduced into the cord at T6. The total number of HRP-labelled cells was counted in the brain stem and motor cortex and a computer-assisted morphometric analysis of axons at the injury site was performed.

The inclined plane scores ($p<0.0001$), MEP amplitude ($p<0.02$), counts of HRP-labelled neurons ($p<0.0001$), and axon counts at the injury site ($p<0.01$) were significantly greater in the group treated with a normal polarity DC field than in the sham or reversed polarity groups. These data confirm that DC fields produce functional neurological and anatomical recovery in rats with acute cord injuries and show, for the first time in mammals, that the mechanism of action of DC stimulation is critically dependent on field polarity.

253.10

TRAUMATICALLY INDUCED DEAFFERENTATION AND REORGANIZATION IN THE LATERAL VESTIBULAR NUCLEUS OF CAT. D.E. Erb* and J.T. Povlishock. Department of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA. 23298.

Previous investigations have demonstrated that diffuse axonal injury (DAI) is a consistent finding in traumatically brain-injured man and animals. As such DAI also triggers diffuse deafferentation with sparing of related fiber systems, this situation provides a unique opportunity to assess the potential for posttraumatic neuronal reorganization and regeneration. To explore this issue we examined synaptic terminal loss and recovery in the lateral vestibular nucleus, known to undergo traumatic deafferentation. This nucleus offered a further advantage in that it possesses a relatively homogeneous neurotransmitter population. Specifically, we have examined moderately, traumatically brain-injured cats, at 2-211 days postinjury. In this study we employed both LM and EM immunocytochemistry for detection of Gabaergic terminals within the lateral vestibular nucleus. Through such an approach we again confirmed the association of traumatic brain injury with DAI, particularly within the vestibular complex where the lateral vestibular nucleus appeared most susceptible to axonal damage, associated with reactive axonal swelling, Wallerain degeneration and terminal loss. Such terminal loss was related to the focal loss of immunoreactive punctae. Following this loss of immunoreactivity preliminary analysis suggests a return of Gabaergic terminals in an "adaptive fashion". The source of such terminals is unknown, however, they are assumed to originate from related intact axons. The relation of these phenomena to both the morbidity and recovery seen in brain-injured humans will be discussed. This research was supported by grant NS 20193.

GENE STRUCTURE AND FUNCTION II

254.1

ACTIVE-SITE-DIRECTED INHIBITION OF Ca^{++}/CaM-DEPENDENT KINASE II (CK-II) BY A BIFUNCTIONAL CALMODULIN-BINDING PEPTIDE. P. Kelly, R. Weinberger* and M. N. Waxham*, Dept. of Neurobiol. and Anat., and Neurology, Univ. of Tex. Med. Sch., Houston TX 77225.

Recent recombinant DNA and synthetic peptide studies have shown that the calmodulin (CaM)-binding domains of CK-II reside between two consensus phosphorylation sequences, RQET and RNFS at its N- and C-terminus, respectively. CK-II activity is initially dependent on Ca^{++}/CaM, however, following autophosphorylation its activity becomes independent of Ca^{++}/CaM. Recent studies suggest that the autophosphorylation of a Thr residue accompanies the generation of Ca^{++}/CaM-independent kinase activity. We report here that a synthetic peptide containing the CaM-binding domain and consensus phosphorylation sequence at its N-terminus contains bifunctional inhibitory properties. The peptide acts as a potent antagonist of Ca^{++}/CaM-dependent activation of CK-II by competing for Ca^{++}/CaM. More importantly, this peptide inhibits (IC_{50}= 2.5 um) synapsin I phosphorylation and continued autophosphorylation of Ca^{++}/CaM-independent CK-II. A second peptide, identical to the one just described but lacking the Arg of the N-terminal consensus phosphorylation site did not inhibit Ca^{++}/CaM-independent CK-II activity. These results suggest that the phosphorylation sequence just N-terminal to the CaM-binding domain of CK-II can bind to or near the enzyme's active site and inhibit activity. We suggest that this active-site-directed inhibitory domain naturally resides in the active site of Ca^{++}/CaM-dependent CK-II. Furthermore, binding of Ca^{++}/CaM and subsequent autophosphorylation of this N-terminal phosphorylation sequence may cause disinhibition of the active site thus facilitating kinase activation and substrate phosphorylation.

254.2

NEURON SPECIFIC EXPRESSION OF BACTERIAL β-GALACTOSIDASE UNDER THE REGULATION OF THE MOUSE THY-1.2 PROMOTER IN TRANSGENIC MICE. K. A. Kelley* and K. Herrup (SPON: S. Zaremba). The Mount Sinai School of Medicine, New York, NY 10029 and E.K. Shriver Center, Waltham, MA 02254.

In an attempt to produce a novel neuronal marker in transgenic mice, the bacterial β-galactosidase gene was fused to 5'-flanking sequences from the mouse Thy-1.2 gene. Five transgenic mice were produced and their offspring were subsequently examined for the presence of transgene mRNA. Expression was observed in only two of the five lines, and was restricted to the CNS. Although the endogenous mouse Thy-1 gene is expressed in thymus as well as brain, expression of the Thy-1/β-galactosidase transgene was never detected in the thymus. These results suggest that the regulatory sequences which are necessary for neuronal or thymic expression of the endogenous Thy-1 gene may be discrete elements. The Thy-1 regulatory sequences that were used for this study were sufficient to direct neuronal expression; however, transcription appears to be integration dependent since only two of the five lines express the transgene. This suggests that the Thy-1 sequences may contain a weak neuron-specific promoter/enhancer. The bacterial protein was easily detected in many types of neurons throughout the CNS, with particularly high levels observed in neurons of area CA2 of the hippocampus, the septal nucleus and large neurons of most cranial nerve nuclei. Expression of this heterologous protein in the PNS is currently under investigation. The presence of bacterial β-galactosidase in the CNS of mice provides a novel marker to examine the lineages of various neurons during the development of the CNS.

Supported by the NIH (NS-20591 and NS-18381) and March of Dimes Birth Defects Foundation (KH), and American Cancer Society Postdoctoral Fellowship (PF-2744) and Swebilius Cancer Research Award (KAK).

254.3

HOMOLOGUES OF RAT BC1 RNA ARE EXPRESSED IN MICE AND MEN. Henri Tiedge*, Katarina Eisinger*, Robert T. Fremeau, and Jürgen Brosius. Center for Neurobiology and Behavior, Columbia University CPS, 722 W 168th St., New York, NY 10032.

We have previously shown that neural BC1 RNA is heterogeneously expressed in the rat central and peripheral nervous system. This small, non-messenger RNA is comprised of three domains: (1) the 5' ID element which includes an internal RNA polymerase III promoter, (2) a central A-rich region, and (3) a 3' unique sequence. If BC1 RNA serves a specific function in the mammalian nervous system we would anticipate that it is conserved in evolution. Thus we have used probes complementary to the 3' most 60 nucleotides of rat BC1 RNA to analyze expression of BC1 homologues in species other than rat.

We now report that in addition to other rodents such as mouse and hamster, a BC1 homologue is also expressed in human nervous tissue. Furthermore, when the rat probe was used to localize the murine homologue by hybridization *in situ* to mouse brain sections, a high degree of conservation of the overall expression pattern was observed between mouse and rat, again suggesting a functional role for BC1 RNA. Specifically, high levels of expression were observed in the mouse cerebral cortex, thalamic and hypothalamic nuclei, the zonal and superficial gray layer of the superior colliculus, the central gray, and the amygdaloid complex. Only background labelling was observed in white matter areas such as the corpus callosum, the anterior commissure, and the optic nerve. The putative human BC1 homologue was detected in cerebral cortex by Northern blot analysis at low stringency. This RNA-species is barely detectable in non-neural human organs such as lung or kidney. Like rat BC1 RNA, the human brain homologue cofractionates with the poly-A fraction on an oligo-(dT) column. The human and murine BC1 homologues are currently being cloned, and sequence analysis should reveal the degree of homology with rat BC1 RNA.

254.4

MOLECULAR CLONE OF A RAT BRAIN cDNA HOMOLOGOUS WITH THE *raf/mil* FAMILY OF PROTO-ONCOGENES. R.M. Lewis. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh, Sch. of Medicine, Pittsburgh, PA 15261.

Proto-oncogenes are believed to be normally occurring genes which are involved in regulating cell growth or differentiation. When altered, these genes (now referred to as oncogenes) are capable of causing malignant transformation. Oncogenes are frequently associated with cell lines and tumors of neuronal origin. The expression of oncogenes also appears to be regulated during normal neuronal development. The *raf* oncogene is among the oncogenes which recently have been identified.

The cDNA clone XRAF1 was obtained while screening a rat brain cDNA expression library with antibodies specific for neuronal proteins. XRAF1 had a high degree of homology with a portion of the nucleotide sequence for the human c-*raf*-1 gene. The nucleotide sequence of XRAF1 was not homologous to *pks*, another *raf*-related gene. Unlike *raf* and *pks*, the derived peptide sequence of XRAF1 did not appear to code for any known serine/threonine protein kinases. The XRAF1 mRNA was detected as a single species of ~2.9 kb on Northern blots of rat brain poly A^+ RNA. Probes generated from XRAF1 bound to specific restriction fragments of rat, mouse, and human genomic DNA on Southern blots. The ontogeny and tissue specificity of the mRNA for XRAF1 is currently under investigation.

Supported by IN-58Z from the ACS and NIH RR05416-25.

254.5

DETECTION OF ANTI-MBP RNA IN MYELIN DEFICIENT MUTANT MICE. H. Okano*, K. Ikenaka* and K. Mikoshiba*. (SPON:Y. Oda). Institute for Protein Research. Osaka Univ., Suita, Osaka 565, Japan.

A hereditary dysmyelinating mutation, named myelin deficient (mld), is characterized by hypomyelination in the central nervous system and the reduced myelin basic protein (MBP) gene expression. In mld, the MBP gene was duplicated and its reduced expression was mainly determined by the level of mRNA. The reduced mRNA level was not attributed to the defect of the promoter region, as promoter activities of 1.3 kb 5'-flanking regions from respective genes of mld measured by cell-free transcription assay and transfection studies were indistinguishable from that of the control MBP gene. Chromosomal mapping by in situ hybridization suggested that the duplicated MBP genes were located closely to each other at the distal part of chromosome 18. Exon 3 was present in an inverted orientation 10 kb upstream of the 5' end of the gene-2. Recently, Popko et al. (Neuron in press) demonstrated that the MBP gene is duplicated tandemly in mld, and exon 3 to 7 of the upstream copy is inverted. We detected antisense RNA complementary to exons 3 and 7, which correspond to the inverted segment. This abnormal transcript was also shown to elongate through the inverted segment to reach the transcription initiation site of the downstream gene.

254.6

MORPHOMETRIC ANALYSIS OF MYELIN IN THE OPTIC NERVE OF TRANSGENIC SHIVERER MICE H.D. Shine, C. Readhead*, B. Popko*, L. Hood* and R. L. Sidman. Ctr. for Biotech., Baylor College of Med., The Woodlands, TX 77381; Depts. of Neurosci., Children's Hospital and Neuropath., Harvard Medical School, Boston, MA, 02115; Div. of Biol., Calif. Inst. of Tech., Pasadena, CA 91125.

The mutation Shiverer (shi) is a large deletion in the Myelin Basic Protein (MBP) gene. Homozygotes (shi/shi) have no MBP, have severely hypomyelinated CNS, shiver, convulse, and die at 4-5 months. The MBP gene was introduced into the shi germline by microinjecting the cloned gene into fertilized eggs. Homozygous transgenic mice (shi/shi;MBP^1/MBP^1) had approximately 25% of normal amounts of MBP mRNA and protein and did not shiver, convulse or die early. A detailed morphometric analysis of the myelin structure in optic nerve of normal, shi, Myelin Deficient (mld, an allele to shi), and transgenic mice was performed using computer assisted quantitative methods at the electronmicroscopic level. As the amount of MBP mRNA increases the percentage of myelinated axons increases and myelin thickness increases. Axons with larger calibre are myelinated before smaller calibre axons.

Supported by NIH grants NS 22223, NS 20820, NS 14069 and postdoctoral grant NS 07724; Multiple Sclerosis grant MS RG 1683-A-1; the Seaver Foundation; the Retina Research Foundation.

254.7

A DIVERSE POPULATION OF NEURAL ADHESION MOLECULE TRANSCRIPTS IS GENERATED BY ALTERNATIVE SPLICING D. Barthels[1]*, M.-J. Santoni[2]*, G. Vopper[1]*, C. Goridis[2]* and W. Wille[1]. [1]Institut für Genetik der Universität zu Köln, D-5000 Köln 41, F.R.G. and [2]Centre d'Immunology INSERM-CNRS, F-13288 Marseille Cedex 9, France

The name 'neural cell adhesion molecule' (NCAM) refers to a class of cell surface glycoproteins which play a role in developmental and adult processes of cell-cell contact formation and maintenance. Three major isoforms NCAM-180, -140, & -120 have been identified in the mouse. All three isoforms are encoded by a single gene with a length of more than 80kb. At least four mRNA species are detected in rodents, of which the 7.4 kb transcript encodes NCAM-180, the 6.7 kb mRNA NCAM-140, and the 2.9 & 5.2 kb species code for NCAM-120. The two latter mRNA only differ by the length of their 3'-non-coding region.

The three isoforms have very similar N-terminal (extracellular) domains and differ mainly by the size of their transmembrane and cytoplasmic regions. While NCAM-180 & -140 contain membrane spanning domains, NCAM-120 lacks a transmembrane segment and is anchored to the membrane by lipids.

We isolated and analyzed the complete cDNA sequences of all transcripts encoding the three NCAM isoforms and established the splice pattern on the genomic and cDNA level leading to the major NCAM proteins. Here we report data which demonstrate that in addition to alternative splice sites in the codin region for the cytoplasmic domain at least two more alternative splice sites, a & π, exist in the coding region for the extracellular domains which are involved in posttranslational modification generating a much more complex NCAM family than anticipated.

254.8

EXPRESSION OF TRANSFECTED GENES BY DIFFERENTIATED, POSTMITOTIC NEURONS AND PHOTORECEPTORS IN CULTURE. **Marc Werner*, Paul Lieberman*, Steven Madreperla* and Ruben Adler, The Wilmer Institute and Department of Pharmacology, The Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD.**

Using purified cultures of chick embryo retina neurons and photoreceptors (Prog. Ret. Res., 6:1-27), we have investigated whether gene transfection techniques, which are frequently used with dividing cells, are equally applicable to postmitotic, differentiated cells. Marker genes for chloramphenicol-acetyltransferase (CAT) or β-galactosidase (β-gal) were driven by either a Rous sarcoma virus or a cytomegalovirus promoter. Cultured cells were exposed to DNA for 18-48 hrs in cultured medium plus a transfection solution containing Na_2HPO_4 and $CaCl_2$, and returned to normal medium for up to 30 hr. β-gal histochemistry and a autoradiographic CAT assay showed that up to 15% of the neurons and photoreceptors expressed these genes with efficiencies depending on DNA concentration and length of transfection. When compared to control cultures, calcium phosphate-treated cultures showed relatively similar cell numbers, modest (10-30%) decreases in aspartate uptake and choline acetyltransferase activity, and approximately 60% decrease in GABA uptake. Thus, although different metabolic activities are selectively affected by the transfection treatment, it is possible to obtain expression of genes transfected into primary, postmitotic cells such as neurons and photoreceptors.

254.9

MOLECULAR CLONING AND SEQUENCE ANALYSIS OF cDNAs FOR A UNIQUE GFAP FROM THE GOLDFISH VISUAL PATHWAY. S. Giordano*, E. Glasgow*, P. Tesser*, N. Schechter. Departments of Psychiatry and Biochemistry, SUNY, Stony Brook, New York 11794.

We have characterized cDNA clones corresponding to a glial fibrillary acid protein (GFAP) expressed in goldfish optic nerves. A comparison of its amino acid sequence with those of intermediate filaments (IFs) from higher vertebrates reveal highly conserved and unique regions, for this protein. The goldfish proteins ON_3 and ON_4 are synthesized in glial cells associated with the optic nerve. Biochemical data and immunohistochemical localization studies suggested that ON_3 and ON_4 correspond to a unique GFAP.

A lambda gt11 expression library prepared from optic nerve total RNA was screened with anti-ON_3/ON_4 polyclonal antisera. Ten positive clones between 1.5 and 2Kb in length were characterized and appear to be derived from the same mRNA species. Evidence that we have cloned a cDNA for ON_3/ON_4 comes from in vitro transcription and hybrid select experiments. These clones have been subcloned into an MI3mp18 vector and sequenced.

All IF proteins contain a highly conserved 40K core region. Sequence data from our clones reveals the presence of this 40K core. The carboxy region of the core in these clones matches exactly the amino acid sequence for that region in most other IFs. In the less conserved amino end of the core, the ON_3 amino acid sequence more closely resembles that of vimentin than mammalian GFAP. The diversity of IF proteins is generated by variable regions on either side of the core, which may be involved in cell specific functions of IF proteins. The amino acid sequence in the carboxy terminal region of ON_3 does not resemble the amino acid sequence for other known IF proteins. In addition, the ON_3 carboxy variable region contains a sequence rich in Ser, Gly, and Tyr. This sequence is repeated at least four times and may represent phosphorylation sites. Supported by EY05212 to N.S.

254.10

STRUCTURE, NGF-INDUCIBILITY AND NEURON-SPECIFIC EXPRESSION OF THE RAT SCG10 GENE ENCODING A NOVEL 22kD PROTEIN: THE 5' UPSTREAM REGION CONTAINS NEURON-SPECIFIC TRANSCRIPTIONAL SIGNALS. N.Mori*, C.W.Wuenschell, R.Stein*†, and D.J. Anderson*. Div. of Biology, Calif. Inst. of Technology, Pasadena, CA 91125, and †Tel Aviv Univ., Tel Aviv, Israel.

We have sequenced a rat cDNA, SCG10, originally isolated as a marker for neuronal derivatives of the neural crest. It encodes a novel 22kD protein. Structural features suggest the protein may function in mediating membrane-cytoskeletal interactions, possibly related to neurite extension. The rat SCG10 gene contains at least five exons and spans 40kb of DNA. Primer extension and S1-mapping studies show multiple transcriptional start sites and identify a putative promoter. To study the transcriptional regulatory region, sequences upstream of the SCG10 gene were fused to the bacterial CAT gene. In DNA-mediated transfer experiments using various cell lines and in transgenic mice, expression of a construct bearing 4kb of SCG10 upstream sequence (pCAT37) was largely restricted to neuronal cells. However, a 0.5kb SCG10 upstream-CAT construct (pCAT4) was expressed in all cell lines and tissues studied except melanoma cells. The pCAT37 was also inducible by NGF in PC12 cells, while pCAT4 was not. These data suggest that the 4kb upstream sequence includes necessary and sufficient information for NGF responsiveness and neuron-specific expression of the SCG10 gene.

254.11

TRANSFECTION OF NEURONS WITH A DEFECTIVE HSV-1 VECTOR AND EXPRESSION OF *B*-GALACTOSIDASE. A.I. Geller, A. Freese, J.F. Gusella*, and X.O. Breakefield, Lab. Of Neurogenetics, Mass. Gen. Hosp., Boston, MA

We are developing HSV-1 virus vectors to deliver genes into neurons in vitro and in vivo. This approach offers the potential to perform gene therapy on neuronal disease, and to modify neuronal physiology in vivo to study learning and memory. pHSVlac, a 8.1 kb defective HSV-1 vector, contains a transcription unit that places the E. coli Lac Z gene, which encodes a *B*-galactosidase, under the control of the HSV-1 immediate early 4/5 promoter. pHSVlac was packaged into HSV-1 virus particles using temperature sensitive (ts) mutants of HSV-1 as helper virus. Virus was grown at the permissive temperature of 31C. Infection of cells at the restrictive temperature of 37C results in expression of *B*-galactosidase without cell death. Expression of *B*-galactosidase following infection with pHSVlac has been observed in mitotic cells: CV1 monkey fibroblasts, LM tk^- mouse fibroblasts, N1E-115 mouse adrenergic neuroblastomas, NS-20Y mouse cholinergic neuroblastomas, PC12 rat pheochromocytomas, AtT-20 mouse pituicytes, GH 4 rat pituicytes, SK-N-BE(2) human neuroblastomas, and in nonmitotic cells: PC12 cells differentiated with NGF, and N1E-115 cells differentiated with dibutyryl cAMP. Expression also occurs in neurons in primary culture from superior cervical ganglia, dorsal root ganglia, spinal cord, cerebellum, thalamus, striatum, hippocampus, occipital cortex, temporal cortex, and frontal cortex. pHSVlac is stably maintained, and *B*-galactosidase is stably expressed for at least two weeks in differentiated PC12 cells, differentiated N1E-115 cells, and sensory neurons. HSV-1 ts mutants have been shown to establish a latent infection following injection into the mouse cerebral cortex. These results form a foundation for using HSV-1 vectors to deliver genes into neurons in adult animals. The Lac Z gene in pHSVlac can be replaced with genes whose products affect neuronal physiology, including components of second messenger systems and neurotransmitter metabolism.

254.12

THE OLFACTORY MARKER PROTEIN GENE IS NON-CLASSICAL. E. Danciger* and F.L. Margolis. (SPON: R. Wurzburger) Roche Inst. Molec. Biol., Nutley, N.J. 07110.

Genomic DNA encoding rat olfactory marker protein (OMP) was isolated from a charon 4A lambda phage library using OMP-cDNA as a probe. A 10 Kb EcoRI fragment (λ-OMP) was subcloned into Bluescript plasmid. A restriction map was constructed. Upon hybridization to OMP cDNA a 2.6 Kb HindIII-SacI fragment was found to contain the entire cDNA information. Southern blot of rat genomic DNA, digested with EcoRI, XbaI and BamHI was hybridized to OMP-cDNA. The bands obtained agreed with the restriction map of λOMP. Bands that hybridized less intensely were also seen. These related fragments remain to be investigated. The HindIII-SacI fragment was subcloned into M13Mp18 and sequenced by the Sanger method. Deletions were generated to provide a series of overlapping clones. The sequence obtained was co-linear with the cDNA. To look for the promoter, 860 bp upstream of the ATG translation start site, was sequenced by the Sanger method using synthetic primers and double stranded DNA. Primer extension studies with an 18-mer synthetic primer complimentary to the 5'end of the coding region indicated the transcription start site. Beyond the start site, no TATA or CAAT boxes were found. The OMP gene, seems to lack introns and to be missing typical promoter boxes. We intend to use the genomic upstream region to study promoter activity and specificity.

254.13

MOLECULAR AND IMMUNOLOGICAL CHARACTERIZATION OF GLYCOPROTEINS OF THE POSTSYNAPTIC DENSITY. B. Ni, J.W. Gurd and I.R. Brown. Departments of Zoology and Biochemistry, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada M1C 1A4.

We have previously identified three high molecular weight glycoproteins (gp180, gp116 and gp110) which are concentrated in postsynaptic densities (PSDs) purified from the mammalian brain (Gurd, Can. J. Biochem., 58, 941-951, 1980). In order to further characterize these glycoproteins, antibodies were raised by intrasplenic immunization of rabbits with protein blots of gp180 and gp116 purified from rat PSDs. Immunoblots against whole brain homogenates demonstrated that the antibodies raised against gp180 recognized a prominent protein of app. Mr 65K and a minor species of app. Mr 180K whereas the antibodies against gp116 reacted with a protein of app. Mr of 116K. Both antisera detected immunoreactive species in adult rat brain but not in liver, spleen or kidney. In both cases the immunogens were enriched in synaptic glycoprotein fractions and were highly concentrated in isolated PSDs. The concentrations of the proteins recognized by both antisera changed during early postnatal development at a time when synapses are actively being formed. The antisera have been utilized to screen a rat brain gt11 expression library. (Supported by grants from NSERC to I.B. and MRC to J.G.)

254.14

CHARACTERIZATION OF cDNA CLONES ENCODING PUTATIVE SYNAPTIC GLYCOPROTEINS. I.G. Johnston, T. Paladino, J.W. Gurd and I.R. Brown. Departments of Zoology and Biochemistry, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada M1C 1A4.

Glycoproteins are important components of the mammalian synapse. We have previously reported the isolation of clones from a rat brain cDNA expression library based on immunoreactivity with a mixed polyclonal antibody raised against synaptic glycoproteins (Johnston et al., Soc. Neurosci. Abstr. 13:561, 1987). Briefly, mice were injected with concanavalin A-binding glycoproteins isolated from bovine synaptic junctions and the resultant antiserum used to screen the library. Clones recognizing two messages have been isolated in this fashion. The clone designated SC1 recognizes a 3.2 kb message which is expressed at high levels in brain and much lower levels in muscle and lung. It is absent in liver and spleen. Interestingly, DNA sequence data reveal extensive homology with the collagen-binding protein osteonectin. The clone designated SC2 recognizes a 1.3 kb message which is abundant in brain and is expressed at lower levels in all other tissues examined. In situ hybridization reveals a neuronal pattern of expression for both clones although glial expression is also observed for SC2. Efforts are currently underway to establish the subcellular location of proteins encoded by SC1 and SC2. (Supported by grants from NSERC to I.B. and MRC to J.G.)

254.15

HYPERSENSITIVE SITES AND CHROMATIN ORGANIZATION OF GENES EXPRESSED IN CORTICAL NEURONS. T.R. Ivanov and I.R. Brown. Department of Zoology, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada, M1C 1A4.

Nuclei purified from cortical neurons of the adult mammalian brain exhibit an atypically short nucleosomal repeat length of approximately 165-170 base pairs whereas glial and liver nuclei possess nucleosomal repeat lengths of 200 base pairs. This unique chromatin conformation appears during the first week of postnatal development in mouse, rat and rabbit, concomitant with an increase in overall DNase I sensitivity of total cortical neuronal chromatin. Within this relatively uncoiled, 'short repeat' chromatin, hierarchies of DNase I sensitivity exist reflecting genes which are active (i.e., neurofilament and neuron-specific enolase) or inactive (i.e., albumin) in neuronal cells. Developmental studies reveal the presence of three DNase I hypersensitive sites associated with the 68 kDa neurofilament gene and at least one hypersensitive site associated with the neuron-specific enolase gene. Availability of a mouse genomic neurofilament probe (Lewis and Cowan, 1986, Mol. Cell. Biol. 6: 1529-1534) allows for the mapping of the neurofilament gene hypersensitive sites. (Supported by grants from NSERC).

254.16

EXPRESSION OF A NOVEL TRANSCRIPT OF THE MOUSE MBP GENE A. Campagnoni, S. Newman*, K. Kitamura*, and C. Campagnoni* UCLA School of Medicine, Los Angeles, CA 90024.

An MBP cDNA with an usually long 5'-UT region was isolated from a mouse brain cDNA library. The sequence of the clone indicated that it corresponded to a full-length 14 K MBP mRNA plus an additional ∿350 bp upstream of the CAP site thought to represent the 5' end of all MBP mRNAs. The 350 bp sequence is encoded by two genomic regions, one of which is immediately upstream of exon 1 of the mouse MBP gene, and the other of which is more than 25 kb upstream of exon 1. A probe specific for the unique 5'-UT region was prepared and used to examine the expression of this transcript in the developing mouse brain. The probe hybridized to two bands of ∿2.7 and ∿5.4 kb on Northern blots. The developmental expression of these mRNAs was unlike that of the major MBP mRNAs, with greatest expression occurring much earlier than that of the transcripts expressed from the "normal" promoter. The 2.7 kb band was missing from Northern blots of mRNA isolated from shiverer mice, which contain a deletion of exons 3-7 of the MBP gene. These results indicate that the clone corresponds to the 2.7 kb mRNA and that the gene encoding the 5.4 kb mRNA lies outside the genomic piece deleted in shiverer, but shares genomic regions in common with the MBP gene. The data also suggest that the MBP gene is more complex than previously thought and that there exists a subpopulation of MBP mRNAs, under separate developmental regulation, that arises from a previously unsuspected transcription start site.

254.17

ALLELIC HYPOMYELINATION MUTATIONS jp AND jp^{msd} INTERACT DIFFERENTLY WITH qk. S. Billings-Gagliardi, J.-B. Gow*, and M.K. Wolf. Dept. Cell Biol., U.Mass. Med. Sch., Worcester, MA 01655.

Individual mice whose genomes contain both CNS hypomyelination mutations quaking (qk/qk) and myelin synthesis deficiency ($Tajp^{msd}/Y$) (abbreviation: $qk*jp^{msd}$) have been bred. The morphology of their optic nerves, posterior columns, and cerebellar white matter is indistinguishable from that of animals with genotypes qk/qk or qk/qk*Ta+/Y (qk*Ta control for effects of Ta marker). In P20 optic nerve the proportion of axons myelinated relative to wild type is 25% qk; 24% $qk*jp^{msd}$; 22% qk*Ta. All three phenotypes are characterized by axons and oligodendrocyte processes associated with whorls of myelin-like membranes, inappropriate myelin targeting, and macrophages. Myelin sheaths are thin but compacted. Thus in $qk*jp^{msd}$ the distinctive morphology of jp^{msd} appears completely suppressed by qk, although the shorter lifespan of jp^{msd} is not. By contrast, the allelic double mutant qk*jp has about half as many myelinated axons as $qk*jp^{msd}$ with thicker, more regularly formed sheaths grouped in clusters. This morphology is truly intermediate between qk and jp. We had previously found that shi suppresses jp more completely than jp^{msd}; now we show that qk suppresses jp^{msd} more completely than jp! Grant support: NINCDS (Javits Award).

254.18

PROTEOLIPID PROTEIN (PLP) GENE EXPRESSION AND PROMOTOR ACTIVITY IN C6 GLIOBLASTOMA AND CULTURED SCHWANN CELLS. K.-A. Nave, G. Weinmaster*, C. Lai*, and G. Lemke.
Molecular Neurobiology Lab, The Salk Institute, La Jolla, CA 92037.

PLP is the major structural protein in central nervous system myelin, and has recently also been detected in Schwann cells of the peripheral nerve, where it appears not to be incorporated into the myelin sheath (Puckett, C. et al., J. Neurosci. Res.,18: 511, 1987). In order to functionally define the PLP promotor and to test its tissue specificity, we have isolated the rat PLP gene and constructed a plasmid in which 1200 basepairs of DNA upstream of the PLP transcription start site are linked to the bacterial chloramphenicol acetyltransferase (CAT) reporter gene. Calcium phosphate mediated DNA transfection showed that the PLP promotor region was active in the C6 glioblastoma cell line as well as in NIH3T3 and rat2 fibroblasts. C2C12 myoblastoma and B78H1 melanoma cells did not respond to the PLP promotor. In C6 cells CAT activity was about 5 fold higher when the transiently transfected cells were allowed to grow into stationary phase.
Using a full length rat PLP cDNA (p27) as a probe, Northern blot analyses confirmed transcription of the PLP gene in rat sciatic nerve and in cultures of primary rat Schwann cells. Forskolin, which stimulates the cAMP dependent expression of the P0 and MBP mRNAs in Schwann cells, also increases the steady state level of PLP mRNA. This suggests that genes encoding the major myelin proteins are coordinately regulated in Schwann cells. The PLP promotor region will be used to direct the expression of foreign genes into myelinating glial cells *in vivo*.

254.19

STRUCTURE OF THE GENE ENCODING DROSOPHILA TYROSINE HYDROXYLASE. W. S. Neckameyer.* (SPON: T. Tully). Dept. of Biology, Brandeis University, Waltham MA 02254.

Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in the sysnthesis of catecholamines. Since catecholamines are believed to modulate many behaviors in both vertebrates and invertebrates, it would be interesting to compare TH regulation in diverse species.

cDNA homologues to rat TH were isolated from Drosophila third instar- and head-specific libraries using a probe (gift of D. Chikaraishi) derived from the conserved 3' coding sequence of the rat gene. The longest cDNA clone, λcDTH.2, contains 90 bp of 5' noncoding, 1525 bp of coding sequence, 1605 bp of 3' noncoding and a 60 nucleotide poly(A) tail. Recently, Drosophila genomic TH clones have been obtained. The genomic clone λgDTH.1 contains all sequences corresponding to λcDTH.2. It is therefore believed that λgDTH.1 contains the entire coding sequence for the enzyme, as well as up to 4 kb of 5' upstream sequences.

Similar to the rat and human TH genes, Drosophila TH is single-copy and spans approximately 8 kb. Preliminary sequence analysis of λgDTH.1 indicates that none of the 5' intron/exon junctions are conserved between the mammalian hydroxylases and the Drosophila gene, but that at least one junction within the 3' end of the genes is conserved. Complete genomic analysis of Drosophila TH is in progress.

This work was supported by NIH Postdoctoral Fellowship GM11060 and was done in the laboratory of W. G. Quinn at MIT, Cambridge MA.

RESPIRATORY REGULATION II

255.1

FIRING PATTERNS OF PHASE-SPANNING GENIOHYOID MOTOR UNITS (MU) IN CATS. E. van Lunteren and T.E. Dick. Department of Medicine, Case Western Reserve University and University Hospitals, Cleveland, OH 44106.

The geniohyoid, an upper airway dilating muscle, is supplied by the hypoglossal nerve, and is phasically active with respiration. To compare its neural regulation during inspiration (I) and expiration (E), electrical activity was recorded from 23 MU which were active during both phases of the respiratory cycle in anesthetized tracheostomized cats. The relative duration of I activity (92 ± 2% Ti) was significantly longer than the relative duration of E activity (68 ± 5% Te; $P < 0.00001$). Furthermore the mean frequency of I firing was significantly higher than the mean frequency of E firing (23.5 ± 1.6 versus 15.7 ± 1.3 impulses/second; $P < 0.00001$), and the number of impulses per I exceeded the number of impulses per E (16 ± 2 versus 10 ± 1; $P < 0.0001$). The influence of volume-related vagal afferents was tested by occluding the airway at end-expiration. MU activity during I was significantly increased in relative duration ($P < 0.02$), firing frequency ($P < 0.001$) and number of impulses ($P < 0.0001$), whereas MU activity during E was significantly decreased in relative duration ($P < 0.00002$), firing frequency ($P < 0.001$) and number of impulses ($P < 0.0001$). These results suggest that during unobstructed breathing the firing intensity of phase-spanning geniohyoid MU during I exceeds that during E, and that lung-volume related afferents inhibit I but stimulate E activity of geniohyoid MU. NIH HL-38701, HL-01600.

255.2

CHOLINERGIC RETICULAR MECHANISMS CAUSE STATE-DEPENDENT HYPOTONIA IN UPPER AIRWAY MUSCLES. R. Lydic, H.A. Baghdoyan, K. Gilbert* and C.W. Zwillich*. Pulmonary Div., Penn. State Univ. College of Med. Hershey, PA 17033.

Microinjection of the cholinergic agonist carbachol into the medial pontine reticular formation (mPFR) of intact, unanesthetized cats produces a state (D-CARB) which is similar to rapid eye movement (REM) sleep. Since D-CARB and REM sleep are both characterized by postural muscle atonia, this study is using the D-CARB model to evaluate the hypothesis that cholinergic mechanisms also mediate state-dependent changes in upper airway muscle tone. To date, over 30 microinjections have been made into 6 mPFR sites in 4 cats. EMG recordings were made from the posterior cricoarytenoid (PCA) muscles in the larynx. Analysis of PCA EMG activity for 200 breaths before and 200 breaths at 4 min after carbachol revealed that every drug injection produced significant decreases in PCA EMG activity. During D-CARB, inspiratory (I) PCA activity was 44.5% of PCA I activity during waking (W) ($p<0.001$). Expiratory PCA activity declined from 37% to 14.5% of IW PCA values ($p<0.001$). The direction and magnitude of these changes parallel the PCA hypotonia of physiological REM sleep. Since these carbachol-induced effects are blocked by centrally administered atropine, these data specify for the first time that cholinergic reticular mechanisms can causally mediate state-dependent hypotonia in the upper airway.

Supported by: Research Initiation Grant from The Pennsylvania State University.

255.3

RESPONSE OF CERVICAL INSPIRATORY NEURONS TO INTERCOSTAL NERVE AFFERENT STIMULATION. R. Shannon, Y.M. Hernandez* and B.G. Lindsey. Dept. Physiol. & Biophysics, Col. Med., Univ. South Florida, Tampa, FL 33612.

Experiments were conducted to determine if intercostal nerve afferents have intersegmental effects on inspiratory (I) neurons located in the cervical spinal cord (C1-C2). We compared the latencies of response of extracellularly recorded evoked unit activity in the dorsal caudal medulla, cervical I-cell activity, and phrenic (C5) efferent activity (PA) to electrical stimulation of internal intercostal nerve afferents (IINAs). Studies were performed on 8 anesthetized (Dial), thoracotomized, paralyzed, ventilated cats. Stimulation of low threshold T5,6 IINAs which reduces PA only by inhibition of medullary I-cells (i.e., disfacilitation), resulted in a decrease in cervical I-cell (19) activity at approximately the same time (3/19) or after 16/19) the decrease in PA; PA decreased after medullary evoked activity. Stimulation of low threshold T9,10 IINAs which excites PA through intersegmental pathways, resulted in an increase in cervical I-cell activity (6/12 cells) at approximately the same time (4/6) or after (2/6) the increase in PA; evoked activity in the medulla occurred at approximately the same time as the increase in PA. These results suggest that: a) intercostal nerve afferents (e.g., from tendon organs) do not inhibit cervical I-cells through intersegmental pathways, and b) lower intercostal afferents excite cervical I-cells intersegmentally. (NIH Grant HL17715)

255.4

Phrenic Afferent Fibers (PAFs) Excite Cervical Spinothalamic Tract (STT) Neurons in the Monkey. **D.C. Bolser, S.F. Hobbs, M.J. Chandler and R.D. Foreman. Dept. of Physiol. and Biophys., OUHSC, Okla. City, OK 73190.**

Pain associated with diseases of the diaphragm, such as diaphragmatic pleuritis, can be referred to the shoulder. The neurophysiological basis for this pain referral is unknown. Referred visceral pain can be explained by excitatory convergence of visceral and somatic afferent input onto the same STT neurons. This theory of referred visceral pain also may be applicable to the diaphragm, a somatic organ. We hypothesized that STT neurons in the cervical spinal cord are excited by stimulation of somatic fields in the shoulder and proximal forelimb and by activation of PAFs. Six monkeys (Macaca fascicularis) were anesthetized with chloralose and paralyzed with pancuronium. Extracellular single unit activity was recorded from the left grey matter of the C5-C8 spinal cord. All cells were antidromically activated from the ventroposterolateral nucleus of the thalamus and had excitatory somatic fields in the left shoulder and/or proximal forelimb. Electrical stimulation of the left phrenic nerve just rostral to the diaphragm (2-34 V, 20 Hz, 0.1 ms pulse) increased the activity of 13/16 neurons. Three neurons were unaffected. We conclude that activation of PAFs can increase the activity of cervical STT neurons that have excitatory somatic fields in the shoulder and forelimb. This excitatory convergence can explain referral of pain to the shoulder in diseases involving the diaphragm. (Supported by NIH grants HL22732, HL07930, NS08150 and the American Heart Assoc.).

255.5

GRAVITATIONAL REPRESENTATION OF RESPIRATORY NEURON ASSEMBLIES B.G. Lindsey, R. Shannon, and G.L. Gerstein. Dept. Physiol., Univ. S. Florida, Tampa, FL 33612 and Dept. Physiol., Univ. Penn. Philadelphia, PA 19104.

We have used the gravity method (J. Neurosci. 5:881) to assess the cooperative behavior of respiratory modulated medullary neurons in raphe nuclei and lateral respiratory groups. Twelve sets of 5-9 simultaneously recorded neurons from 8 anesthetized (Dial), paralyzed, bilaterally vagotomized, artificially ventilated cats were studied. Neurons were represented as particles that acquire charges as a function of the cells' activities. 1) Rates of particle aggregation a) were related to interaction strengths derived from cross-correlograms, and b) varied with time. 2) Putative shared inputs and excitatory interactions were more readily detected than inhibitory interactions. 3) Some midline neurons exhibited multiple concurrent associations with both midline cells and the lateral groups. Cross-correlation analysis of midline neurons recorded in n. raphe obscurus or at the pontine-medullary border revealed joint increases in activity that resulted in symmetrical and asymmetrical correlogram peaks spanning up to 100 ms. These preliminary results establish that midline neurons may be elements of spatially distributed neural assemblies, suggest fluctuations in the relationships among some cells, and confirm conclusions about the method derived from network simulations. Supported by NS19814, SDF-0013, and ONR N00014-87-K-0766.

255.6

INHIBITION OF MEDULLARY EXPIRATORY NEURONS IN THE CAT BY RETROFACIAL INSPIRATORY NEURONS. P.M. Lalley, K.Anders*, D.Ballantyne‡ A.M. Bischoff*, and D.W. Richter. I. Institute of Physiology, Univ. Heidelberg, D6900 Heidelberg,FRG

The discharge of expiratory neurons in the caudal medulla is terminated by decrementing waves of IPSP's during inspiration (I) and post-inspiration(PI). We wished to determine if I neurons near the retrofacial nucleus (RFN) are involved in the depression of caudal expiratory (CE) neurons. RFNI and CE neurons were recorded from. Comparisons were made between the discharge patterns of RFNI neurons and the membrane potential profiles (MPP's) of CE neurons. Spike - triggered averaging (STA) of IPSP's in CE neurons by RFNI firing was used to detect synaptic connectivity. Of 51 RFNI neurons, 46 had incrementing discharges, 6 had plateau and 3 had decremental patterns;thus most patterns did not match the MPP's of CE neurons. However, a later wave of IPSP's was often seen, accompanied by increased RFNI firing frequency. STA of 30 pairs revealed IPSP's with latencies and shape indicies which were not consistent with a monosynaptic connection; at least one inhibitory neuron may be interposed between RFNI and CE neurons. We conclude that RFNI neurons inhibit CE neurons throughout I, in concert with other types of medullary I neurons. The inhibition by RFNI neurons is relatively moderate , but is augmented at times during late inspiration, leading to a second wave of IPSP's in CE neurons.

255.7

THE NUCLEUS RAPHE MAGNUS: A SITE FOR PRODUCTION OF ACUTE RESPIRATORY FAILURE IN THE RAT. M.K. Carruth*, A.A. Fowler*, G.R. Leichnetz, D.J.Mayer (SPON: D. Bossut). Departments of Anatomy, Medicine and Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Acute respiratory failure was produced in rats by injection of the excitotoxin, ibotenic acid into the nucleus raphe magnus (NRM). Pulmonary arterial pressure, blood gas tensions, systemic arterial pressure, core body temperature, lung water, and bronchoalveolar lavage fluid (BALF) protein levels were monitored and compared to controls. NRM injection produced a dramatic rise in pulmonary arterial pressure (128%), acute hypoxia ($p < 0.01$), metabolic acidosis ($p < 0.01$), and a widened arterial to alveolar oxygen gradient in the absence of a rise in systemic arterial pressure. Following injection we also observed a significant decrease in core body temperature ($p < 0.01$). There was no significant change in pCO2 levels, lung water, or BALF protein content compared to controls.

This is the first study suggesting that NRM participates either directly or indirectly in neural regulation of pulmonary arterial tone. Electrolytic lesioning of this nucleus does not produce similar respiratory effects. We suggest that prolonged excitation of the NRM, not death of constituent neurons, produces rapid onset of pulmonary failure. We further conclude that pulmonary arterial hypertension with profound ventilation/perfusion mismatching is responsible for the hypoxemia which results following neural excitation of the NRM.

255.8

DISCHARGE DEPENDENCIES OF PERIAQUEDUCTAL GREY (PAG) NEURONS TO THE RESPIRATORY CYCLE DURING SLEEP-WAKING STATES. H Ni*, J Zhang, RR Terreberry and RM Harper. Dept of Anatomy & Brain Res Inst, UCLA, Los Angeles, CA 90024.

The PAG projects to brainstem structures regulating respiratory patterning, especially the n. tractus solitarius, and rostral ventrolateral medulla including the n. ambiguus, and receives projections from rostral limbic structures that can alter respiratory patterning. Since the PAG could modulate descending influences on respiratory patterning, particularly during different states, we examined PAG neuronal discharges in cats during sleep and waking. In 6 cats chronically instrumented with microelectrodes in the PAG and diaphragmatic leads, we correlated tonic PAG neuronal discharge with T(tot) and determined phasic discharge dependencies with the respiratory cycle in waking (AW), quiet sleep (QS) and active sleep (REM) states. Of 38 cells recorded in the PAG, 10 showed a tonic discharge correlation and 8 showed a breath-by-breath dependency to the respiratory cycle. The greatest number of tonic relationships (5) were observed during AW, followed by REM and QS. Breath-by-breath correlations were observed equally in AW and QS, but less often in REM. These results suggest that a subset of PAG cells discharge with aspects of the respiratory cycle, and that these discharge correlations are state related. The PAG may mediate these state-related differences, or PAG cell discharges may be altered by descending state-related influences. Supported by HL-22418-10

255.9

NUCLEUS RETICULARIS GIGANTOCELLULARIS MEDIATES RESPIRATORY INHIBITION DURING TRANSIENT HYPERTENSION. RW Stremel*, CA Richard, KW Lemmons* and TG Waldrop* (SPON: JP Porter). Depts of Physiology & Biophysics, Univ of Louisville, Louisville, KY 40292 & Univ of Illinois, Urbana, IL 61801.

Activation of cell bodies within the nucleus reticularis gigantocellularis (NGC) causes inhibition of tidal volume and ventilatory frequency. Since the NGC is known to receive baroreceptor information, we hypothesized that this same inhibitory mechanism is responsible for the suppression of ventilation during transient hypertension (TH). In anesthetized cats, respiration was quantified by either C5 phrenic recording or pneumotachographic integration. Phenylephrine (PE) was injected IV (5-40 µg in saline) before and after either electrolytic or chemical (0.1 µg/ml kainic acid in 500 nl saline) NGC lesion. The inhibition of breathing (e.g., apnea) during TH was significantly reduced by bilateral NGC lesion; TH induced by PE caused frequency to be significantly reduced before lesion while after lesion frequency was only minimumly influenced. Normalizing the breath frequency response as change per mm Hg pressure change provides the same result. Indices of tidal volume indicated similar suppression due to TH while lesion has less effect on the inhibition of this variable. The NGC appears to be critically involved, particularly for frequency changes, in the inhibition of breathing during transient hypertension. Supported by Kentucky Heart Association.

255.10

AGE DIFFERENCES IN RESPONSES OF RAT BRAINSTEM RESPIRATORY CHEMOSENSORY NEURONS. Pan Y.*, Whittaker*, J.A., Bernard D.G., and Trouth C.O. Dept. Physiol, Coll. Med. Howard Univ., Washington, D.C. 20059 (SPON: J.A. Holloway).

Central respiratory chemosensitivity has been ascribed to neurons in 3 areas (rostral, intermediate, caudal) located within the marginal glial layer of the ventral medullary surface (VMS). This study examines the hypothesis that the superficial brainstem chemosensory mechanism, might from a functional point of view, not be fully developed at birth. In 3 age groups (newborn 1-4 days, young pups 5-11 days, and adult over 6 months) of spontaneously breathing rats, neuronal activity in the caudal zone (Area L) on the VMS was studied for responsiveness to increased inspired CO_2 and decreased inspired O_2. Units were also tested for responsiveness to other modalities of sensation such as touch, pressure, joint manipulation and temperature. In young and adult rats, increased inspired CO_2 (5% CO_2) increased the firing rate of chemosensitive neurons, and increased respiratory frequency. In newborns the response was slight; 1 and 2 day old rats were least sensitive to CO_2 stimulus. Decreased inspired O_2 (12 % O_2) increased neuronal activity in newborns and young pups, whereas no significant change was noted in the adult. These results suggest (1) central CO_2 chemoreceptor mechanism might not be fully developed functionally at birth (2) a central hypoxic drive to respiration appears to be mediated via the central chemoreceptor elements in newborns. (Supported by MBRS-2-S06-RR-08016-16).

255.11

BRAINSTEM NEURONS DEVELOP AT DIFFERENT RATES IN EARLY LIFE: IN-VITRO ELECTROPHYSIOLOGIC STUDIES. G.G. Haddad and P.A. Getting. Dept. of Pediatrics, Columbia Univ., New York, NY, and Dept. of Physiology, Univ. of Iowa, Iowa City, IA 52242.

We have previously shown that the electrophysiologic properties of neurons in the ventral region of the Nucleus Tractus Solitarius (NTS) develop postnatally. In order to determine whether the cellular properties of other neuronal groups involved in cardiorespiratory control mature postnatally, we studied dorsal vagal (DMNX) and Hypoglossal (HYP) motoneurons in newborn (3-12d) and adult rats. Transverse, in vitro brainstem slices were used for intracellular recording. DMNX neurons of both the adult (n=24) and newborn (n=20) displayed profound spike frequency adaptation (SFA) with steady-state to peak firing frequency ratios of 0.31 and 0.37 respectively. In addition, both adult and newborn DMNX cells exhibited delayed excitation (DE) which was manifested as a delay between the onset of depolarization and the first spike. HYP neurons of both the adult and newborn showed much less SFA (steady-state to peak ratio = 0.6-0.9) than DMNX cells but no DE was observed. The major difference between newborn and adult DMNX or HYP neurons was a higher input resistance and longer time constant for the newborn cells.

We conclude that besides differences in passive properties that can be attributed to soma size, the electrophysiologic properties of DMNX and HYP neurons mature early suggesting that the developmental schedule of DMNX or HYP neurons is different from that of NTS neurons.

255.12

ROLE OF OPIOID PEPTIDES IN LUNG DEVELOPMENT: EFFECTS OF CNS BETA-ENDORPHIN (BE) ON ORNITHINE DECARBOXYLASE (ODC) ACTIVITY AND ON DNA SYNTHESIS. N.L. Greer*, J.V. Bartolome* and S.M. Schanberg* (SPON: F. Menitti). Dept. Pharmacol., Duke Univ., Durham, N.C. 27710.

It is well established that neonatal lung maturation is controlled by various hormones including catecholamines and insulin. Accumulated evidence indicates that endogenous opioids may also have an important role in lung development. Premature infants of narcotic addicts rarely show Respiratory Distress Syndrome. Naloxone, an opiate antagonist, retards lung maturation. The specific opioid(s) involved and its mechanism of action is unknown. Recent studies suggest BE may participate in this process. Fetal and postnatal levels of BE are elevated in neonates of opiate exposed mothers. We have reported that CNS BE alters basal ODC activity as well as its responsivity to trophic factors in liver and heart. ODC is the rate-limiting enzyme in the synthesis of the polyamines which regulate nucleic acid and protein syntheses. The present study examined the effects of postnatal BE on basal lung ODC activity and lung DNA synthesis as well as lung ODC responsivity to insulin and isoproterenol.

Central BE markedly lowered lung ODC activity in the neonatal rat. ODC inhibition had clear developmental consequences as indicated by significant reductions in DNA synthesis. In addition, BE treatment suppressed lung ODC responsivity to insulin and isoproterenol in an age-selective manner. These findings strongly support a modulatory role for CNS BE in neonatal lung development. (Supported by grants: NIH R01-NS25738 and NIH 5R01-MH13688)

NEUROENDOCRINE CONTROLS: PITUITARY III

256.1

EVIDENCE FOR INHIBIN β-CHAIN LIKE-PEPTIDE MEDIATION OF SUCKLING-INDUCED OXYTOCIN SECRETION. P.M. Plotsky, P.E. Sawchenko, and W. Vale. The Salk Institute, La Jolla, CA, 92037.

The recently identified inhibin-β-subunit immunopositive projection, ascending from the nucleus of the solitary tract (NTS) to oxytocin-immunoreactive (irOT) magnocellular perikarya of the paraventricular (mPVN) and supraoptic (SON) nuclei, may represent an important regulatory pathway for parturition- and suckling-induced irOT secretion. This hypothesis was tested in several rat models. First, we were able to demonstrate a 2.0-fold ($p < 0.001$) elevation of irOT in the systemic circulation following bilateral microinfusion of inhibin β-chain homodimer into the mPVN of urethane-anesthetized male rats (36 fmoles/1 µl per side infused over 15 min). Conversely, infusion of carrier or inhibin α-subunit peptide was without effect. Second, activation of endogenous inhibin β-subunit containing pathways via electrical stimulation of the NTS (100 µA, 1 msec duration paired pulses, 1 Hz) in intact or 6-OHDA treated (2 µg/1 µl bilaterally into mPVN 5 days earlier) rats also resulted in elevation of systemic irOT levels. Finally, bilateral mPVN microinfusion of antiserum to the inhibin β_A-subunit attenuated both suckling-induced increases in intramammary pressure and irOT secretion anesthetized dams on day 10-12 of lactation; infusion of carrier was without effect. These observations provide strong support for the hypothesis that the NTS-to-mPVN inhibin β-chain peptide-containing projection represents a physiologically significant afferent system mediating the milk ejection reflex. [Supported by NIH grant DK26741]

256.2

EVIDENCE FOR INVOLVEMENT OF AN ADRENAL CATECHOLAMINE IN THE β-ADRENERGIC INHIBITION OF OXYTOCIN RELEASE IN LACTATING RATS. S.-L. Song*, C.E. Grosvenor, and W.R. Crowley. Departments of Physiology & Biophysics and of Pharmacology, University of Tennessee College of Medicine, Memphis, TN 38163.

Adrenergic systems exert both excitatory (α-adrenoceptors) and inhibitory (β-adrenoceptors) influences on oxytocin (OT) release. Because suckling also releases epinephrine from the adrenal medulla, the present experiments tested whether adrenal catecholamines participate in the adrenergic regulation of OT release during lactation. In two experiments, adrenal demedullation in midlactation did not alter the basal plasma levels of OT, but markedly enhanced the suckling-induced release of OT. OT release in response to suckling in both sham and adrenal demedullated rats was blocked by stimulation of β-adrenergic receptors with isoproterenol. Blockade of β-adrenergic receptors with propranolol prevented the inhibitory action of isoproterenol and when given alone, mimicked the effect of demedullation to enhance suckling-induced OT release. Stimulation of α-adrenergic receptors with phenylephrine did not affect OT release, but blockade of α-receptors with phentolamine blocked release of OT by suckling. These findings support the concept that stimulation of β-adrenergic receptors inhibits OT secretion and suggest that this may be due to an action of an adrenal catecholamine, which may act centrally and/or at the neurohypophysis.

256.3

SUPRAOPTIC VASOPRESSIN CELL EXCITATION FOLLOWING SOLITARY NUCLEUS STIMULATION IS MEDIATED VIA THE A1 NORADRENALINE CELL GROUP. Trevor A. Day and John R. Sibbald*, Department of Physiology, University of Otago Medical School, Dunedin, New Zealand.

Nucleus tractus solitarius (NTS) stimulation excites supraoptic (SON) vasopressin (AVP) cells via a catecholaminergic projection. Although a direct NTS input to SON involving A2 noradrenaline (NA) cells exists, this projection is sparse, suggesting the possibility that NTS effects on AVP cells are relayed through the A1 NA cell group. This possibility is consistent with evidence that NTS projects to the A1 region, and that A1 stimulation excites SON AVP cells.

SON cells recorded in pentobarbital anaesthetized rats were classified as AVP-secreting on the basis of activity patterns and responses to baroreceptor activation. Medial NTS stimulation excited 82% (latency 51+/- 1 ms) and A1 region stimulation 77% (38 +/- 1 ms) of putative AVP cells tested (n=83). Most NTS responsive units (90%) were excited by A1 stimulation. Bilateral A1 lesions, or contralateral A1 lesions combined with ipsilateral GABA injection (100 nl, 0.75 M), abolished NTS stimulation effects on AVP cells.

The present data suggest that, despite a direct projection to SON from NTS, visceral afferent information reaching the brain via NTS influences SON AVP cells indirectly, through the A1 NA cell group.

256.4

RESPONSES OF NEUROSECRETORY NEURONS IN THE RAT PARAVENTRICULAR NUCLEUS TO FASTIGIAL NUCLEUS STIMULATION STUDIED BY INTRACELLULAR RECORDINGS. T. Katafuchi* and K. Koizumi. (SPON: C.M. Brooks) Department of Physiology, SUNY, Health Science Center at Brooklyn, Brooklyn NY 11203

It has been reported that the fastigial nucleus of the cerebellum (FN) is involved in the regulation of the sympathetic nervous system and vasopressin release in relation to the orthostatic reflex. However, the influence of the FN on secretion of vasopressin is still controversial. In the present study, responses of neurons in the paraventricular nucleus (PVN) of the hypothalamus to electrical stimulation of the FN were examined by extracellular and intracellular recordings in anesthetized rats. About one half of spontaneously firing PVN neurosecretory cells identified by the pituitary stalk stimulation responded to contralateral FN stimulation (0.1-0.3mA, 0.2 ms, 1Hz), of which 70% were inhibited after a short latency. Intracellular recordings of neurosecretory neurons in the PVN showed that the membrane resistance was about 30 MΩ and FN stimulation evoked IPSPs. Most of them were considered to be monosynaptically evoked, since their latencies were constant when FN stimulus intensity was changed. Polysynaptic IPSPs and EPSPs were also observed. These results suggest close functional connections between the PVN and the FN which may contribute to hypothalamic modulation of autonomic and neuroendocrine systems.
(Supported by NIH, USPHS NS- 00847)

256.5

HYPERTONIC SALINE IS AN EFFECTIVE STIMULUS FOR ALTERING SPINAL CORD OXYTOCIN AND VASOPRESSIN IN SHR. D. Lukic*, J. Vrba* and J. Haldar Department of Biological Sciences, St. John's University, Jamaica, NY 11439

Recently we have demonstrated that immobilization stress (Brain Res. In Press) as well as hypertonic saline stress (Neurosc. Abst. #186.4, 1987) alter Oxytocin (OT) and Vasopressin (VP) content of the spinal cord in Long Evans (LE) rats. Since spontaneously hypertensive rats (SHR) are known to be particularly sensitive to some stressors, this study was conducted with three groups of 12 weeks old male SHR. Rats were decapitated 15 mins. after an ip injection of either isotonic (0.85%, 20ml/kg) or hypertonic saline (1M, 20ml/kg). Untreated rats served as control. OT and VP content of cervical, thoracic, and lumbosacral cord segments of all three groups of rats were determined by RIA in sep-pak extracted sample. Our results demonstrate 1) hypertonic saline increases OT content but decreases VP content of the spinal cord 2) isotonic saline decreases primarily VP content of the spinal cord. 3) changes observed for either hormone are similar throughout the cord 4) saline-induced effect is more pronounced in the SHR than in LE. In conclusion, these data suggest that extrahypothalamic neuropeptide of SHR respond differently to stress.

256.6

VASOPRESSIN AND OXYTOCIN RELEASE IN RESPONSE TO HYPOTONICITY. C.Yagil* and C. D. Sladek. University of Rochester School of Medicine, Rochester, NY 14642.

Vasopressin (VP) release in vivo and from cultured explants of the hypothalamo- neurohypophyseal system (HNS) is inhibited by decreases in osmolality (OSM). In the current study, we utilized HNS explants to examine the time course of the response to a decrease in OSM and the effect of the rate of decrease on VP and oxytocin (OT) release. HNS explants were perifused at 2.1ml/hr. Basal OSM was 301-306mosm/kg H20. A 15mOsm decrease in OSM was achieved by decreasing the concentration of NaCl in the medium over a 2 hour period (2% rate of decrease) or a 1 hour period (5% rate of decrease). Both 2% and 5% rates of decrease in OSM significantly inhibited VP release (75±8% and 88±4% of basal respectively, $p<0.025$; n=4 and n=7 resp.) and OT release (66±10% and 89±4% of basal resp., $p<0.05$). During the 2% rate of decrease, inhibition of VP and OT release was evident 30-40 minutes after initiation of the decrease when OSM was reduced by 4-5mOsm. During the 5% rate of decrease, a significant reduction in VP and OT release was observed in the first 10-20 minutes of hypotonicity corresponding to a 3-5 mOsm reduction in OSM. These data do not provide evidence of rate sensitivity in the inhibition of VP and OT release by a decrease in OSM, in contrast to our previous demonstration that the response to an increase in OSM is rate sensitive. During both the 2% and the 5% rate of decrease, inhibition of hormone release was present only during the portion of the pulse which corresponded to 10 mosm reduction in OSM. This was followed by an increase in VP and OT release which exceeded basal release (2%: 131±4% of VP basal release, $p<0.01$ and 169±8% of OT basal,$p<0.025$. 5%: 127±14 of VP, $p<0.025$ and 151±25%, $p<0.05$ of OT). This rebound phenomenon was previously observed when OSM was returned to basal levels after a period of hypertonicity. The mechanisms underlying the rebound hormone release remain to be elucidated. Supported by NIH RO1 DK 19761.

256.7

OXYTOCIN IS NATRIURETIC AT PHYSIOLOGICAL PLASMA CONCENTRATIONS. J.G. Verbalis, M. Mangione* and E. M. Stricker. Departments of Medicine and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

Oxytocin (OT) is known to stimulate natriuresis at high plasma levels. We examined the potential physiological effects of lower plasma OT levels by infusing graded doses of OT subcutaneously in adult male rats maintained on sodium-deficient (NaD) diet. Our results demonstrated a clear dose-related increase in urinary Na+ excretion during the initial day of OT infusion (**p< 0.01 compared to 0 dose):

OT Infusion Rate (mU/h)	Plasma OT (uU/ml)	Urinary Na+ (uEq/24h)
0 (n = 14)	2.1 ± 0.3	195 ± 21
1.0 (n = 15)	2.7 ± 0.7	194 ± 21
5.0 (n = 12)	6.4 ± 0.9**	618 ± 100**
10.0 (n = 20)	6.7 ± 0.9**	927 ± 86**
50.0 (n = 11)	39.5 ± 5.3**	1102 ± 100**

The minimal plasma OT levels associated with significant increases in natriuresis (5-10 uU/ml) were well within the range of physiological OT secretion in rats in response to stimuli such as dehydration and food intake. However, the OT-induced natriuresis was not sustained during subsequent days on NaD diet, suggesting that this effect can be overridden by opposing antinatriuretic factors such as aldosterone. These results are consistent with the hypothesis that peripherally and centrally secreted OT act in concert in rats to promote sodium excretion and to inhibit sodium appetite.

256.8

REGIONAL ANALYSIS OF VASOPRESSIN mRNA BY IN SITU HYBRIDIZATION IN WATER DEPRIVED RATS **R.B. Meeker, R.S. Greenwood and J.N. Hayward, Dept of Neurology and Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599**

A method was developed for semi-quantitative regional and single cell analysis of vasopressin (VP) mRNA transcription in the magnocellular neuroendocrine system in response to water deprivation. A 26-mer oligonucleotide probe to VP mRNA was 3' end labeled to a specific activity of 1-3 X 10^7 CPM/ug with ^{125}I-dCTP using deoxynucleotide terminal transferase. The probe was hybridized to 20 um sections of 4% paraformaldehyde perfused rat brain. These sections were then coated with NTB-2 emulsion, exposed for 2-5 days at 4° C, developed and counterstained with acridine orange. Silver grain densities were analyzed over fluorescent cells utilizing an image analysis system (Bioquant IV). Each magnocellular neuroendocrine region showed an increase in probe hybridization in response to three days of water deprivation. The increase in hybridized probe within the supraoptic nucleus (SON) was twice that of the paraventricular nucleus (PVN) and 56% greater than the nucleus circularis complex (NC). Analysis of single cells within each of these regions, however, revealed substantial heterogeneity in the hybridization signal. Labeled cells within the SON fell within a single population, whereas, two populations of cells were observed within the PVN and NC. This diversity of mRNA expression may represent different functional categories of vasopressin magnocellular neurons.

Supported by Javits Award NS-13411.

256.9

INHIBITORY EFFECTS OF κ-AGONISTS ON SUPRAOPTIC NEURONS IN HYPOTHALAMIC SLICE PREPARATIONS. H. Yamashita[1], K. Inenaga[1*], H. Kannan[1], S. Uesugi[1*], K. Nakao[2*] and H. Imura[2*]. [1]Dept. of Physiol., Occup. & Environ. Health, Sch. of Med., Kitakyushu & [2]Dept. of Med., Univ. of Kyoto, Kyoto Japan

Existence of opioide peptides in the hypothalamic supraoptic nucleus(SON) suggests functional importance on the neural activity. Recently it has been observed that a κ-agonist, Leumorphin (LM), administered cerebroventricularly inhibited release of vasopressin in the plasma. This inhibitory mechanism, however, is not known. To clarify the mechanism, intra and extracellular recordings were made from rat SON neurons in the slice preparations. LM decreased the spontaneous neural activities in a half of both phasic and non-phasic SON neurons with dose-related manner. The threshold was about 10^{-8}M. Another κ-agonist, dynorphin inhibited both phasic and non-phasic neurons as LM did. The κ-agonist-induced inhibition was suppressed by κ-antagonist, MR2266. LM decreased excitatory post-synaptic potentials with membrane hyperpolarization and a small increase of membrane resistance. Morphine, μ-agonist, and DADLE, δ-agonist, inhibited non-phasic neurons more preferentially than phasic neurons. From these results, we suggest that κ-agonists inhibit presynaptically the neural activities of both vasopressin and oxytocin secreting neurons.

256.10

INTRAVENOUS VASOPRESSIN INFUSION INCREASES PLASMA CORTISOL CONCENTRATION IN DEXAMETHASONE-TREATED, CONSCIOUS DOGS. V.L. Brooks* and L.J. Blakemore*. (SPON: L. Pubols) Dept. of Physiology, Oregon Hlth Sci Univ., Portland, OR 97201.

Although vasopressin (AVP) is considered a major stimulator of ACTH secretion, we recently found that AVP infusion decreases plasma ACTH concentration (ACTH), and only transiently increases plasma cortisol concentration (CS) in dogs. To determine if AVP causes an early undetected increase in ACTH that mediates the initial increase in CS, dogs (n=5) were pretreated with dexamethasone (DEX; 4 mg S.C.), to inhibit endogenous ACTH release, 135 min before the start of a 90 min AVP (1 $ng \cdot kg^{-1} \cdot min^{-1}$) infusion. In DEX-treated dogs, AVP infusion did not increase CS (5 ± 1 to 4 ± 1 ng/ml; $p > 0.10$), suggesting that ACTH is necessary for the CS rise. ACTH can enhance the direct adrenal effect of factors that increase CS. Therefore, it was then determined whether AVP increases CS in dogs that were pretreated with DEX and a dose of α1-24 ACTH (0.3 $ng \cdot kg^{-1} \cdot min^{-1}$) that clamped ACTH and CS at basal levels (DEX+0.3 ACTH), or a dose of ACTH (0.5 $ng \cdot kg^{-1} \cdot min^{-1}$) that elevated ACTH and CS (DEX+0.5 ACTH). In DEX+0.3 ACTH dogs, AVP infusion produced a sustained increase in CS from 22 ± 3 to 49 ± 8 ng/ml ($p < 0.001$). In DEX+0.5 ACTH dogs, AVP infusion produced a similar increase in CS (from 32 ± 6 to 61 ± 10 ng/ml; $p < 0.001$). These results suggest that AVP can increase CS independently of an increase in ACTH, but that normal basal ACTH is required for this effect. Supported by UPS HL35872 and AHA, OR affiliate.

256.11

SUCKLING DOES NOT AFFECT GASTRIC MOTILITY IN RATS. D.L. Helmreich, E. Thiels, J.G. Verbalis, E.M. Stricker. Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

After rats are given LiCl or cholecystokinin (CCK) ip, pituitary oxytocin (OT) secretion (reflecting magnocellular activity in hypothalamic paraventricular nucleus (PVN)) is highly correlated with decreases in gastric motility (reflecting parvocellular PVN activity). We now report the effects on gastric motility of suckling, another known stimulus of OT secretion. Gastric motility, measured in 11 lactating rats 8-14 days postpartum, was 94 ± 14% (M ± SE) of baseline values when the pups were attached but no milk ejections occurred, and 97 ± 13% of baseline values during the milk ejections. Gastric motility during suckling and milk ejection also was not altered by pretreatment with naloxone (2.5 mg/kg), which enhances OT secretion and inhibits gastric motility in rats after injection of LiCl or CCK (Flanagan et al., Soc. Neurosci. Abstr., 1988). Moreover, suckling and milk ejection did not influence the decrease in gastric motility caused by CCK in rats. These results demonstrate that suckling does not affect gastric motility in rats, and thus it appears to be a stimulus that selectively activates magnocellular rather than parvocellular neurons in PVN. (Supported by Research Grant MH-25140.)

256.12

EFFECTS OF VASOPRESSIN OR OCYTOCIN ANTISERUM ON ANTERIOR PITUITARY HORMONE SECRETION IN THE RAT.

C.F. Franci*, J. Anselmo-Franci*, G. P. Kozlowski and S.M. McCann, Department of Physiology, UT Southwestern Medical Center, Dallas, Texas 75235 and *Department of Physiology, Medical School of Ribeirao Preto-USP, 14049-Riberao Preto, SP-Brazil.

Adult female Sprague-Dawley rats were used for experiments 5 weeks after ovariectomy. Stainless steel cannulae were implanted into the third ventricle and silastic cannulae were introduced into the external jugular 1 week and 24 hours before the experiment, respectively. Blood samples were collected immediately before (time 0) and 15,30,60,120 and 180 min or 1,2,3,4,5,6 and 24 hours (h) after intracerebroventricular microinjection (i.c.v.m.) of normal rabbit serum (NRS), vasopressin antiserum (AB-VP) or oxytocin antiserum (AB-OT). There was no difference in the plasma LH, FSH, PRL and TSH between control groups (NRS) and groups submitted to the i.c.v.m. of AB-VP or AB-OT. Plasma GH was not changed with i.c.v.m. of AB-VP but increased significantly by 1-24 h after i.c.v.m. of AB-OT. Thus, endogenous oxytocin (OT) may play a role in the control of GH release by stimulating somatostatin secretion or inhibiting CRF secretion or by both actions. Surprisingly, a previous study showed that i.c.v.m. of OT increased GH release. However, the i.c.v.m. of OT could have blocked the action of endogenous OT by ultra short loop negative feedback. Elimination of ultra short loop feedback of OT by the AB-OT could explain why i.c.v.m. of AB-OT could have the same effects on GH secretion as i.c.v.m. of OT.

256.13

ULTRA-RAPID FREEZE TECHNIQUES ACHIEVE SUPERIOR ULTRASTRUCTURAL PRESERVATION AND SPECIFIC IMMUNOGOLD LABELING IN RAT NEURAL LOBE. W.E. Armstrong, M. Tian* and J. Reger* (SPON: R. Stiles). Dept. of Anat. Neurobiol., Univ. Tenn., Memphis, The Health Science Center, Memphis, TN 38163.

Ultra-rapid freeze fixation provides a theoretical means of more accurately preserving molecular structure. With the eventual goal of describing relatively fast morphological events which might accompany secretion of vasopressin and oxytocin, we rapidly froze freshly dissected neural lobes on a Med-Vac device, then freeze-substituted with acetone and osmium prior to plastic embedding and conventional electron microscopy. Post-embedding immunogold techniques were used to localize neurophysin (antibody kindly provided by A. Robinson).

Excellent ultrastructural preservation was consistently observed from the point of initial freezing to a depth of 15 μm. Neurosecretory terminals and their associated dense core granules, clear vesicles, mitochondria, microtubules and other tubular structures were clearly resolved. In thinner sections, immunogold labeling of dense core granules for neurophysin was achieved without etching the plastic or removing the osmium, thus allowing retention of well preserved ultrastructure. As ultra-rapid freeze fixation of neural lobes is useful in describing normal ultrastructure and hormone distribution, it offers a preferred means of identifying morphological changes associated with neurosecretory events.

Supported by NIH grant #NS23941 (WEA).

256.14

GALANIN CONCENTRATIONS IN THE MEDIAN EMINENCE (ME) AND ANTERIOR PITUITARY ARE INFLUENCED BY THYROID STATUS. S.C. Hooi* J.I. Koenig, S.M. Gabriel and J.B. Martin. Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Galanin (GAL) is a 29 amino acid peptide implicated in neuroendocrine regulation. In order to determine if GAL has a role in the regulation of TSH secretion, we studied the effects of both chemical and surgical thyroidectomy on GAL concentrations in the hypothalamus and anterior pituitary (AP). Adult male rats were thyroidectomised either chemically using 5-propyl-2-thiouracil (PTU) or surgically. T4 replacement in PTU treatment and T4 treatment in control rats were achieved by daily sc T4 injections for 3 weeks. PTU treatment significantly decreased GAL concentration 39% in the ME and 67% in the AP. The effects of PTU treatment in both regions were reversed by daily T4 injections. However, T4 injections alone did not influence GAL concentrations in control animals. Similarly, surgical thyroidectomy significantly decreased GAL concentrations in the ME and AP by 54% and 64% respectively. No significant changes were seen in the rest of the hypothalamus. This study demonstrates that GAL concentrations in the ME and AP are responsive to changes in thyroid status of the animal and suggests that GAL may be involved in thyroid hormone regulation.

256.15

NEUROENDOCRINE REGULATION OF GALANIN IN RAT HYPOTHALAMUS AND PITUITARY. S.M. Gabriel, J.I. Koenig, S.C. Hooi*, D.M. Maiter*, L.M. Kaplan, J.B. Martin. Departments of Neurology and Medicine, Massachusetts General Hospital, Boston MA 02114. Galanin is a 29-amino acid peptide present in brain and peripheral tissues. We have studied galanin-like immunoreactivity (Gal-IR) in the hypothalamus and pituitary gland under a variety of biologic circumstances by radioimmunoassay. Gal-IR is affected in a tissue-specific manner by several endocrine and autonomic stimuli. Anterior pituitary (AP) gland Gal-IR is modulated by reproductive endocrine treatments and is under the potent stimulatory influence of estrogen. Thyroid hormone and adrenal steroids are also necessary for the maintenance of normal AP levels of Gal-IR. High concentrations of prolactin and GH in plasma exert an inhibitory influence on AP intrinsic Gal-IR. Two nerve terminal beds, the median eminence and the neurointermediate lobe, contain high concentrations of Gal-IR. In these sites, Gal-IR is under the permissive influence of thyroid hormone. In addition, these tissues respond provocatively to alterations in fluid balance. These studies indicate that multiple neuroendocrine inputs influence Gal-IR in the hypothalamus and pituitary gland.

256.16

NEONATAL MONOSODIUM GLUTAMATE (MSG) TREATMENT CAUSES A CHRONIC REDUCTION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) LEVELS IN THE ANTERIOR PITUITARY OF RATS. P.N. Riskind, S.C. Hooi*, J. I. Koenig and J. Audet-Arnold*. Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

VIP is a putative prolactin (PRL)-releasing factor which is found in high concentration in both the hypothalamus and anterior pituitary gland. It has not been clearly established whether pituitary VIP is synthesized locally within the gland or sequestered from the portal blood after hypothalamic secretion. As a first step in evaluating the hypothalamic contribution to pituitary VIP *in situ* we have evaluated the effect of chronic neurotoxic lesions of the hypothalamus with MSG. This treatment is known to result in an 80-90% loss of neurons in the arcuate nucleus of the hypothalamus without damage to axons of passage. In adult male rats, MSG-treatment reduced anterior pituitary VIP content from 178 $\pm$ 45 pg/gland to less than 20 pg/gland, and decreased VIP concentration from 220 $\pm$ 52 pg/mg protein to less than 90 pg/mg protein, but did not change medial basal hypothalamic VIP content. These results indicate that the hypothalamus normally has a stimulatory effect upon anterior pituitary VIP levels, and suggest that releasing factors or neurotransmitters contained in the arcuate nucleus may participate in this phenomenon. It is not possible to exclude a hypothalamic source of pituitary VIP from these studies.

256.17

CO-LOCALIZATION OF TYROSINE HYDROXLASE (TH) AND SEROTONIN (5-HT) IMMUNOREACTIVITY IN RAT PITUITARY INNERVATION. L.C. Saland, J.A. Wallace, A. Samora*, L. Gutierrez* and M. Williams*. Dept. of Anatomy, Univ. of New Mexico Sch. Medicine, Albuquerque. NM 87131.

Fibers containing catecholamines, 5-HT, gamma aminobutyric acid (GABA) as well as a variety of peptides have been demonstrated within the neural and intermediate lobes with immunostaining methods. Recently, GABA and TH have been co-localized with EM in pituitary nerves. We have compared fiber staining patterns in neurointermediate lobes (NILS) for TH and 5-HT on adjacent sections, which suggested that both substances might co-exist in specific fiber locations (Saland et al, '87, Soc. Neurosci. Abst. 13:418). Here, we demonstrate co-localization of TH and 5-HT fibers, using immunofluorescence. Adult male Sprague-Dawley rats were ether anesthetized, perfused with buffered 4% paraformaldehyde, and the pituitaries prepared for paraffin sectioning. Sections were consecutively incubated with sheep anti-TH, donkey anti-sheep IgG-FITC, then rabbit anti-5-HT and goat anti-rabbit IgG-TRITC. Co-localization of staining was observed in areas of both lobes, especially along border zones. No staining was observed if the inappropriate 2° fluorescent antibody (Ab) was matched with either 1° Ab alone, or if the 1° Ab was omitted. These findings suggest that multiple classic neurotransmitters co-exist in nerve fibers to regulate NIL endocrine secretion. Supported by NIH NS 21256, RR 08139 (LS) and NSF BNS-8511079 (JW).

256.18

NEUROPEPTIDE Y POTENTIATES EXCITATORY ACTIONS OF NORADRENALINE ON SUPRAOPTIC NEUROSECRETORY CELLS. B.K.J. Wilson*, J.R. Sibbald* and T.A. Day (SPON: R.J. Harvey), Department of Physiology, University of Otago Medical School, Dunedin, New Zealand.

Supraoptic (SON) vasopressin (AVP) cells receive an excitatory input from the A1 noradrenaline (NA) cell group. In addition to NA, however, A1 neurons also contain neuropeptide Y (NPY), and application of either substance excites SON AVP cells examined in vivo. In the present experiments we have used an in vitro preparation in order to more accurately determine the dose responsiveness of SON cells to NA and NPY, and to examine possible interactions between these two substances.

Using rat hypothalamic slices, extracellular recordings were obtained from spontaneously active SON cells. Bath application of NA (10^{-6} - 10^{-3} M) elicited dose dependent increases in cell discharge, phasically discharging units displaying marginally greater responsiveness than non-phasic units. Bath applications of NPY ($10^{-7}$and 10^{-6}M) also increased SON cell discharge rates and additionally produced a 40-60% increase in the excitatory effects of low concentrations of NA (10^{-5}M).

These data are consistent with the possibility that NPY released from A1 NA cells acts on SON AVP cells to both mimic and potentiate the actions of NA.

256.19

DISTRIBUTION OF LAMININ IN THE MURINE PITUITARY GLAND. R.F. Payette*, M.D. Gershon, E.A. Nunez* (SPON: C. Noback). Dept. of Anatomy and Cell Biology, Columbia Univ., College of P & S, New York, N.Y. 10032.

Previous studies have demonstrated immunological heterogeneity among the secretory granules of gonadotrophs. All adult male gonadotroph granules contain luteinizing (β-LH) hormone, but only 43% of the granules contain follicle stimulating hormone (β-FSH) and 9% have serotonin (5-HT) immunoreactivity. Moreover, β-FSH and 5-HT immunoreactivities do not co-exist in the same secretory granule (Anat. Rec. 219: 1987). In the present study, the localization of the extracellular matrix glycoprotein, laminin, was investigated in the adult murine pituitary gland and compared with that of 5-HT. EM immunocytochemistry was employed with 3 different laminin antisera, applied to aldehyde-fixed sections embedded in L.R. White. Immunoblots confirmed that laminin was the only immunoreactive protein in the adult murine pituitary gland. Sites of binding of primary antisera to laminin were identified with affinity-purified secondary antisera coupled to 20 nm colloidal gold particles. The patterns of immunostaining by the 3 antisera raised against laminin were compared and found to be identical. Laminin immunoreactivity was found extracellularly only in formed basal laminae in all three lobes of the pituitary. Laminin immunoreactivity was found intracellularly in gonadotrophs but in no other endocrine or non-endocrine cells of the anterior lobe. Within gonadotrophs, only secretory granules were labeled. The majority of secretory granules (64%; n=1089) contained laminin immunoreactivity in all gonadotrophs examined. None of the laminin-immunoreactive granules of gonadotrophs contained 5-HT, and none of the 5-HT-immunoreactive granules contained laminin. No intracellular laminin immunoreactivity was detected in the intermediate or neural lobes of the pituitary. These observations confirm that the secretory granules of gonadotrophs are heterogeneous. All granules contain β-LH, but a subset also contains laminin and another non-overlapping subset also contains 5-HT. Supported by NIH grants AM19743 and NS12969.

256.20

COMPUTER 3D RECONSTRUCTION OF MEDIAN EMINENCE MICRO-CAPILLARIES. L.S. Hibbard, B.J. Dovey-Hartman* and R.B. Page. The Pennsylvania State University College of Medicine, Hershey, PA 17033.

The microvasculature of the median eminence (ME) of the hypothalamus modulates communication between the brain and the pituitary. To understand the structural details of that functional relationship, we have developed computer 3D reconstruction (3DR) methods to observe microcapillary modules and attendant cellular detail from transmission electron micrographs (TEM).

Currently, a survey 3DR is underway to map capillaries in a block of ME tissue from low magnification (100X) TEM images. New feature extraction and alignment techniques have been developed to supplement those reported previously (Hibbard, et al., Comput. Biol. Med., 16:411, 1986). Capillary lumen edges are extracted by algorithms based on pixel neighborhood gray level variance, and the images were aligned by alternate stages of translation and rotation refinement using Fourier crosscorrelation (Hibbard, et al., Science, 236:1641, 1987). This low magnification 3DR will survey the largest capillaries in a 100-micron tissue block to guide the choice of features to be re-imaged at higher magnification (1650X) in the same tissue sections. The high magnification 3DR will seek to place cellular details onto a capillary network mapped at low magnification. (Support: NSF BNS-8506479 and NIH NS15926.)

257.1

PREPARATION OF PURIFIED BASAL FOREBRAIN CHOLINERGIC NEURONS FROM THE DEVELOPING RAT. A. Fine and P.J. Richardson*. Dept. Physiology & Biophysics, Dalhousie Univ. Med. School, Halifax, NS, Canada B3H 4H7, and *Dept. Clin. Biochemistry, Univ. Cambridge Clinical School, Cambridge U.K.

The extrinsic cholinergic innervation of much of the neocortex and hippocampus arises from cell bodies in several basal forebrain nuclei. Transplantion and tisssue culture of "cholinergic" neurons from these heterogeneous regions have inevitably been impure and problematic; for such studies, it would be useful to be able to purify the cholinergic cells. We have developed gentle immunoaffinity methods for purification of cholinergic neurons from developing rat brain, using solid-phase immunoadsorbants and cholinergic cell surface-specific antibodies. These methods are effective, simple, inexpensive, and less damaging than FACS methods. Using polyclonal sheep antiserum to the cholinergic cell membrane-specific ganglioside Chol-I, with an anti-sheep IgG-derivatized cellulose immunoadsorbant, we have achieved 20- to 25-fold enrichment (as determined by ChAT/LDH and AChE/LDH enzyme activity ratios) of viable cholinergic cells, dissociated by papain proteolysis from the basal forebrain of 20-35 day old rats, with yields of >100,000 cholinergic cells, representing approximately 1% of the total initial cells. Using monoclonal mouse antibodies to NGF receptor with anti-mouse IgG-derivatized magnetizable microspheres, we have achieved 6-fold enrichment of viable cholinergic cells from 18-day rat fetuses. These methods should be useful for purification of other cell types by substitution of appropriate primary antibodies. Transplantation studies and studies of gene expression using these purified cells are underway.

257.2

EXOGENOUS CHOLINE INDEPENDENT AND INTRACELLULAR CHLORIDE DEPENDENT ACETYLCHOLINE SYNTHESIS IN THE RAT BASAL FOREBRAIN SLICES. T. Suzuki* and K. Kawashima. Department of Pharmacology, Kyoritsu College of Pharmacy, Tokyo 105, Japan.

Acetylcholine (ACh) content of basal forebrain slices (including the basal nucleus of Meynert) of the male Wistar rat (8- to 10-weeks-old) under various incubation conditions was determined by radioimmunoassay. ACh content of slices incubated with artificial cerebrospinal fluid (ACSF) was elevated significantly during incubation with time and reached to the plateau level by 60 min incubation. The increase in the ACh content was not affected by the exogenous choline supply. After preincubation with high-K^+ ACSF (30 mM, 30 min) which depletes releasable ACh and choline in the nerve, subsequent incubation with and without exogenous choline supply caused the same degree of increase in ACh content. Incubation with low-Cl^- ACSF (4.8 mM Cl^-, 142 mM I^- or CH_3COO^-) did not affect the ACh synthesis. However, preincubation with high-K^+ and low-Cl^- ACSF diminished subsequent increase in ACh content. These findings suggest that some portion of the ACh synthesis is independent on extracellular choline, but is dependent on intracellular Cl^-.

257.3

RELEASE OF ENDOGENOUS ACETYLCHOLINE (ACh) FROM THE RAT MEDIAL SEPTUM/VERTICAL LIMB (ms/vdB) OF THE DIAGONAL BAND. R.H. Metcalf*, R.J. Boegman, R.J. Riopelle & S.K. Ludwin. Departments of Pharmacology and Toxicology, Medicine, and Pathology, Queen's University, Kingston, Ontario, K7L 3N6.

In the present study, K^+-stimulated release of endogenous ACh from tissue slices prepared from the ms/vdB of adult rat brain was determined by gas chromatography - mass spectrometry (GC-MS). Choline acetyltransferase(ChAT) activity in the ms/vdB region was 150.21 ± 11.25 nmoles ACh formed/mg protein/hr (n=5). Multipolar neurons approximately 20-30 μm in diameter stained intensely with ChAT monoclonal antibody. The basal release of ACh in the ms/vdB was 0.14 ± 0.02 nmoles/g wet wt/min on the right side and 0.16 ± 0.03 nmoles/g wet wt/min on the left side. Upon 35 mM K^+ depolarization, this release increased to 3.73 ± 0.19 nmoles/g wet wt/min on the right side and 3.83 ± 0.16 nmoles/g wet wt/min on the left side. Neither basal nor K^+-induced ACh release differed significantly between hemispheres ($p > 0.05$, n=6). Omission of Ca^{2+} resulted in a 91% decrease in ACh release in the presence of 35 mM K^+. ACh release in the presence of 4 μM atropine resulted in a 48.22 ± 4.76 (n=4) increase in release above that seen in the absence of atropine. These results indicate that depolarization-induced ACh release, which is Ca^{2+}-dependent and atropine-sensitive, can be demonstrated in tissue sections prepared from the ms/vdB of rat brain. (Supported by the Medical Research Council of Canada)

257.4

MEDIAL HYPOTHALAMIC AFFERENTS TO FOREBRAIN CHOLINERGIC PROJECTION NEURONS. W.E.Cullinan and L. Zaborszky Neuroscience Program and Dept. of Otolaryngology, Univ. of Virginia School of Medicine, Charlottesville, VA 22908

The distribution of medial hypothalamic projections to forebrain areas containing cholinergic neurons was examined in the rat using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) in combination with choline acetyltransferase (ChAT) immunocytochemistry. For the simultaneous detection of PHA-L fibers and ChAT neurons, the avidin-biotin peroxidase (ABC) technique was utilized with either the nickel-enhanced DAB/DAB (Hsu and Soban,1982) or BDHC/DAB (Levey et al., 1986) method.

Discrete iontophoretic PHA-L injections were delivered to the medial preoptic area, anterior hypothalamic area, paraventricular hypothalamic nucleus, and the ventromedial hypothalamic nucleus. PHA-L labeled fibers and terminals were found in varying amounts approximating ChAT labeled cell bodies and dendrites in the lateral part of the vertical limb of the diagonal band nucleus, medial portion of the horizontal limb of the diagonal band nucleus, bed nucleus of the stria terminalis, sublenticular substantia innominata, peripallidal region, and nucleus ansa lenticularis. The density and distribution of these afferents in relation to the cholinergic projection neurons was dependent upon the location of injected sites. Electron microscopic analyses are underway to determine synaptic contact. Supported by USPHS Grant NS 23945, 17743.

257.5

LAMINAR TERMINATION PATTERN OF EFFERENTS OF THE NUCLEUS BASALIS OF MEYNERT. M.L. Voytko, C.A. Kitt and D.L.Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182.

The major pathways by which neurons in the nucleus basalis of Meynert (nbM) project to targets in the telencephalon were delineated recently using a combination of choline acetyltransferase and autoradiography [Kitt et al., Brain Res. 406:192, 1987]. However, laminar and regional patterns of nbM efferent projections have not been characterized fully. In order to address this issue, electrophysiologically guided, stereotaxically placed injections (1-2.5 μl) of [^{3}H] amino acids were made into the nbM of three rhesus monkeys. Projections to the temporal pole were examined because it is one of the few cortical regions that shares a reciprocal relationship with the nbM. Autoradiographic studies revealed labeled fibers exiting the nbM laterally and entering the white matter of the temporal lobe, within fasicles of the temporal stem, before terminating within superficial cortical layers of the temporal pole. There is heavier labeling along the medial aspect of the temporal pole in one animal, suggesting regional specificity in nbM projections to this cortical region. The distribution pattern of nbM efferents to temporal pole suggests that the nbM may be involved in modifying associative functions of this polysensory cortical area, which has been implicated in learning and memory [Voytko, Behav. Brain Res. 22:25, 1986].

257.6

IMMUNOCYTOCHEMICAL VISUALIZATION OF CHOLINERGIC FIBERS IN MONKEY NEOCORTEX: ENHANCED VISUALIZATION USING SILVER NITRATE. C.A. Kitt, A.I. Levey, D.P. Friedman†, L.C. Walker, V.E. Koliatsos, L.S. Raskin*, and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182 and †Neuropsychology Lab., NIMH, Bethesda, MD 20892 and Div. Preclinical Research, NIDA, Rockville, MD 20857.

Acetylcholinesterase (AChE) has been used to visualize putative cholinergic fibers and cell bodies in the nervous system, but AChE is not a specific cholinergic marker. The development of anti-choline acetyltransferase (ChAT) monoclonal antibodies allowed detection of cholinergic somata in monkey forebrain and brainstem; however, ChAT-positive axons and terminals within cortical and/or subcortical structures have proved difficult to image. To address this issue, rhesus monkeys were anesthetized deeply with sodium pentobarbital and perfused quickly with 2% paraformaldehyde, followed by sucrose in PO_4. Frozen sections were processed for ChAT peroxidase-antiperoxidase immunocytochemistry and intensified with silver nitrate, according to a modification of the Fontana-Masson method [Masson, Am. J. Pathol. 4:181, 1928]. ChAT-positive fibers and cell bodies had a black "Golgi"-like appearance. Within premotor cortex, a distinct laminar pattern of ChAT-positive fibers are seen: layers II and V had dense bands; labeled fibers in layers I and IV were sparse.

257.7

COMPARATIVE LAMINAR DISTRIBUTIONS OF CHOLINERGIC FIBERS AND RECEPTORS IN MONKEY PREMOTOR CORTEX. D.P. Friedman[1,3], A.E. Levey[4], J.B. O'Neill[1], P.B.S. Clarke[2], D.L. Price[4], and C.A. Kitt[4]. Lab Neuropsychology[1] & Biol Psychiatry Section[2], NIMH, Bethesda, MD 20892, Div Preclinical Res[3], NIDA, Rockville, MD 20857, Neuropath Lab[4], Johns Hopkins Medical School, Baltimore, MD 21205.

The distribution of fibers stained for ChAT was compared to the distributions of muscarinic (M) and nicotinic (N) cholinergic receptors in rhesus monkey cortex. Cholinergic fibers were revealed using a monoclonal antibody raised against ChAT as described in the accompanying abstract (Kitt et al). ^{3}H QNB and ^{3}H nicotine were used to reveal M and N binding sites as described previously (O'Neill et al., Soc Neurosci Abs 11:0153, 1985). All animals were deeply anesthetized with barbiturate before perfusion or brain removal.

ChAT positive fibers were seen in all cortical layers but were densest in layer II and the upper part of layer V. They were least dense in layer IIIb. Fibers stained for AChe were similarly distributed. M receptors were distributed in two bands. A dense band was seen in layers I-IIIa and a second, less dense band was seen in layers V-VI. Layer IIIb was largely unlabeled. N receptors were only seen in the lower part of layer IIIa.

Despite the overlap of high densitites of ChAT positivity and M receptors, neither the receptor nor the fiber distributions could predict the other.

257.8

GLUTAMIC ACID DECARBOXYLASE mRNA IN NEURONS OF THE PRIMATE NUCLEUS BASALIS OF MEYNERT. L.C. Walker, D.L. Price, and W.S. Young III§. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182. §Lab. of Cell Biology, NIMH, Bethesda, MD 20892.

The primate basal forebrain magnocellular complex (BFMC), which includes the nucleus basalis of Meynert (nbM) and nucleus of the diagonal band of Broca, has been defined by the presence of large cholinergic neurons. In the rat, a significant population of neurons in the BFMC contain the inhibitory transmitter γ-aminobutyric acid (GABA) as determined by immunocytochemical detection of glutamic acid decarboxylase (GAD) [Brashear et al., Neurosci. 17:439, 1986]. However, GABAergic neurons have not yet been reported in the BFMC of primates. Therefore, we used in situ hybridization to localize putative GAD-producing cells in the nbM of a 21-year old female rhesus monkey (Macaca mulatta). A [^{35}S]-labeled synthetic DNA probe to bases 1982-2029 of human GAD mRNA was hybridized [Young et al., Proc. Natl. Acad. Sci. USA 83:9827, 1986] to 20-µm thick coronal sections through the anterior nbM. Emulsion-coated slides were exposed for four months, developed, and counterstained. Hybridization was present in many small- to medium-sized neurons, as well as some large neurons, scattered throughout the anterior nbM. These data suggest that some neurons of the primate nbM may use GABA as a neurotransmitter. The authors thank Drs. Y. Kobayashi and A.J. Tobin for providing information on the human GAD sequence.

257.9

IBOTENIC ACID LESIONS OF THE SUBSTANTIA INNOMINATA AND MEDIAL SEPTUM RETARD AMYGDALOID KINDLING. D.P. Carey, D.J. Stewart and D.P. Cain, Depts. of Clin. Neuro. Sci. and Psychology, University of Western Ontario, London, Ontario, CANADA.

Cholinergic agonists facilitate and cholinergic antagonists retard but do not prevent the development of amygdaloid kindling. The amygdala receives cholinergic inputs from both the substantia innominata (SI) and medial septum (MS). The role of these inputs in amygdaloid kindling was studied after ibotenic acid lesions of both the SI and MS in the rat.

Combined SI and MS lesions significantly retarded the rate of amygdaloid kindling. Lesioned rats took a mean of 16.7 (plus or minus 2.1) after discharges (ADs) to reach a stage 5 fully kindled seizure. Control rats required 9.9 (plus or minus 0.5) ADs to reach a stage 5 seizure. Cell loss was evident in the SI and MS and acetylcholinesterase staining was decreased relative to controls in the basolateral amygdala of the ibotenic acid treated rats. The data support previous pharmacological studies implicating cholinergic mechanisms in kindling. These data also suggest that the basal forebrain cholinergic system may be involved in the developmemt of amygdaloid kindling.

Supported by a Natural Sciences and Engineering Research Council of Canada grant awarded to D.P. Cain.

257.10

SINGLE NEURONS IN THE MAGNOCELLULAR BASAL FOREBRAIN AND MESOPONTINE TEGMENTUM PROJECT TO BOTH RETICULAR THALAMIC NUCLEUS AND CORTEX: A DOUBLE RETROGRADE STUDY IN THE RAT. A. Jourdain, K. Semba and H.C. Fibiger. Div. Neurol. Sci., Univ. of British Columbia, Vancouver, B.C., Canada, V6T-1W5.

Both magnocellular basal forebrain (MBF) and mesopontine tegmentum (MPT) provide a cholinergic input to the reticular thalamic nucleus (RTN) and cerebral cortex (CX). We have examined the degree to which single neurons in the MBF and MPT have axons that diverge to innervate both the RTN and CX using a double retrograde technique. Each rat received a unilateral injection of fluoro-gold (FG) in the RTN followed by 6-9 ipsilateral cortical injections of propidium iodide (PI) or true blue (TB). The results indicate that the MBF and MPT contain individual neurons that innervate both the RNT and CX. In the MBF, double labeled cells (15-20% of total FG labeled cells) were mainly localized in the caudal part of the MBF, particularly in the nucleus basalis magnocellularis. In the MPT, double labeled cells (10-15% of total FG cells) were primarily observed in the region of the pedunculopontine tegmental nucleus.

The present data may be relevant in light of the proposed role of cholinergic transmission in regulating arousal and sleep in that some MBF and MPT neurons appear to modulate RTN and CX activity simultaneously.

257.11

CHOLINERGIC INNERVATION OF DEEP CEREBELLAR NUCLEI IN RAT ORIGINATES FROM THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS. T.L. Dellovade*, L.J. Martin*, V.E. Koliatsos, and D.L. Price. SPON: (T.C. Hain). Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182.

Although reports suggest that acetylcholine may be a transmitter within the mammalian cerebellum, cholinergic neurons have not been visualized intrinsic to cerebellum, and extrinsic sources of cholinergic innervation have not been identified. Cholinergic projections to cerebellum were examined by combining retrograde tracing with choline acetyltransferase (ChAT) immunocytochemistry. The fluorescent dye Fluoro Gold was injected into deep cerebellar nuclei (DCN) and cerebellar cortex (CC). Retrogradely labeled neurons were identified bilaterally in the pedunculopontine tegmental nucleus (PPN) following DCN but not after CC injections. Texas Red-ChAT immunofluorescence revealed that ca. 40% of the total number of PPN neurons projecting to DCN were cholinergic. Although label was present in cuneate, pontine, reticulotegmental, vestibular, olivary, and red nuclei, forebrain cholinergic groups were not labeled. After the anterograde tracer Phaseolus vulgaris leukoagglutinin was iontophoresed into PPN, terminals were observed in DCN, particularly in lateral and interpositus nuclei. These findings indicate that the PPN, a recipient of basal ganglia efferents, projects to DCN and may interrelate basal ganglia and cerebellar circuits and that the PPN provides a source of cholinergic innervation to thalamus and cerebellum.

257.12

TIME OF ORIGIN OF CHOLINERGIC NEURONS IN THE MESOPONTINE TEGMENTUM OF THE RAT. Kazue Semba and Hans C. Fibiger. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5 Canada.

The neurogenesis of cholinergic neurons in the magnocellular basal forebrain has been shown to occur from embryonic day (E) 12 to 16 in a caudal to rostral manner (Semba & Fibiger,'88). In the present study, the timing of neurogenesis of cholinergic neurons in the mesopontine tegmentum was examined. Timed pregnant rats were injected with tritiated thymidine, and the brains of their progeny as young adults were processed for immunohistochemistry for choline acetyltransferase, serotonin, tyrosine hydroxylase, or dopamine-β-hydroxylase followed by autoradiography. The data indicated that cholinergic neurons in the pedunculopontine tegmental nucleus and the laterodorsal tegmental nucleus become postmitotic mostly on E12 and 13. This paralleled the neurogenesis of serotonergic neurons in the dorsal and median raphe nuclei, whereas the noradrenergic neurons in the locus ceruleus underwent final mitosis about one day earlier, on E11-12. These results indicate earlier completion of cholinergic neurogenesis in the mesopontine tegmentum as compared to the magnocellular basal forebrain, and, in combination with previous studies, suggest the presence of a caudal to rostral gradient in the neurogenesis of cholinergic and aminergic projection neurons in the brain. (Supported by the MRC)

257.13

AFFERENT CONNECTIONS OF THE PEDUNCULOPONTINE AND LATERODORSAL TEGMENTAL NUCLEI IN THE RAT. Hans C. Fibiger and Kazue Semba. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5 Canada.

The cholinergic neurons in the mesopontine tegmentum have been shown to innervate various structures of the forebrain as well as the brainstem. To obtain a better understanding of the organization and function of the mesopontine cholinergic system, afferents to two subregions of the mesopontine tegmentum, i.e., the pedunculopontine (PPT) and the laterodorsal (LDT) tegmental nuclei, were investigated using standard anatomical techniques. The results indicate that both PPT and LDT receive heavy projections from the reticular formation throughout the brainstem, the midbrain central gray, and the lateral hypothalamus. Less heavy projections arise in the central nucleus of the amygdala, somatosensory relay nuclei in the medulla, the dorsal horn of the spinal cord (to the PPT), lateral habenula, and medial prefrontal cortex (to the LDT), as well as the dorsal raphe nucleus and zona incerta (to both PPT and LDT). In addition, bilateral and rostrocaudal interconnections appear to exist between the PPT and LDT. These anatomical data are consistent with the concept that the cholinergic neurons in the mesopontine tegmentum are involved in the regulation of behavioral states and sensorimotor functions. (Supported by the MRC)

257.14

DEVELOPMENT OF BRAIN CHOLINERGIC SYSTEMS. L.L. Butcher, E. Gould*, T.W. Farris, N.J. Woolf, Dept. of Psychology, UCLA, L.A., CA.90024.

Several biochemical reports indicate that brain cholinergic systems differentiate in the postnatal period. To better understand such developmental phenomena, ChAT immunohistochemistry, AChE pharmacohistochemistry and Golgi impregnation were performed on brains from postnatal rat pups. Light microscopic analysis revealed that cholinergic forebrain neurons undergo cell body hypertrophy and subsequent shrinkage during the third and fourth postnatal week, respectively. The number of dendrites and degree of dendritic branching on Golgi-impregnated basal forebrain neurons correlated positively with cell body size. Cholinergic neurons of the pontomesencephalic tegmental complex undergo a similar developmental pattern but at an earlier period than cholinergic neurons of the forebrain. Cholinergic neurons of cranial nerve nuclei do not undergo cell body hypertrophy and shrinkage during postnatal development but, rather, steadily increase in size through the fifth postnatal week. As cell body size increases, numbers of dendrites and degree of dendritic branching also increases. These findings suggest that brain cholinergic neurons undergo patterns of postnatal development specific to certain brain regions yet, for each group, cell body size increase correlates with increased number of dendrites and dendritic branching. Cell body size decreases are accompanied by pruning of dendrites and branches. (NS 10928)

257.15

BASAL FOREBRAIN NEURONS ARE SENSITIVE TO THYROID HORMONES. E. Gould*, T.W. Farris, C. Lee* and L.L. Butcher, Dept. of Psychology, UCLA, L.A., CA 90024.

To determine the effects of thyroid hormones on the development of basal forebrain cholinergic neurons, brains from hyperthyroid, hypothyroid and euthyroid rat pups were examined from the neonatal period through adulthood. Brain tissue from each group of rats was processed for ChAT immunohistochemistry and Golgi impregnation and quantitatively analyzed via light microscopy. Hypothyroid treatment resulted in ChAT+ basal forebrain neurons which were significantly smaller and less immunoreactive than untreated controls. Hypothyroid treatment also revealed Golgi-impregnated basal forebrain neurons with stunted dendrites and significantly less dendritic branching. Hyperthyroid treatment resulted in ChAT+ basal forebrain neurons which undergo cell body hypertrophy at an earlier period than untreated controls. Excess thyroid hormone also resulted in fine filopodia extending off Golgi-impregnated basal forebrain somata and dendrites. These findings suggest that thyroid hormones stimulate cell body hypertrophy and extension of dendritic processes on cholinergic basal forebrain neurons. It is of particular interest that morphological profiles seen in the basal forebrain of hyperthyroid animals were similar to regenerative dendritic changes and increased cell body sizes reported in the same neuronal population of Alzheimer's disease brains (Arendt et al., 1986). (NS 10928)

BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT II

258.1

IDENTIFICATION AND CHARACTERIZATION OF CALCIUM-DEPENDENT, ZINC-INHIBITED METALLOPROTEASES IN NEONATAL AND ADULT RAT BRAIN. R. B. Nelson and R. Siman, Neuroscience Group, The DuPont Company, Wilmington, DE 19898.

We investigated brain protease activities separated on substrate-containing polyacrylamide gels, with particular interest in calcium-sensitive proteases. We have identified three calcium-dependent proteases in rat brain which become active at micromolar and millimolar calcium concentrations. In contrast to the calcium-dependent neutral thiol proteases (calpains), all three activities appear to be metalloproteases on the basis of their inhibitor profiles. The three metalloproteases were designated MP-92, MP-70, and MP-65 on the basis of apparent molecular weights in kilodaltons. MP-70, the major activity detected, required 25-50 μM calcium for activity and was maximally stimulated at 1-5 mM added calcium. It was predominantly soluble and showed much higher activity in 7-day neonate forebrain than adult forebrain. MP-65 was similar to MP-70 in distribution, but had a magnitude greater sensitivity to calcium. MP-92, which had an intermediate calcium-sensitivity, contrasted with MP-65 and MP-70 due to its predominance in particulate fractions of adult brain. All three activities were: 1) inhibited by low levels of zinc (but not magnesium or manganese) even in the presence of 50-fold greater calcium; and 2) detectable in particulate fractions after detergent extraction of membrane, indicating a possible association with cytoskeletal or other structural proteins. The present results identify a new class of calcium-dependent brain proteases of potential importance in calcium-regulated neuronal functions. By analogy with their function in peripheral tissues, these enzymes may alter neuronal morphology by degrading intracellular and extracellular structural proteins.

258.2

EFFECT OF CALCIUM ON GLUCOSE TRANSPORT IN RAT BRAIN GLIAL CELLS. L.M. Roeder*, J.T. Tildon* and I.B. Hopkins*. (SPON: J. Krikorian) Dept. Ped., Univ. of MD Sch. of Med., Baltimore, MD 21201.

The signal transduction systems involved in the regulation of glucose transport in neural cells have not been fully delineated. There is evidence that the glucose transporter is phosphorylated under the influence of a variety of agents. Since calcium (Ca^{++}) plays a role in membrane-associated phosphorylation reactions, experiments were carried out to determine whether Ca^{++} influenced the transport of 2-deoxyglucose (2DG) in glial cells cultured from neonatal rat brain. Cells were maintained in MEM with 10% FBS and the net uptake of ^{3}H-2DG measured as previously described (J. Neurochem. 45:1653, 1985). The rate of transport of 2DG (1mM) in these cells (28-29 days in culture) was stimulated by addition of calcium in a concentration-dependent manner, reaching 62% above control (no Ca^{++}) with 2mM Ca^{++}. The addition of dBcAMP (0.25mM from day 14 to 29) increased the uptake of 2DG under conditions with no added Ca^{++}; addition of Ca^{++} also produced a dose-dependent enhancement in these dBcAMP-treated cells. These results indicate that the glucose transport system in brain glial cells is stimulated by Ca^{++} and this effect is still present after net glucose uptake is increased as a result of dBcAMP treatment. Furthermore, the data are consistent with Ca^{++}-mediated phosphorylation as part of glucose transport regulation in neural cells. (NICHD-16596)

258.3

DEVELOPMENT OF BASAL GANGLIA-SPECIFIC PHOSPHOPROTEINS IN REAGGREGATE CULTURES OF THE MOUSE CAUDATOPUTAMEN. N.L. Rosen, I.A. Shalaby, J.-A. Girault*, A. Horiuchi,* H.H. Hemmings, Jr., and P. Greengard. The Rockefeller University, New York, NY 10021. We have been investigating the ontogeny of the basal ganglia-specific phosphoproteins that are substrates for cyclic AMP-dependent protein kinase: DARPP-32, ARPP-21 and ARPP-16. Measured with a quantitative western blot, these phosphoproteins increased in concentration in the mouse caudatoputamen (CP) during the first three postnatal weeks concurrently with tyrosine hydroxylase [a marker of dopaminergic innervation from the substantia nigra (SN)]. ARPP-19, closely related to ARPP-16 in structure, but more generally distributed in the CNS, did not undergo this ontogenetic increase in the CP. To investigate the influence of innervation from the SN and other regions of the brain, we have grown cells from the fetal mouse CP in reaggregate culture for 4 weeks. In reaggregate culture the phosphoproteins underwent a time dependent increase in concentration that mirrored the in vivo development. Co-culturing CP with SN did not stimulate the synthesis of DARPP-32 or ARPP-16. Co-culturing with cortex, thalamus, or mesencephalic tectum did not stimulate the synthesis of DARPP-32. It appears that the basal ganglia specific phosphoproteins develop in a manner which is independent of the innervation from brain regions extrinsic to the CP. Supported by USPHS NS00988, MH40899, Hereditary Disease Fdn. and the American Parkinson's Disease Assoc.

258.4

DEVELOPMENTAL CHANGES IN NEURONAL CALMODULIN-BINDING PROTEINS J.W. Polli,* R.L. Kincaid,* and M.L. Billingsley. Hershey Medical Center, Penn State Univ. Herhsey, PA, 17033 and Section on Immunology, NIAAA, Rockville, MD 20852.

Developmental changes in calmodulin-dependent phosphodiesterase (PDE), calcineurin (CN) and other rat brain calmodulin binding proteins (CaM-BPs) were analysed. Cytosolic and synaptosomal preparations from embryonic day 6 (E6) through adult exhibited rapid postnatal increases in expression of CaM-BPs. Several CaM-BPs were expressed only during development. One embryonic protein was an isoform of CN which differed from adult CN in molecular weight and isoelectric point. Another embryonic protein was a putative isoform of PDE; it had immunoreactivity against affinity purified anti-PDE but exhibited an Mr of 75 KDa. This PDE isoform was found on day E6, and was absent by postnatal day 7; the 60 KDa "adult" PDE was expressed from E17 onward. Immunocytochemistry indicated developmental expression of this PDE isoform; hippocampal CA3 cells and hypoglossal nucleus exhibited PDE immunoreactivity only in embryonic brain while other regions (neocortex) exhibited no immunoreactivity at this time. Supported by AG-06377

258.5

A NOVEL PROCEDURE TO ILLUSTRATE KINETIC CHARACTERISTICS OF Ca^{2+} UPTAKE BY RAT BRAIN MITOCHONDRIA: DEVELOPMENTAL CHANGES AND REGIONAL DIFFERENCES. J.R. Jensen, G. Lynch and M. Baudry. Dept. of Psychobiology, and Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA 92717.

We have previously reported that spermine, an organic polyamine, stimulates mitochondrial Ca^{2+} uptake by allosterically activating the Ca^{2+} uniporter; cooperativity of uptake is decreased and the affinity for Ca^{2+} is increased in the presence of spermine. Analysis of mitochondrial Ca^{2+} uptake kinetics indicated the cooperativity of uptake is greatest in mitochondria isolated from developing telencephalon, lower in adult telencephalon, and lower still in adult non-telencephalic mitochondria. Correspondingly, spermine stimulation of mitochondrial Ca^{2+} uptake is greatest in developing telencephalon, lower in adult telencephalon and lowest in adult non-telencephalic brain regions.

In the present experiments, isolated brain mitochondria were subjected to repetitive low Ca^{2+} additions, closely spaced in time, and the extramitochondrial Ca^{2+} concentration ($[Ca^{2+}]_e$) measured by a Ca^{2+}-selective electrode. With these procedures, differences in kinetic parameters of Ca^{2+} uptake were markedly more apparent than with traditional methods of assessing mitochondrial Ca^{2+} buffering by utilizing the measurement of $[Ca^{2+}]_e$. The differences found were consistent with the results of the $^{45}Ca^{2+}$ uptake kinetics cited above.

In summary, we present a new method that amplifies the changes in $[Ca^{2+}]_e$ as a result of temporal summation and thus enables the detection of more subtle differences in mitochondrial Ca^{2+} buffering characteristics. Furthermore it might also reflect better the *in situ* properties of mitochondria, as Ca^{2+} is added in a presumably more physiological fashion. We use these methods to illustrate developmental changes and regional differences that suggest important differences in intracellular Ca^{2+} regulation during development and between telencephalic and non-telencephalic brain regions.

Supported by NIA #AG-00538 to G.L. and #NS-18427 to M.B.

258.6

CHANGES IN LAMINAR DISTRIBUTION OF ORNITHINE DECARBOXYLASE DURING DEVELOPMENT OF THE CEREBELLAR CORTEX. L. Schweitzer, I.V. Nadler and T.A. Slotkin. Departments of Anatomy and Pharmacology, Duke University, Durham, NC 27710.

Ornithine decarboxylase (ODC) is the first enzyme in the biosynthesis of the polyamines, which control macromolecular synthesis during cellular development. Polyamines appear to play a critical role in the development of the cerebellar cortex, since postnatal treatment with the specific irreversible ODC inhibitor alpha-difluoromethylornithine (DFMO), arrests cell division and migration in this region. To determine whether the distribution of ODC within the developing cerebellar cortex correlates with specific maturational events, tritiated-DFMO, a specific marker for ODC activity, was localized autoradiographically in 3 to 13 day old rats. The density of autoradiographic grains within the cerebellar cortex as a whole paralleled the postnatal rise and fall of biochemically-determined ODC activity. Superimposed on this pattern was a selective laminar distribution of label which indicated specific association of ODC with cell replication, as shown by preferential labeling of the superficial (mitotic) zone of the external granule cell layer. In addition, ODC activity was temporally associated with regions in which post-mitotic cells were undergoing migration, axonogenesis and dendritic elongation, as shown by the patterns obtained in the deep layers. In contrast, there was no evidence for an association between ODC activity and synaptogenesis, gliogenesis or myelination. These results support the view that the ODC/polyamine system play an important role in both mitotic and post-mitotic events within the nervous system. Supported by HD-09713 and NS-20162.

258.7

VITAMIN K STIMULATION OF BRAIN SULFATIDE SYNTHESIS AND SULFOTRANSFERASE ACTIVITY. Meir Lev and K.S. Sundaram* CUNY Medical School, New York, NY 10031.

We have shown that the synthesis of sphingolipids in brain and other organs of young mice is inhibited by L-cycloserine and warfarin. Warfarin, the vitamin K antagonist, primarily affects sulfatide synthesis. 16 day old mice, treated with Na warfarin for 10 days showed a reduction of c. 20% in 3-ketodihydrosphingosine synthase activity; a reduction of 40% in sulfatide levels ($p<0.005$) whereas levels of other glycosphingolipids and sphingomyelin were minimally affected. To investigate the increase in sulfatide inhibition, cerebroside sulfotransferase (EC 2.8.2.11) activity was assayed and showed a 45% reduction. Aquamephyton administration to warfarin-treated mice resulted in a >50% increase in [^{35}S] incorporation into sulfatides and a recovery in sulfotransferase activity. Mice, fed a normal diet, which were treated with aquamephyton or menadione for 1 week showed a significant increase in brain sulfotransferase activity (70% and 100% respectively). These results demonstrate a major role for vitamin K in the biosynthesis of sulfatides and possibly other sphingolipids in the very young brain. Supported by NSF grant BNS 85-0395.

258.8

ONTOGENY OF RAT BRAIN PCP AND SIGMA RECEPTORS. G.A. Paleos, Z.W. Yang, AND J. C. Byrd. Developmental Neurobiology Program, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Phencyclidine (PCP) binds principally to two CNS receptors - the PCP receptor, and the Sigma receptor. We have investigated the ontogeny of these two receptors in Sprague-Dawley rats using the selective radioligands [^{3}H]TCP and [^{3}H] (+) PPP. PCP receptors are present at prenatal (PrN) day 2 (B_{max} = 0.35 pmol/mg) and increase steadily after birth, reaching adult levels by PoN 28 (B_{max} = 1.82 pmol/mg). In contrast, Sigma receptor levels are highest on PrN day 2 (B_{max} = 2.0 pmol/mg), and decrease to adult levels by PoN 28 (B_{max} = 0.6 pmol/mg). The K_d of [^{3}H]TCP remains constant during development (9 nM), while the K_d of [^{3}H] (+) PPP declines from 49 nM (PoN day 1) to 27 nM (PoN 28). On PoN 6, the pharmacology of the PCP receptor is esentially equivalent to that of the adult (e.g. IC_{50}(+)NANM = 157 nM; (-)NANM = 292 nM). Sigma receptor pharmacology on PoN 6 also appears similar to that of the adult receptor (e.g. IC_{50} (+)NANM = 223 nM; (-) NANM = 2089 nM).

258.9

DIFFERENTIAL EFFECTS OF PERINATAL EXPOSURE TO DIAZEPAM ON THE TWO SUBTYPES OF BDZ RECEPTORS. B.Frieder and V.E.Grimm* Center of Neurosciences and Behavioural Research,The Weizmann Institute, Rehovot,ISRAEL,76100.

Rats were exposed to diazepam in the last prenatal and the first postnatal weeks of life.The drug was given in a dose of 15mg/kg per day in the drinking water of the pregnant and lactating mothers.Binding of H3-flunitrazepam was measured in the presence and in the absence of 1ul of CL-218872 in various brain regions of the offspring at 20 and 90 days of age.The rsults showed that perinatal exposure to DZP did not affect binding to BDZ_2(specific binding non displaced by CL218-872).Binding to BDZ_1 (binding displaced by CL-218872) was affected only in the cerebellum. In females there was a significant decrease(by 20%) in this binding.In males the binding to BDZ_1 was increased by 16%,but this increase was found only at 20 days of age.At 100 days of age, the animals were injected by 5mg/kg of DZP ip.After this injection,the females exposed perinatally to vehicle showed hyperactivity in an open field. This hyperactivity was abolished in the females exposed perinatally to DZP.In males there was an opposite effect,but it was less significant.Time to sedation was not affected by perinatal DZP.

258.10

CYTOSOLIC ESTROGEN RECEPTOR LEVELS IN THE BRAIN OF MALE AND FEMALE FETAL RHESUS MONKEYS. S.A. Sholl and K.L. Kim*. Wisconsin Regional Primate Research Center, Univ. of Wisconsin, Madison, WI 53715.

We have measured estrogen receptor (ER) levels in brain tissue cytosol from Day 100 and Day 160 (posconception) fetal rhesus monkeys (4-5/group). The brain regions which were examined included medial basal hypothalamus (MBH), amygdala (AMG), cerebral cortex (CTX) and cerebellum (CB). Tissues were dissected and homogenized as previously described (S.A.Sholl and S.M. Pomerantz, *Endocrinology* 119:1625,1986). Cytosol was passed through a microcolumn of Lipidex 1000 to remove interfering lipids and incubated with ^{3}H-moxestrol (4 nM) in the presence or absence of 500 nM moxestrol. Incubations were carried out for 24 hr. at 4°C, and free and bound ligand separated by Sephadex LH-20 gel filtration. In one case (Day 160 male fetus), saturation analysis yielded an estimate of apparent K_a of 2.2 x 10^9 M^{-1} and indicated that maximal specific binding was achieved at a ligand concentration of less than 4 nM. There was no sex difference at either stage of development (ANOVA). A significant tissue effect ($P < 0.001$) and an age x tissue interaction ($P=0.005$) were noted, however. These differences could be explained by significantly higher ER levels in the MBH of older fetuses than in any other tissue-age group (4 vs 1-3 fmoles/mg protein). The AMG of younger fetuses also contained significantly higher ER levels than the CB of older ones. These studies revealed that the ER is present during brain development. Thus any estrogens derived from the aromatization of circulating fetal androgens could potentially exert an influence upon brain differentiation, particularly upon the MBH and AMG. The lack of any definitive sex difference suggests that the availability of ER receptor sites is not determined by the hormonal milieu. (Supported by NIH Grant HD-18865 to SAS.)

258.11

ONTOGENETIC PROFILE OF (^{3}H)NITROBENZYLTHIOINOSINE AND (^{3}H) DIPYRIDAMOLE BINDING SITES IN GUINEA PIG BRAIN. J.Deckert, P.F.Morgan, J.L.Daval*, T.Nakajima* and P.J.Marangos. Unit on Neurochemistry, BPB, NIMH, Bethesda, Md. 20892.

The ontogeny of adenosine uptake sites has recently been described in rat brain using (^{3}H)Nitrobenzlthioinosine ((^{3}H)NBI) as a radioligand probe (Geiger, J.D., Brain Res. 436:265, 1987 and Morgan P.F. et al., J.Neurochem. 49:852, 1987). In guinea pig brain heterogeneity of adenosine uptake sites has been proposed based on the additional use of (^{3}H)Dipyridamole ((^{3}H)DPR) as a probe (Deckert, J. et al., Arch. Pharmacol. 335:660, 1987).

A differential ontogenetic profile for (^{3}H)NBI and (^{3}H) DPR binding sites was now observed in guinea pig cerebral cortex. (^{3}H)NBI binding was highest around E50 and decreased by 50% until P28 while (^{3}H)DPR binding continuously increased by 25% from E40 to P28. Accordingly, (^{3}H)NBI labeled as many sites as (^{3}H)DPR at E44 while at P28 (^{3}H)DPR labeled double as many sites and NBI inhibited a greater part of (^{3}H)DPR binding at E44 than at P28.

These findings suggest that the NBI-insensitive part of (^{3}H)DPR binding sites develops later during ontogeny than the NBI-sensitive one and (^{3}H)NBI binding sites themselves in guinea pig cerebral cortex.

258.12

LOCALIZATION OF ADENOSINE AND ADENOSINE A_1 RECEPTORS IN THE DEVELOPING CHICK RETINA AND IN PURIFIED RETINAL CELL CULTURES. (Spon: A.M. Suburo) R. Paes de Carvalho*, K.M. Braas, S.H. Snyder & R. Adler. The Wilmer Institute and Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Neurotransmitters appear to play important roles as developmental signals in the CNS. For example, adenosine (ADO) has been shown to modulate dopamine-induced cAMP increases in the embryonic chick retina via A_1 receptors. The functional significance of this developmentally-regulated interaction cannot be adequately studied in intact tissues, and requires preparations of isolated cells in which these properties are expressed. Purified cultures of chick embryo retinal neurons and photoreceptors appear to have several of the desired features. Thus, although in post-hatched retinas in vivo ADO immunoreactivity was restricted to the ganglion cell layer, in embryonic retinas it is also apparent in inner and outer nuclear layers, but not on the plexiform layers. Similarly, in 5 day cultures ADO was observed in the cell bodies of 30% of the cells among neurons and photoreceptors, but not on nerve fibers. The cultured cells also showed an uptake mechanism for ADO that could be blocked by dipyridamole. Experiments are now in progress to determine whether A_1 receptors, localized in the plexiform layers in vivo, can also be detected in the cultures, which appear as a useful model for studying the role of adenosine in retinal cell differentiation.

258.13

AN ANALYSIS OF CHOLESTEROL AND CEREBROSIDE IN DEVELOPING RAT BRAIN, SPINAL CORD, AND SCIATIC NERVE. N. Mann* and W.E.Edmonston, Jr. Colgate University, Hamilton, NY 13446.

Brains, spinal cords and sciatic nerves of 14, 21, 28 and 35 day old Long Evans rats were assayed for cholesterol and cerebroside content. Brain and spinal cord cholesterol increased significantly from 14 to 21 days, significantly decreased to 28 days, and significantly increased again to 35 days. Sciatic nerve cholesterol also increased significantly from 14 to 21 days, but significantly decreased thereafter. There were no significant changes in brain and spinal cord cerebroside during the period tested, but sciatic nerve cerebroaide significantly decreased after 28 days. The relationship of these measures to CNS regeneration is discussed.

258.14

EXPRESSION OF C-SRC$^+$ mRNA BY GANGLION AND AMACRINE CELLS OF CHICK NEURAL RETINA REVEALED BY IN SITU HYBRIDIZATION. C.A. Ingraham and P.F. Maness. Dept. of Biochem., UNC Sch. of Med., Chapel Hill, NC 27514.

One form of the c-src proto-oncogene product ($pp60^{c-src}$) is expressed in the central nervous system and contains a 6 amino acid insert as a consequence of alternative mRNA splicing. To identify cells expressing this form, we have carried out in situ hybridization in embryonic chick neural retina using an ^{125}I-labeled oligonucleotide probe complementary to the specific sequence. Grains were observed over ganglion and amacrine neuronal cell bodies. Few grains were observed over the ganglion fiber layer, and inner and outer plexiform layers, which contain primarily neuritic processes, or over undifferentiated, proliferating precursors. In control hybridizations with a non-specific oligomer as probe, little signal was detected. Expression of the c-src$^+$ mRNA occurs when these neuronal cells have ceased mitosis and are undergoing terminal differentiation and neurite extension.

258.15

A DEVELOPMENTALLY REGULATED NUCLEAR ANTIGEN IN NEURAL CELLS.
K. Schilling* and Ch. Pilgrim* (SPON: European Neuroscience Association). Abt. Anat. u. Zellbiol. d. Universitaet, D-7900 Ulm, FRG

We investigated expression of a nuclear antigen recognized by a monoclonal antibody (SMI 31) to phosphorylated neurofilaments (NF) in developing brain tissue.

Nuclear staining elicited by SMI 31 could be observed in brain tissue from fetal rats and was especially pronounced in those cells presumed to be in an early postmitotic phase but was absent in adult rat brain. In primary dissociated cultures of fetal rat brain, antibody SMI 31 elicited nuclear staining in both, neuronal and glial cells. Nuclear staining was also seen in C6 glioma and PC-12 cells.

Extracts of the above cells were subject to western blotting. Antibody SMI 31 recognized, besides NF, four additional proteins with apparent molecular weights of 35, 37, 52/54 and 250 KD. These were exclusively found in developing cells, but not in adult tissue. Neuronal differentiation, as indicated by quantitative increases of neuron-specific enolase, synaptophysin (p38), and NF, resulted in a decreased expression of these proteins. This was paralleled by a decreased nuclear staining in immunocytochemical preparations.

A search of protein data banks for proteins sharing the epitope recognized by antibody SMI 31 did not yield any protein of known nuclear localization that show the solubility properties and/or the same molecular weights of the additional proteins recognized by antibody SMI 31 in embryonic tissue. We conclude that we have come across hitherto unknown nuclear proteins that, in neural cells, are developmentally regulated.

Supported by DFG grant Pi 68/4-1

ALZHEIMER'S DISEASE: AMYLOID

259.1

BRAIN AMYLOID PRECURSORS IN DOWN SYNDROME (DS)
G. Giaccone‡[1] F. Tagliavini‡[1] C. Bouras‡[2] B. Frangione‡[3] and O. Bugiani‡[1] (SPON: V. Olgiati). [1] Istituto Besta, Milano, Italy, [2] IUPG, Genève, CH, [3] NY University, New York.

Patients with DS invariably develop Alzheimer changes. We studied frontal and temporal samples of 5 DS patients aged 6,12,39,46,55, by means of silver salts, Congo red, thioflavine S and immunohistochemistry with an antiserum to a 28 residue peptide homologous to the NH_2 terminal region of β-protein (anti-SP28). Patients aged 6 and 12 did not show Alzheimer changes. In the 39 ys patient, senile plaques (SP), neurofibrillary tangles (NFT) and congophilic angiopathy (CA) were absent, but anti-SP28 revealed several cortical deposits of immunoreactive material, lacking the tintorial and optical properties of amyloid. Similar deposits and a few SP, not CA or NFT, were found in the 46 ys patient and, with many SP, NFT and CA, in the patient aged 55. Noncongophilic anti-SP28 immunoreactive deposits might consist of β-protein not yet in β-pleated sheet conformation. The presence of noncongophilic anti-SP28 immunoreactive deposits in the brain of the 39 ys DS patient without any other Alzheimer change suggests that they represent the earliest step of β-protein deposition in Alzheimer disease and related conditions.

259.2

STUDIES ON THE LOCALIZATION OF THE ALZHEIMER AMYLOID PRECURSOR PROTEIN USING SYNTHETIC PEPTIDE IMMUNOCYTOCHEMISTRY.
J.R. Currie, M. Barcikowska, D.L. Miller, P. Mehta, K.S. Kim, and H.M. Wisniewski. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Three different cDNA sequences provide the only clues so far to the possible structure of the Aging-Alzheimer-Amyloid-Precursor protein (AAAP). Polyclonal and monoclonal antibodies raised against 7 subsequences of the Kang et al. 695 aa sequence have been used on tissue sections (Vector ABC method) from the CNS and other autopsied organs from young, old, and Alzheimer disease cases as well as from rat and monkey tissues and on some cell lines in an attempt to localize the cells which produce and/or process the putative AAAP. The various antibodies did not all label the same cell type or extracellular structure. Following several different pretreatments, multiple structures were still labeled by the antibodies. The labeled CNS elements included: some astrocytes and microglia; vascular media and adventitia; perivascular areas in subpial, cortical, ependymal and white matter regions; amyloid plaques and cores; large neurons and their processes; and synaptic arborizations. The distribution of the labeling often suggests that different, but related, adhesive proteins are being labeled in the various cell types. Other alternately spliced products (containing the beta-peptide sequence) having different functions may also exist.

259.3

cDNA CLONING OF DIFFERENT BETA AMYLOID PEPTIDE PRECURSORS IN ALZHEIMER'S DISEASE.
J.N. Octave*, F. de Sauvage*, A.F. Macq* and J.M. Maloteaux* (SPON: C.G. Veraart). Lab. Neurochimie, Université Catholique de Louvain, B-1200 Brussels.

A cDNA library from a 54 years old patient with anatomopathological confirmed Alzheimer's disease (AD) has been constructed, and different clones coding for the beta amyloid peptide precursor (BAPP) have been isolated. From the sequence of the cDNA clones, different BAPP are expressed in the cerebral cortex of that patient. First, we have isolated BAPP cDNA clones which are identical to those found in the fetal brain (Kang et al., Nature, 325:733, 1987). Second, we have observed that some BAPP mRNA use a different polyadenylation site in the 3' non coding sequence. Third, we have isolated BAPP mRNAs which contain domains homologous to serine proteinase inhibitors either reported by Ponte et al. and Tanzi et al. or by Kitaguchi et al. (Nature, 331:525-532, 1988).

These results confirm that in AD, different RNA species coding for the BAPP can be obtained by alternative splicing of a single transcriptional unit. The expression of the different BAPP mRNA are being studied in AD as compared to control brains.

259.4

STUDIES OF β-AMYLOID PRECURSOR GENE EXPRESSION IN BRAINS OF AGED MONKEYS.
E.H. Koo, D. Goldgaber†*, S.S. Sisodia*, M.D. Applegate*, D.C. Gajdusek§, and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182. †NIH, Bethesda, MD 20892. §CNS Studies Lab., NINCDS, Bethesda, MD 20892.

Nonhuman primates provide a useful model for studying the age-associated deposition of amyloid (antigenetically similar to that occurring in humans) within plaques and blood vessels. Quantitative in situ hubridization with a cDNA probe from the region encoding the β-amyloid peptide of the precursor (APP) gene was used to map the distribution of APP mRNA transcript in four regions of brain (frontal, temporal/hippocampus, occipital cortices, and cerebellum) in rhesus monkeys (15-24 years of age). mRNA transcripts of the APP gene were most abundant within large pyramidal neurons of monkey cortex, but significant levels were also detected in neurons of other regions, including cerebellum. With aging, levels of mRNA in regions vulnerable to the deposition of amyloid (frontal and temporal cortices) were not altered relative to regions not susceptible to the formation of plaques (occipital cortex and cerebellum). In this setting, the pattern of APP expression was not altered with age and did not correlate with the development of plaques. Recent reports suggest the existence of alternative mRNA transcripts of the APP gene, and studies in progress will compare the patterns of expression of other transcripts in brains of nonhuman primates.

259.5

EXPRESSION OF AMYLOID-ß-PROTEIN PRECURSOR (APP) mRNA TRANSCRIPTS IN DOWN SYNDROME AND ALZHEIMER'S DISEASE. #Gerald A. Higgins, Linda B. Dawes and Rachael L. Neve. #Univ. Rochester Med. Ctr., Rochester, NY 14642 and Children's Hospital, Boston, MA 02115.

Amyloid deposition in neuritic plaques and the cerebrovasculature, occur both in Alzheimer's disease, and in older patients with Down syndrome. As part of a larger effort to characterize the relationship between neuronal pathology and APP gene expression in the human central nervous system, we have used *in situ* hybridization and northern blotting to study the expression of different APP mRNA transcripts in the human central nervous system, in Down syndrome, and in Alzheimer's disease. Recent studies show that at least 3 different APP mRNAs are generated from the human APP gene: a 695 amino acid precursor (APP-695), and 2 forms which contain an inserted protease inhibitor domain (APP-751 and APP-770). For these studies, we have used synthetic oligonucleotide probes to discriminate APP-695, APP-751 and APP-770 mRNA transcripts.

In situ hybridization shows that all 3 APP mRNAs have a similar distribution in the normal aged human hippocampal formation and neocortex. Similarly, *in situ* hybridization shows that both APP-695 and APP-751 have similar regional and cellular patterns of distribution in brains from patients with Down syndrome and Alzheimer's disease. However, RNA blotting studies of three Down syndrome brains (10 months, 37 years and 40 years of age) shows changes in the relative expression of APP mRNAs in Down syndrome versus normal aged brain. For example, in association areas of neocortex, levels of APP-695 mRNA appears to be decreased in aged Down syndrome compared to normal. In addition, in the hippocampal formation and caudate-putamen of aged Down syndrome brain, the abundance of APP-751 mRNA is markedly greater than that of APP-695 mRNA when compared to the normal adult brain or to young Down syndrome CNS.

259.6

EXPRESSION OF THE ALZHEIMER AMYLOID PRECURSOR PROTEIN IN THE BRAIN. W.R. Rodriguez*, R.L. Neve, D. Schenk*, D. Davis*, L.I. Benowitz (SPON: J. Gilbert). Harvard Medical School, Boston, MA 02115; Athena Neurosciences, Inc., San Carlos, CA 94070.

We examined Alzheimer amyloid precursor protein (APP) immunoreactivity in adult human brain using two different polyclonal antisera raised against amino acids 479-497 and 687-695 of the amyloid precursor, respectively. The most prominent feature of the immunoreactivity in the neocortex was intense staining of pyramidal cell bodies in cortical layers III and V. The antisera did not stain cortical white matter, with the exception of occasional, exceptionally dark axonal staining in motor and premotor cortex. In addition to the somatic staining, a prominent band of immunoreactivity was observed in layer II of most cortical areas examined. This staining sometimes appeared to overlie neuronal somata, but often appeared to be distributed diffusely in the neuropil and may correspond to the terminals of APP-positive intracortical neurons in layer II. A lower density of punctate staining was seen in the neuropil of cortical layers III-V, and was also present throughout the grey matter in the caudate nucleus, and in the hippocampus. Within the hippocampus, both antisera decorated cell bodies of granule cells in the dentate gyrus and pyramidal cells in Ammon's horn. In addition, a dense, diffuse staining was displayed in the molecular layer of the dentate gyrus. (HD18658, NINCDS NS25830)

259.7

REGULATION OF THE ALZHEIMER AMYLOID PRECURSOR RNA IN PRIMARY CELL CULTURE. L.R. Dawes, M.P. Mattson, S.B. Kater, R.L. Neve. The Children's Hospital, Boston, MA 02115 and Colorado State University, Fort Collins, CO 80523.

Recent work has demonstrated the presence of two forms of the Alzheimer amyloid precursor protein (APP) RNA. We have constructed molecular probes that distinguish between these two RNAs. The culture paradigm provides a means of characterizing the response of APP RNAs to environmental cues, relative to other genes. We have used our differential probes to show that both forms of APP message are expressed in dissociated cell culture of E18 rat hippocampal neurons. We have observed a decrease in the expression of both APP transcripts in the presence of kainate. This may be relevant to previous work, which has shown that glutamate prunes the dendritic tree of cultured hippocampal neurons. We have also observed a differential response of the two APP mRNAs to potassium-induced depolarization.

The pathology of Alzheimer's disease preferentially involves the cholinergic system. Further work, therefore, involves culture of rat post-mitotic sympathetic neurons which remain plastic with regard to transmitter phenotype. Culture conditions may be manipulated to cause these neurons to differentiate. Levels of APP expression will be monitored upon cholinergic differentiation.

Supported by NIH grant HD18658.

259.8

AMYLOID PROTEIN PRECURSOR GENE EXPRESSION IN TANGLE-BEARING NEURONS OF ALZHEIMER'S DISEASE. T. Golde*, M. Palmert*, R. Repasky*, M. Usiak*, L. Younkin, and S. Younkin. Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106.

We are correlating the changes in amyloid protein precursor (APP) mRNA that we have observed in Alzheimer's disease with other changes that occur in this disorder. To evaluate APP mRNA levels in neurons undergoing neurofibrillary degeneration, we analyzed APP mRNA separately in tangle-bearing and tangle-free neurons. In the nucleus basalis of Meynert (where, in earlier studies, we observed a two-fold increase in total APP mRNA due to an increase in APP mRNA lacking the Kunitz protease inhibitor (KPI) domain) the probes for both total and KPI-containing APP mRNA consistently produced fewer specific grains over the tangle-bearing neurons than over the tangle-free neurons. In the subiculum (where we observed no changes in total or KPI-containing APP mRNA) both the probe for total APP mRNA and the probe for KPI-containing APP mRNA produced the same number of specific grains over tangle-bearing and tangle-free neurons. We are currently analyzing our cases to determine if the changes that we have observed in APP mRNAs are correlated (1) with changes in other relevant mRNAs (e.g. tau, ubiquitin) or (2) with the severity of neuronal loss, the extent of senile plaque or neurofibrillary tangle formation, or the degree of amyloid deposition. In addition, we are pursuing our observations on APP mRNAs using junctional probes capable of recognizing individual APP mRNAs. (Supported by USPHS grants AG06656, MH43444, and a grant from the state of Ohio.)

259.9

THE ß-AMYLOID PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE (AD) OCCURS AS A GROUP OF 110-135 kD MEMBRANE-ASSOCIATED PROTEINS IN BRAIN AND NON-NEURAL TISSUES. D. Selkoe, M.B. Podlisny*, E. Vickers*, A. Reynolds*, L. Fritz, and T. Oltersdorf*., Harvard Medical School, Brigham & Women's Hospital, Boston, MA 02115, and Athena Neurosciences, San Carlos, CA 94070.

Cerebral deposition of filaments composed of ß-amyloid protein (ßAP) is a constant feature of AD. Since the gene encoding the ßAP precursor (ßAPP) appears unaltered in AD, changes in posttranslational processing may underlie accelerated ßAP deposition in AD. Detection of the native precursor in mammalian tissues has not been reported. Using 2 antibodies to the predicted C-terminus, we have identified the precursor in brain and non-neural tissues as a group of 110-135 kD proteins found in membrane-rich subcellular fractions. These proteins are distributed throughout the brain, abundant in fetal brain and detected in non-neural tissues containing ßAPP mRNA. Similar proteins occur in bovine and rat brain and cultured human HL-60 and HeLa cells. Confirmation that the immunodetected tissue proteins are forms of ßAPP was obtained when mouse L cells and human 293 cells transfected with full-length ßAPP cDNAs showed increased expression of 110-135 kD proteins. Both C-terminal antibodies label some amyloid plaques in AD brain. Thus, the ßAPP molecule exists in tissue as a heterogeneous group of membrane-associated ~120 kD proteins. Detection of the C-terminus within plaques suggests that proteolysis of ßAPP into insoluble filaments occurs locally in cortical regions developing amyloid in AD.

259.10

DISTRIBUTION IN RAT OF THE PRECURSOR PROTEIN FOR ß-AMYLOID. L.G. Davis, J.P. Card, C.M. Reid*, R.A. Lampe*, R. Meade* and R.W. Manning*. Medical Products Dept., E.I. Dupont de Nemours & Co., Inc., Wilmington, DE 19898.

The isolation and sequencing of the core peptide (ß-amyloid) found in the plaques of patients with Alzheimer's disease has allowed the identification of a cDNA corresponding to the precursor protein. Using a human cDNA clone for the amyloid precursor, we have identified homologous mRNAs (ca. 3.8kb) in eight species. We have determined the distribution of this mRNA in seven brain regions and twelve organs obtained from rodents. This mRNA is abundantly expressed in all brain regions and detectable in most tissues. Interestingly, a prominent, second mRNA species (ca. 2.3kb) has also been identified in non-neuronal tissues.

Synthetic peptides based on the sequence of the human precursor were used to raise polyclonal antibodies for immunohistochemical localization of the amyloid protein in rat brain. These data demonstrate that the amyloid precursor protein is consistently found in discrete cell groups throughout the neuraxis. Most prominent among these areas are the olfactory bulb, the medial septum and diagonal band of Broca, the cortex and cerebellum. Amyloid-like immunoreactivity also surrounds blood vessels in a number of areas of the CNS. Preliminary electron microscopic analysis has demonstrated that the perivascular immunoreactivity is concentrated in glial processes while that in the remainder of the CNS is found in both neurons and glia.

259.11

AMYLOID MESSENGER RNA IN THE ALZHEIMER FRONTAL CORTEX. INTRANEURONAL LEVELS, RELATIONSHIP TO CELL SIZE AND LAMINAR DISTRIBUTION. W.-G. Chou*, R.E. Majocha*, F. M. Benes, B. Tate-Ostroff, S. Rehman*, M. Stoler*, B.S.Zain*, and C.A. Marotta. Harvard Med. Sch., Mass. General Hosp., Boston, MA 02114; McLean Hosp., Belmont, MA 02178; U. Rochester Med. Ctr., Rochester, NY 14642.

cDNA containing the A4 region (Zain, et al., P.N.A.S. 85:929, 1988) was subcloned into the pGEM plasmid and both anti-sense and sense probes were labeled with tritium. The probes were hybridized to AD frontal cortices containing varying levels of immunologically demonstrable amyloid. The mRNA was present at similar levels in all cortical layers except the first where it was less abundant. In layers III and V of a case distinguished by large numbers of amyloid deposits, mRNA was higher per layer, per neuron and per unit area of each neuron, relative to controls. We have previously shown that amyloid deposits detected by anti-A4 antibodies exhibit a laminar-specific pattern (Majocha, et al, P.N.A.S., in press). The lack of a parallel distribution of amyloid mRNA and amyloid protein deposits according to individual layers implicates factors other than, or in addition to, neuronal levels of mRNA as probably contributing to amyloid deposition. AG02126, CA11198, CA36432, AG05134, MH00423, Am. Health Assist. Found., Familial AD Res. Found.

259.12

IMMUNOCYTOCHEMICAL DEMONSTRATION OF DIFFERENTIAL LOCALIZATION OF ALZHEIMER AMYLOID PRECURSOR PROTEIN SUBDOMAINS: A4 vs. A NON-A4 REGION. R.E. Majocha*, B. Tate-Ostroff and C.A. Marotta (SPON: R.A. Nixon), Harvard Med. Sch., Massachusetts General Hosp., Boston, MA 02114; Mailman Res. Ctr., McLean Hosp., Belmont, MA 02178.

To gain insight into the processing of the amyloid precursor protein (APP), we examined the distribution of A4 and pre-A4 epitopes. Monoclonal (mab) and affinity-purified polyclonal (pab) antibodies were prepared to a pre-A4 site, with a unique sequence, considered to be in the extracellular domain. Mabs and pabs to the A4 site were previously characterized (Benes, et al., P.N.A.S., in press). In the Alzheimer's Disease (AD) prefrontal cortex and hippocampus, the A4 antigen was selectively localized to neuritic plaques. By contrast, high concentrations of pre-A4 immunoreactive product were found over neuronal cell bodies. In addition some non-cellular punctate deposition was observed. Neurologically normal control brains were devoid of A4 immunoreactivity. However, both controls and AD cases stained with pre-A4 antibodies were similar. The differential distributions of APP epitopes imply either processing events and/or a membrane orientation that are unique to the AD brain. Supported by AG02126, American Health Assistance Found. and Familial Alzheimer's Disease Res. Found.

259.13

ELEVATED EXPRESSION OF A SPECIFIC AMYLOID-ß-PROTEIN (APP) mRNA TRANSCRIPT IN THE BEHAVORIALLY-IMPAIRED AGED RAT. George A. Oyler[1], Fred H. Gage[2], Karen Chen[2], and Gerald A. Higgins[3], [1]Hershey Medical College, Hershey, PA 17033, [2] U.C.S.D., La Jolla, CA 92093, and [3]Univ. Rochester Med. Ctr., Rochester, NY 14642.

The behavorially-impaired aged rat may be a model system in which to study cellular and molecular changes which underly learning and memory deficits associated with the aging process. Cholinergic and NGF-receptor containing neuronal populations of the basal telencephalon, which send widespread projections to the neocortex and hippocampal formation, have been shown to undergo atrophy during aging of the rat CNS. In order to better understand the molecular basis of such changes, we have examined the expression of: (1) alternate transcripts of the amyloid-ß-protein precursor (APP) gene, (2) nerve growth factor receptor mRNA and protein, and (3) choline acetyl transferase (Chat) mRNA and protein, in young, aged, and behavorially-impaired aged Sprague-Dawley rats.

In situ hybridization and RNA blotting have revealed striking increases in the expression of a specific APP mRNA transcript in the aged rat. Levels of APP-751 mRNA, which encodes an amyloid precursor containing an inserted protease inhibitor domain, is elevated at least 3-4 fold in the basal telencephalon of aged rats. Increases in the abundance of this mRNA were observed in the striatum, nucleus basalis, diagonal band, ventral pallidum and medial septum using *in situ* hybridization with APP transcript-specific probes. In contrast, preliminary studies suggest that NGF-receptor mRNA levels are not significantly altered in aged animals. Thus, it appears that increases in a specific form of APP mRNA may be correlated with neuronal atrophy which occurs during the aging process.

259.14

RELATIONSHIP BETWEEN CALPAIN AND AMYLOID PLAQUES IN ALZHEIMER'S DISEASE. J.E. Wu*+, L.S. Perlmutter, M.Baudry+, G. Lynch+, and H.C. Chui* (SPON: B. Newman). Dept. of Neurology, Univ. So. Calif., Los Angeles, CA 90033 and CNLM+, Univ. of Calif., Irvine, CA 92717.

Alzheimer's Disease is a degenerative illness resulting in a progressive loss of cognitive ability. Characteristic lesions include senile plaques and neurofibrillary tangles, both of which contain abnormal accumulations of cytoskeletal proteins. Many of these are substrates of calpain, a calcium-activated neutral protease, which is implicated in muscle, myelin, and axon cytoskeleton degeneration. In fact, a calpain inhibitor, aluminum, has been colocalized with tangles and plaques. The relationship between calpain localization and amyloid plaques was studied. Postmortem brain tissue from controls and Alzheimer's patients were immunocytochemically labelled with an antibody against calpain then counterstained with thioflavine S (marker for amyloid). Brodmann's area 17 was analyzed for colocalization of amyloid and calpain immunostaining. The percentage of amyloid-positive plaques that was associated with calpain immunoreactivity was positively related to plaque and tangle densities, but not to a measure of clinical severity. Control tissue was devoid of amyloid. These data suggest that calpain may be involved in plaque formation and/or maintenance. (AG-07127 and French Foundation to LSP; AG-05142).

259.15

AMYLOID P COMPONENT IN DEMENTIA. D. Saperia, L.S. Perlmutter, J. Athanikar*, H.C. Chui*. Dept. of Neurology, University of Southern Calif., Los Angeles, CA 90033.

To examine components of senile plaques, correlative light and electron microscopic (LM,EM) localization of amyloid P component (APC) was performed. APC is found in serum, binds to amyloid fibrils in a calcium-dependent manner, and has recently been localized to the pathologic lesions of Alzheimer disease (AD). A polyclonal antibody (Dako) was used on tissue from patients with dementing diseases (classic/mixed AD, vascular dementia). Tissue was immersion fixed in mixed aldehydes and immunolabelled for APC with avidin-biotin-peroxidase. Sections were processed for both LM and EM. LM - a spectrum of patterns of APC immunoreactivity (APC-I) was seen: Non-AD samples had more prominent capillary staining; some also had plaque-like accumulations of APC-I. ADs also had these accumulations, as well as attenuation of capillary staining with concomitant labelling of neurofibrillary tangles (NFT). Decreased vascular staining appeared to be associated with increased NFT staining. EM - endothelial cytoplasm was stained in patients who displayed LM vascular APC-I. In all subjects, extracellular APC-I was seen near stained arterioles. This APC-I displayed an apparent affinity for myelinated axons, suggesting that APC may be involved in alterations in the neuropil in AD and non-AD brains. (AG-07127 & French Fdn to LSP; AG-05142).

259.16

ALTERATION OF BASEMENT MEMBRANE COMPONENTS IN DEMENTIA. J. Athanikar*, L.S. Perlmutter, D.Saperia, H.C. Chui* (SPON: L.Weiner). Dept. of Neurology, University of Southern California, Los Angeles, CA 90033.

Thickened and redundant basement membrane (BM) is seen in several diseases including Alzheimer (AD). Immunoelectron microscopy was used to localize 2 BM components in AD: Collagen type IV (CIV) spans the BM width providing mechanical support; heparan sulfate proteoglycan (HSPG) lines either side of the BM aiding filtration. Autopsy tissue was immersion fixed in mixed aldehydes, immunolabelled (avidin-biotin-peroxidase) with commercially available antibodies (Chemicon), and prepared for electron microscopy. As expected, the BMs were thickened, with vacuole-like holes and redundancies. CIV immunoreactivity (CIV-I) was deposited along the entire width of both normal and thickened BM in all patients. The holes appeared to be caused by dissection within the CIV domain rather than reduplication of the entire BM. HSPG immunoreactivity (HSPG-I) clearly labelled both the inner and outer BM surfaces in most patients. One severely demented AD patient, however, consistently lacked HSPG-I at the endothelial surface of the BM, which may be related to the reported presence of circulating HSPG antibodies in some AD patients. These abnormalities in CIV and HSPG suggest that alterations in both components may be involved in BM pathologies. (AG-07127 & French Fdn to LSP; AG-05142)

259.17

BASEMENT MEMBRANE PATHOLOGY IN DEMENTIA. L.S. Perlmutter, J. Athanikar*, C. Zarow*, H.C. Chui*. Dept. of Neurology, University Southern Calif., Los Angeles, CA 90033.

The vascular basement membrane (BM) participates in the blood-brain barrier. Thickened and redundant BM is seen in several diseases including Alzheimer's (AD). Quantitative electron microscopy (EM) was employed to accurately describe this pathology. Autopsy tissue from 4 AD and 4 non-AD (vascular dementia, supranuclear palsy, alcoholic) patients was immersion fixed and prepared for EM. Small blood vessels were photographed (3-10 per patient). The full extent of the BM on the micrographs was measured by computerized image analysis to determine the inside (I) and outside (O) perimeters as well as the area (A) of the BM. Two variables were computed: O/I (increased ratio seen with either thickenings or redundancies), and A/I (thickened BM results in a higher ratio, while redundancies of BM yield a lower ratio). Both measures discriminated between AD and non-AD patients. O/I: AD mean (±sd)=1.40 ± 0.1, non-AD mean=1.19 ± 0.1; A/I: AD mean= 0.52 ± 0.1, non-AD=0.36 ± 0.1. ADs scored consistently high on both measures. The one alcoholic patient had a thickened, but not reduplicated BM, perhaps indicative of alcoholic encephalopathy. Thus, these quantitative techniques discriminate between various BM pathologies and confirm a high incidence of overall BM pathology in AD. (AG-07127 & French Fdn to LSP; AG-05142)

ION CHANNELS: LIGAND-GATED I

260.1

FUNCTIONAL PROPERTIES AND DEVELOPMENTAL REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN CHICK SYMPATHETIC NEURONS. B.L. Moss, S.M. Schuetze and L.W. Role§. Dept. Biol. Sci., Columbia Univ., NY, NY 10027, §Dept. Anat. & Cell Biol. and Ctr. Neurobiol. & Behav., Columbia Univ. P & S. NY, NY 10032.

We have examined the channel properties of neuronal nicotinic acetylcholine receptors (AChRs) by recording single-channel currents of cultured sympathetic neurons isolated from the lumbar chain ganglia of chick embryos. To address the role of synapse formation in the regulation of these properties, we removed ganglia before the onset of innervation (ED 8-10) and during the first major wave of synaptogenesis (ED 16-21).

In ED 10 neurons acetylcholine activates three distinct channel amplitude classes, S, M and L, in both cell-attached and outside-out patches. These classes can be distinguished from one another by both slope conductance and mean burst duration. The slope conductances are: S ≈ 20 pS, M ≈ 34 pS and L ≈ 50 pS. The burst distribution of S is fit with two exponentials with time constants of 1 ms and 8 ms (23°C). The burst distributions of M and L are singly exponential with time constants of 6 ms and 3 ms respectively. The three amplitude classes also differ in the frequency with which they occur in cell-attached patches; S and M are the predominant classes.

Our preliminary experiments indicate three developmental changes in channel properties in neurons removed from ED 16-17 animals. In addition to S, M and L, a new channel amplitude class (denoted XL) appears. This new class is also specifically activated by ACh in that in outside-out patches, XL openings are observed only during the application of ACh and in cell-attached patches, the frequency of XL openings increases with increasing ACh concentration. A second change in channel properties is a change in the relative frequency with which the classes occur in patches. In ED 16-17 neurons, M and L are the predominant classes. Finally, the fraction of patches with just one amplitude class increases from ≈11% at ED 10 to ≈ 55% at ED 16-17.

260.2

PHARMACOLOGICAL ANTAGONISM OF ACH AND ATP RECEPTOR-CHANNELS IN RAT CULTURED PARASYMPATHETIC NEURONS. L.A. Fieber* and D.J. Adams* (SPON: P. Strang). Dept. of Pharmacology, Univ. of Miami Sch. of Med., Miami, FL 33101

Acetylcholine- (ACh) and adenosine triphosphate- (ATP) activated responses were investigated in cultured cardiac neurons dissociated from neonatal rat parasympathetic cardiac ganglia. Ionic currents evoked by pressure application of the agonists were obtained using the whole-cell configuration of the patch clamp technique. The current-voltage relationships obtained with a pipette solution containing 140 mM CsCl, 5 mM BAPTA, 2 mM MgATP and 5 mM Cs-HEPES (pH 7.2) were similar for the two agonists, exhibiting marked inward rectification, and a reversal potential of approximately 0 mV. The nicotinic receptor antagonists hexamethonium, d-tubocurarine and mecamylamine inhibited the ACh-evoked response in cardiac neurons, but the P_{2y} purinergic receptor antagonist α,β-methylene ATP had no effect. Block of the ACh-induced current by hexamethonium (50 μM) and d-tubocurarine (2 μM) was voltage dependent whereas block by mecamylamine (3 μM) was voltage independent. ATP-activated whole cell currents were inhibited in a voltage independent manner during bath perfusion of α,β-methylene ATP (10 μM) and were unaffected by d-tubocurarine and 8-phenyltheophylline. This suggests that P_{2y} receptors mediate the ATP response. Bath application of ACh (10 μM) failed to inhibit the ATP-induced current. These data indicate that the action of ACh and ATP are mediated via distinct populations of receptor-channels.

260.3

THE NUCLEOTIDE SEQUENCE AND DEDUCED AMINO ACID SEQUENCE OF RAT NON ALPHA 2 K.E. Isenberg and G. Meyer Washington University School of Medicine, St. Louis, MO 63110

We report the nucleotide sequence and complete deduced amino acid sequence of a new member of the nicotinic acetylcholine receptor (nAChR) gene family. The 2500 bp clone was isolated by screening (3 X 10^5 recombinants) a rat superior cervical ganglion cDNA library (provided by J.P. Merlie) with an oligonucleotide that hybridizes to muscle nAChR subunits. Sequencing was performed by the dideoxy chain-termination method.

The novel clone lacks the pair of extracellular cysteine residues characteristic of acetylcholine binding (alpha) nAChR subunits. Although similar to the previously reported non-alpha nAChR subunit, the amino acid sequence is particularly different in a proposed second cytoplasmic loop. The deduced amino acid sequence suggests that the subunit could function as a non-acetylcholine binding subunit in a ligand gated ion channel.

260.4

PHOTOAFFINITY LABELING OF THE TORPEDO NICOTINIC ACETYLCHOLINE RECEPTOR BY THE NONCOMPETITIVE ANTAGONIST [^{3}H]-TETRACAINE. R.E. Middleton* and J.B. Cohen. Dept. of Anatomy and Neurobiology, Washington University, St. Louis, MO. 63110.

[^{3}H]-Tetracaine ([^{3}H]-Tet) binds to the noncompetitive antagonist site on the Torpedo acetylcholine receptor (AchR) with high affinity (K_d=1 μM) unless AchR is desensitized by agonist when K_d=30 μM. We have studied the photoincorporation of [^{3}H]-Tet into polypeptides of Torpedo postsynaptic membranes by SDS-PAGE. Irradiation at 302 nm resulted in specific labeling of each AchR subunit, i.e. photoincorporation was blocked by noncompetitive antagonists but not by α-bungarotoxin. Histrionicotoxin inhibited up to 90% of the subunit labeling with IC_{50}=2 μM. [^{3}H]-Tet photoincorporation was also inhibited by agonists, but this inhibition was blocked by α-bungarotoxin. Specific labeling was insensitive to the aqueous scavenger glutathione (50 mM). The extent of specific labeling varied with the [^{3}H]-Tet concentration (K=2 μM) and for each subunit the maximal specific incorporation was equivalent to 1-2% of the total subunit protein. The specific photoincorporation into the α-subunit has been localized to a S. aureus V8 protease fragment which begins at Ser-173 and contains the predicted membrane spanning regions M1, M2, and M3. The observed labeling pattern will be compared to affinity labeling by desensitizing non-competitive antagonists. (Supported in part by NS 19522 and a Markey Foundation fellowship to R.E.M.)

260.5

THE C-TERMINUS OF THE TORPEDO ACETYLCHOLINE RECEPTOR δ SUBUNIT IS EXTRACELLULAR. **M. DiPaola,* C. Czajkowski,* M. Bodkin,* and A. Karlin. Depts. Biochem. and Molec. Biophys., and Neurol., Columbia U., New York, NY 10032.**

Both even and odd numbers of membrane crossings of the AChR subunits ($\alpha_2\beta\gamma\delta$) have been supported by theory and experiment. An even number corresponds to an extracellular location of the subunit-C-termini; an odd number, with a cytoplasmic location. Torpedo AChR is a dimer in native membrane, disulfide-crosslinked between δ subunits. We found that this disulfide is readily reduced in sealed right-side-out (RSO) native membrane vesicles by the relatively impermeant reducing agents, glutathione and 2-mercaptoethansulfonate (cf. McCrea et al. (1987) EMBO J., 6, 3619-3626; Dunn et al. (1986) BBRC 139, 830-837). Far less reduction of δS-Sδ is obtained in sealed inside-out (ISO) vesicles. We have also identified the half-cystinyl residue forming δS-Sδ. We alkylated the accessible free SHs of the AChR dimer in Triton X-100 solution , mildly reduced the dimer to monomer, alkylated the newly formed SHs with ^{3}H-NEM, isolated the δ subunit, cleaved it with CNBr, and separated the fragments by reverse-phase HPLC. ^{3}H-NEM was incorporated into only one fragment, which we identified both by amino acid analysis and by sequencing as the C-terminal CNBr fragment. We also isolated a homodimer of this fragment from CNBr-cleaved unreduced δS-Sδ. The one Cys residue in this fragment is in the penultimate position of the δ sequence. Thus, the dimer is crosslinked via δ Cys 500 (cf. Popot and Changeux (1984) Physiol. Rev. 64, 1162-1239), and the C-terminus of δ and presumably of the other subunits is extracellular. Supported by NS07065, NS07258, and MDA.

260.6

EFFECTS OF ORGANOPHOSPHORUS ANTICHOLINESTERASES ON NICOTINIC ION CHANNELS AT ADULT MOUSE MUSCLE ENDPLATES. JEH Tattersall*.(SPON: L.D. LEAKE). Biology Division, Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire SP4 OJQ, UK.

Organophosphorus esters such as diisopropylfluorophosphate and VX have been shown to have direct actions on acetylcholine-activated ion channels at amphibian endplates (Albuquerque,E.X., Deshpande,S.S., Kawabuchi,M., Aracava,Y., Idriss,M., Rickett,D.L. and Boyne,A.F., Fundamen. Appl. Toxicol., 5:S182-S203, 1985). The present study investigated the effects of these compounds on mammalian endplate channels.

Flexor digitorum brevis muscles from adult Porton strain mice were dissociated according to the method of Henderson,L.P., Lechleiter,J.D. and Brehm,P. (J.Gen. Physiol., 89:999-1014, 1987). Single endplate channels were recorded in the cell-attached configuration, using patch pipettes filled either with acetylcholine (ACh, 100-300nM) alone, or with ACh and an organophosphate (1-500μM). Data were analyzed using a microcomputer to determine open and closed lifetimes and single channel conductances.

The results demonstrated that organophosphorus anticholinesterases can indeed have direct effects on mammalian nicotinic endplate channels, such as blocking open channels and reducing single channel conductance.

260.7

NON-COMPETITIVE ANTAGONISTS ACTING AT BRAIN PRESYNAPTIC NICOTINIC RECEPTORS: EVIDENCE FOR A CONSERVED ION CHANNEL. S. Wonnacott, J. Irons*, G.G. Lunt, & E.X. Albuquerque. (SPON: R. Sjodin). Biochemistry Dept., Univ. Bath, Bath BA2 7AY U.K. & Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Structural diversity of neuronal nicotinic acetylcholine receptors (nAChR) is evident at the gene level and this is manifest by at least two classes of nicotinic ligand binding sites in vertebrate brain. Using nicotine-stimulated transmitter release from perfused synaptosomes we have characterized presynaptic nAChR in rat striatum and hippocampus. These functional nAChR best correlate with high affinity [^{3}H]nicotine binding sites. We have now investigated the action of non-competitive antagonists on nicotine-evoked [^{3}H]GABA release from hippocampus, to explore the molecular mechanism of the presynaptic nAChR. In agreement with previous results (FEBS Lett. 212:292, 1987), the nicotinic channel blocker perhydrohistrionicotoxin (10 μM) inhibited the action of nicotine. Phencyclidine (PCP; 10 μM) was also effective in this system. PCP blocks the channel of the endplate and central nervous system (Ramoa and Albuquerque, this meeting). MK801, another non-competitive antagonist at NMDA receptors, also reversibly inhibited nicotine-evoked [^{3}H]GABA release. Antagonism by MK801 increased on repetitive stimulation. These agents did not affect K^+-stimulated release and they did not inhibit radioligand binding to brain membranes. Thus the presynaptic nAChR may operate via an ionophore that is not only similar to that of the muscle nAChR but also related to the NMDA receptor ion channel. This is in accord with the proposal that ligand-gated ion channels, although pharmacologically distinct at the level of their agonist binding site, are members of a highly conserved "super family". (Support: NATO Collab. Res. Grant and NIH Grant NS25296)

260.8

NON-OXIME BISPYRIDINIUM COMPOUND SAD-128 ALTERS THE KINETICS OF ACh-ACTIVATED CHANNELS. **M. Alkondon* and E.X. Albuquerque.** (Spon:Dr.N.Brookes) Dept. Pharmacol. & Exper. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Endplate current (EPC) measurements from frog sartorius muscles in presence of 1,1'-oxybis (methylene)bis 4-(1,1,dimethylethyl)pyridinium dichloride (SAD-128) revealed a nonlinear I-V relationship and a biphasic decay. Using the frog interosseal muscle fibers for patch clamp recordings under cell attached configuration, the kinetics were analysed in detail. SAD-128 (1-40 μM) produced a concentration-dependent reduction in the mean channel open time of ACh (100 -200 nM)-activated channel currents without affecting their conductance. The square wave-like current pulses which appear under control condition were interrupted by many flickers forming bursts of many openings and closings in presence of the drug. Membrane hyperpolarization accelerated the reduction of mean open time but increased the duration of the closed times inside the bursts. The reciprocal of the mean open time followed a linear increase at low concentration range of SAD-128 (1-10 μM), but increased with a parabolic function at higher concentrations (10-40 μM). SAD-128 also reduced the mean total open time of the burst. A simple sequential scheme of channel block does not explain most of the effects of SAD-128. Additional pathways and/or a change in rate constants are needed in the above model to account for the channel blocking effects of SAD-128. (Support: U.S. Army Med. Res. & Devel. Com. Contract. DAMD17-84-C-4219).

260.9

EFFECTS OF PHILANTHUS TOXIN (PTX) ON THE NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR). R. Rozental*, G.T. Scoble*, S. Sherby*, A.T. Eldefrawi, M.E. Eldefrawi* and E.X. Albuquerque. Dept. Pharmacol. & Exp. Ther., Univ. of MD Sch. of Med., Balto, MD, 21201.

PTX is the major neurotoxin present in the venom of the wasp *Philanthus triangulum*. PTX (80 μM) blocked the indirectly elicited twitch of frog sartorius muscle; these effects were reversed upon washing. PTX (20 μM) depressed the peak EPC amplitude (from 0.3 to 0.13 μA at -100 mV) in a voltage-dependent manner, and reduced the linearity of the I-V relationship. The decay time constant of the EPC was shortened, with marked loss of voltage sensitivity at negative membrane potentials. Using cell-attached patch clamp technique of frog interosseal muscle fibers, PTX caused a voltage- and concentration-dependent shortening of burst and channel lifetime of currents activated by ACh. In addition, PTX reduced and blocked the frequency of channel opening at hyperpolarized holding potentials. PTX competitively displaced the specific binding of [^{3}H]H$_{12}$HTX to activated but not to resting *Torpedo* nAChR. PTX also inhibited binding of [^{125}I]α-bungarotoxin to *Torpedo* nAChR, suggesting that PTX binds to the ACh recognition site as well as to the high affinity noncompetitive site associated with the ion channel. The results suggest that PTX interacts with the nAChR as a competitive and a non-competitive open channel blocker. (Support: US Army Med. Res. & Dev. Comm. Cont. DAMD17-84-C-4219 and NIH Grants NS25296 & ES02594.)

260.10

THIOCHOLINE IS AN AGONIST OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. G. Amitai & S. Chapman*, Dept. of Pharmacology, IIBR, P.O. Box 19 Ness Ziona, Israel.

Thiocholine (TCh) mediates the opening of the acetylcholine receptor (AChR) channel in BC3H-1 muscle cells. The functional response of AChR was determined by agonist-mediated $^{22}Na^+$ influx in intact cells under depolarizing conditions. The K_{act} values obtained for TCh and carbamylcholine (Carb) were: 80 and 38μM, and n_H values were 1.3 and 2.7, respectively, implying that TCh interacts by a non-cooperative mechanism. TCh activity was inhibited competitively by d-tubocurarine (IC50=0.25μM, n_H=1.1) suggesting that TCh activates the channel via the agonist/antagonist site. The K_{des} values obtained from the desensitization state-function for TCh and Carb were: 19 and 63 μM, and n_H=0.8 and 1.9, respectively, indicating a non-cooperative mechanism for TCh-induced desensitization. The recovery from TCh-induced desensitized state was 90% of the original response ($\tau_{1/2}$=15 sec). Agonist activity was not induced by dimethylamino ethanethiol, diisoprpyl-amino ethanethiol, mercaptoethanol or choline. Thus, both TCh-elicited activation and desensitization are unrelated to its reductive capability. In addition, the agonist action of organophosphates which release TCh by hydrolysis (e.g. phospholine iodide) should be evaluated with caution. (Supported by USAMRDC, DAMD17-84-C-4016).

260.11

MUSCARINIC AGONISTS DEPRESS CA^{2+}-SENSITIVE CURRENT IN HIPPOCAMPAL PYRAMIDAL NEURONS. K.L. Zbicz and F.F. Weight. Lab. Physiologic and Pharmacologic Studies, NIAAA, Rockville, MD. 20852.

Outward membrane currents in hippocampal CA3 pyramidal neurons were studied using the single microelectrode voltage clamp technique. Neurons were impaled with microelectrodes (15-25 MΩ) containing a CsCl or Cs_2SO_4 solution. Cs^+-loading selectively reduced outward currents elicited by depolarizing voltage steps, allowing visualization of an initial large inward current and a slowly developing outward current which had characteristics of a Ca^{2+}-dependent K^+ current. This slow current was blocked or reduced by Ca^{2+}-channel blockers Co^{2+}(1-3 mM) and Mn^{2+}(2-4 mM), lowered extracellular Ca^{2+}, and by TEA($\geq$10 mM), but was insensitive to 4-AP (200 μM). The slow outward current was also greatly reduced by application of Ba^{2+}(0.2-1.0 mM).

Application of the cholinergic agonists carbacol or muscarine (10-25 μM) significantly reduced the slow Ca^{2+}-sensitive outward current. Application of these drugs also reduced the amplitude of the initial inward current. When the slow outward current was abolished by exposing the neurons to a 0 Ca^{2+} solution containing Mn^{2+}, subsequent application of muscarine did not reduce depolarization activated currents. These results indicate that in hippocampal pyramidal neurons, cholinergic effects are based, at least in part, by effects on Ca^{2+}-dependent outward current. The observed reduction in this current may be secondary to depression of inward Ca^{2+} current.

260.12

A CALCIUM CONDUCTANCE IS INDUCED IN HIPPOCAMPAL PYRAMIDAL CELLS BY MUSCARINIC RECEPTOR ACTIVATION. R.D. Blitzer, O. Gil*, and E.M. Landau. Dept. of Psychiatry, Bronx VA Medical Center, Bronx, NY 10468

Muscarinic agonists depolarize hippocampal pyramidal cells, due at least in part to the blockade of a number of K+ conductances. We have been interested in the possible activation of Ca++ conductances in these neurons by cholinergic stimulation, and have therefore examined the membrane effects of muscarinic agonists in the presence of external Ba++ ions.

Guinea pig hippocampal slices were superfused with artificial CSF and maintained at room temperature or at 31° C. Pyramidal cells from region CA1 were single-electrode voltage-clamped (sampling freq.: 2-6 kHz; holding potential between -70 and -80 mV), and drugs were applied in a medium containing 1-4 mM Ba++ and 0.5 μM TTX. In nearly all cells tested (95/97), carbachol (10 μM) induced a rapid inward current associated with an increase in conductance. This current inactivated within 3 min despite continued carbachol exposure, the period of desensitization lasting 10-20 min. The size of the current depended upon Ba++ concentration (1 mM: 147±34 pA, n=15; 4 mM: 213±17 pA, n=81), and the current was blocked by pirenzepine (0.1 μM) and by external Cd++ (200 μM) and Mg++ (8-10 mM). Oxotremorine (10 μM) only weakly induced the Ba++ current, and antagonized the carbachol effect.

We conclude that M-1 receptor stimulation is linked to the activation of a calcium conductance in pyramidal cells, perhaps through effects on the PI system. This conductance may underlie the calcium-dependent component of muscarinic depolarization reported recently by Alger and McCarren [*Soc. Neurosci. Abstr.*, **13**, 1005 (1987)]. In addition, this conductance may represent an important link between cholinergic activity and memory. Supported by NIH Grant 5-P50-AG05138-02.

260.13

Ca-ACTIVATED K-CHANNEL IN RAT NEUROHYPOPHYSIAL NERVE TERMINALS. P.J. Thorn* and J.R. Lemos. Worcester Foundation for Experimental Biology, Shrewsbury, MA

Nerve endings from the rat neurohypophysis were dissected free from pars intermedia and dissociated by homogenization. The isolated terminals release oxytocin and vasopressin in response to membrane depolarization in a Ca^{2+} dependent manner (Cazalis, et al., 1987, J. Physiol. 390:5). It is possible, using patch-clamp techniques, to directly analyze membrane ionic currents, including Ca^{2+} channels (Lemos and Nowycky, 1987, Soc.Neurosc. 13:793), and their influence on secretion using this preparation.

We have observed several types of cation channels in these isolated nerve endings. One has the characteristics of a maxi-Ca^{2+} activated K^+ channel, hypothesized to exist in the intact neurohypophysis (Parsons, et al, 1986, Bioph.J. 51:428). Reversal potentials in assymetrical salt gradients indicate that it is a cation channel which is more selective for K^+ than for Na^+ ($P_{K/Na}$=9:1). In symmetrical KCl the open probability increases with depolarizing voltages and with $[Ca^{2+}]_{in}$ >1 uM. This activation is very steep between 1 and 3 uM $[Ca^{2+}]_{in}$. The slope conductance of the Ca^{2+} activated K^+ channel is about 240 pS in symmetrical 150 mM KCl and appears to be affected by intracellular Na^+. This and other cation channels in the terminal membrane are being further characterized.

(This work was supported by NIH grant NS22542)

260.14

SINGLE QUININE-SENSITIVE K^+ CHANNELS ACTIVATED BY D_2 DOPAMINE RECEPTORS IN RAT CORPUS STRIATUM. Jonathan E. Freedman and Forrest F. Weight. Section of Electrophysiology, Laboratory of Physiologic and Pharmacologic Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

We have studied the actions of dopamine and antipsychotic drugs at the single channel level, using an enzymatic and mechanical method to acutely dissociate neurons suitable for patch-clamp recording from the corpus striatum (caudate and putamen) of young adult rats (*Proc. Natl. Acad. Sci. USA*, **85**: 3618, 1988). In cell-attached recordings, single channel openings were observed in the presence of dopamine or of quinpirole (a D_2 receptor agonist), but not in the presence of SKF-38393 (a D_1 agonist) or in the absence of drugs. Haloperidol and the D_2 antagonist spiperone were potent antagonists, whereas the D_1 antagonist SCH-23390 was less potent. The channel conductance was about 85 pS in the presence of 140 mM KCl, and the reversal potential varied with the extracellular K^+ concentration as predicted by the Nernst equation, suggesting the opening of single K^+ channels following D_2 dopamine receptor activation.

Quinine (which is known to block various K^+ and other ion channels at 1 μM to 1 mM, and is less toxic than many other channel blockers) potently inhibited D_2-activated channel openings. There was a partial blockade with as little as 5-10 nM quinine, and almost no channel current was observed at 100 nM. Concentrations exceeding 100 nM are known to be attained in human brain at clinical (ie, antimalarial) dosages of quinine. Why extrapyramidal signs, which are characteristic of D_2 receptor blockade, are not prominent quinine side effects is an interesting and unanswered question.

260.15

5-HT-INDUCED CURRENTS IN NCB-20 AND N1E-115 CELL LINES. T.G. Hales*, J.J. Lambert* and J.A. Peters*. (SPON: S.M. Bunt). Dept. of Pharmacology & Clinical Pharmacology, Ninewells Med. Sch., Dundee Univ., DD1 9SY. Scotland. UK.

Neuronal clonal cell lines are useful model systems for the study of neurotransmitter receptor function. Here clonal lines N1E-115 and NCB-20 were used to investigate the molecular properties of the 5-HT_3 receptor (e.g. Neijt, H.C. et al, Eur J. Pharmac., 127: 271, 1986).

Locally applied 5-HT (10μM) induced inward currents on 70% of NCB-20 cells (n=221) voltage-clamped at -60 mV. The response reversed in sign at 0mV ($[Na^+]_o = [Cs^+]_i$). Such currents were suppressed by the 5-HT_3 antagonists GR 38032F (1 nM) and ICS 205-930 (0.3 nM), but were unaffected by the 5-HT_2 antagonist ketanserin (1μM), the mixed "5-HT_1-like"/ 5-HT_2 antagonist methysergide (1μM), or the 5-HT uptake blocker citalopram (1μM).

On isolated outside-out membrane patches from N1E-115 cells (holding potential = -60 mV), 5-HT evoked inward currents of -1 to -20pA, which were reversibly suppressed by GR 38032F (1nM). In common with whole cell currents, the response of the isolated patch demonstrated desensitization and reversed in sign at 0mV. Despite the high resolution of these recordings, discrete single channel events were not discernible during such currents. Qualitatively similar results were obtained on membrane patches from NCB-20 cells. It is possible that 5-HT evokes a channel of small conductance in these cells.

260.16

GABA RECEPTOR cDNAs EXPRESSED IN TRANSFECTED CELLS AND STUDIED BY PATCH-CLAMP AND BINDING ASSAY D. Pritchett*, P. Schofield*, H. Sontheimer*, S. Ymer*, H. Kettenmann*, and P. Seeburg* (SPON: B. Shivers). ZMBH and Dept. of Neurobiology U. Heidelberg, D6900 Heidelberg, FRG.

GABA receptor chloride ion channels were expressed in human cell lines by DNA-mediated transfection. Four different (30% identity) cDNAs encoded GABA A receptor polypeptides; ligand-gated chloride channels expressed in transfected cells were characterized using the whole cell patch-clamp mode. Ligand and ion selectivity was observed even in single subunit homo-oligomeric receptors formed in cells transfected singly with any individual GABA cDNA. GABA receptor subunit cDNAs produced GABA- and muscimol-gated, bicuculline- and picrotoxin-sensitive channels; GABA had no effect on untransfected or vector transfected cells and glycine had no effect on GABA subunit cDNA transfected cells. Pentobarbitol potentiated only the GABA responses including the GABA homo-oligomeric channels. In filter binding assays, α+β transfected cell membranes had 3H muscimol and 35S TBPS binding sites; the addition of the γ cDNA produced a receptor, showing 3H flunitrazepam binding. This expression system offers the opportunity to assess how structural differences alter channel properties and, ultimately, information processing in the CNS. This system is also less labor intensive and seasonally variable than the oocyte expression system. In addition, stable cell lines can be produced for evaluating the therapeutic potential of channel-active drugs.

260.17

DIAZEPAM DOES NOT ALTER THE GATING KINETICS OF GABA RECEPTOR CHANNELS. C.J. Rogers, R.E. Twyman and R.L. Macdonald. Dept of Neurology, University of Michigan, Ann Arbor, MI, 48104.

We have reported previously that diazepam (DZ) increased the frequency of GABA receptor channel currents (Soc Neurosci Abst 12:669,1986). We now report the effects of DZ on the gating kinetics of the GABA receptor channel.

Dissociated fetal mouse spinal cord neurons were cultured for 3-6 weeks prior to use. The recording pipette and bathing media were Hepes buffered and had equal Cl^- concentrations. GABA (2 uM) with or without DZ (20 or 100 nM) was applied to the patch by pressure ejection from blunt micropipettes. When DZ was studied, it was added to the bathing medium. Strychnine was added to all solutions to block glycine receptor currents. Outsideout patches were voltage clamped at the potassium equilibrium potential (-75 mV). Currents were digitized at 8 KHz with a 1 KHz 8-pole Bessel filter and analyzed using a 50 percent threshold for channel detection.

GABA receptor channels opened to at least two conductance states (27 and 19 pS). Gating kinetics of the larger, more frequent 27 pS channel were analysed. DZ (20 or 100 nM) produced a concentration-dependent increase in the frequency of GABA-gated currents. Frequency distributions (F(O)s) of open durations of GABA receptor currents were fit with three exponential functions, suggesting that the GABA receptor channel opened into at least three different open states. After addition of DZ, the F(O) of GABA receptor currents was unchanged. DZ did not alter the time constants or relative areas of the exponential functions. Thus, DZ did not alter the gating kinetics of the GABA receptor channel but increased chloride current by increasing the frequency of channel opening.

260.18

GABA MAIN STATE AND GLYCINE SUBSTATE RECEPTOR CHANNELS HAVE SIMILAR GATING KINETICS - ADDITIONAL SUPPORT FOR A RECEPTOR SUPERFAMILY R.E. Twyman, C.J. Rogers and R.L. Macdonald. Dept of Neurology, University of Michigan, Ann Arbor, MI, 48104.

GABA and glycine (Gly) receptor channels have been shown to have significant sequence homology in their subunit peptides and to open to the same conductance states. The structural holomogy of the receptors suggests that GABA and Gly receptor channels may also have similar gating kinetics. To evaluate this, we recorded single channel currents from outside-out patches obtained from 2-5 week old mouse spinal cord neurons grown in cell culture.

Bathing and intrapipette recording medium consisted of a HEPES-buffered, symmetrical chloride solution. Patches were clamped at the potassium equilibrium potential (-75 mV). Pressure ejection micropipettes were used to apply GABA (1 uM) or Gly (1 uM). Responses were digitized at 20 kHz with a 2 kHz 8 pole Bessel filter and analyzed by computer.

GABA and Gly receptor channels both opened to at least 3 conductance states (19, 27 and 44 pS). The main states for GABA and Gly were 27 and 44 pS, respectively. Since the GABA receptor channel only opened rarely to the 44 pS state, the gating properties of the 27 pS states of the receptor channels were compared. The frequency distributions of open durations (F(O)s) for the 27 pS states were similar. The F(O)s for the 27 pS states were fit with 4 exponential functions whose time constants and relative areas were not significantly different. These results suggest that the gating domains of the 27 pS GABA and Gly receptor channels are similar, consistent with membership in a receptor superfamily.

260.19

ETHANOL ENHANCES A COMPONENT OF GABA-GATED CHLORIDE CHANNEL CURRENTS IN RAT DORSAL ROOT GANGLION NEURONS. M. Nishio* and T. Narahashi (SPON: E.M. Silinsky). Department of Pharmacology, Northwestern University Medical School, Chicago, IL 60611.

Behavioral and electrophysiological studies suggest that the effects of ethanol on CNS are mediated at least in part through interactions with GABA receptor-channel complex. A recent study has shown that GABA-induced ^{36}Cl influx in cultured spinal cord neurons is greatly enhanced by a low concentration (10 mM) of ethanol (Ticku, Brain Res. Bull., 17, 123, 1986). However, the effect of ethanol on the GABA-gated chloride channel current is yet to be determined. Membrane currents in response to bath application of GABA were recorded from primary cultured neurons of rat dorsal root ganglia using the whole cell patch clamp technique. The external and internal solutions were designed to isolate the chloride current. GABA produced an inward current which was composed of a transient peak and a sustained current. Both components were blocked by bicuculline. Ethanol at concentrations of 3 to 100 mM enhanced the initial peak component of the GABA-induced chloride current in a dose-dependent manner without affecting the sustained component. The maximum increase in the current amplitude was approximately 30%. This action appears to contribute to the depressant effect of ethanol on CNS (supported by NIH grant NS14144).

260.20

PROPERTIES OF SINGLE CAPSAICIN-ACTIVATED CHANNELS. C.A. FORBES* and S. BEVAN* (SPON: K. Valentino) Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN, U.K.

Capsaicin is the pungent agent present in the hot Capsicum pepper. It has a specific excitatory action on mammalian polymodal nociceptive neurones. We have shown in whole cell voltage clamp recordings from cultured primary sensory neurones from the dorsal root ganglia of adult or neonatal rats that capsaicin, at sub-micromolar concentrations, causes an increase in the membrane conductance. This results in membrane currents which can be carried by sodium, potassium, caesium, calcium, and possibly other cations (Bevan and Forbes, 1988, J.Physiol. 398 28P).

We have now made recordings in cell-free outside-out membrane patches of single channel currents flowing through membrane channels activated by capsaicin. The channels have a conductance of 25-30pS at -80mV, but the single channel conductance is not linearly related to holding potential, being around 70pS at +40mV. We attribute this to the presence of calcium ions in the medium bathing the external face of the membrane, since in bathing medium containing no added calcium ions much of the non-linearity is lost. We therefore suggest that calcium can permeate the capsaicin-activated channels, but that it does so less well than other cations such as sodium, and hence brings about a reduction in the observed single channel conductance.

ION CHANNELS: CHLORIDE AND OTHER

261.1

A DELAYED COMPONENT OF THE CALCIUM-DEPENDENT CHLORIDE CURRENT IN XENOPUS OOCYTES INJECTED WITH BRAIN MESSENGER RNA. B. Gillo,* T.M. Moriarity,* S. Sealfon,* J. Roberts* and E. Landau (SPON J. Eisenman). Depts. of Psychiatry and Pharmacology and the Fishberg Molecular Neurobiology Center of the Mt. Sinai School of Medicine and the Bronx VA Medical Center, New York, NY

When the membrane potential in oocytes injected with brain RNA was jumped to +10 mV, two peaks of outward chloride current were observed (Tout 1 and Tout 2) with average latencies of 90±10 ms (n=31) and 710±55 ms (n=31) respectively. Both currents had similar time constants of decay and similar amplitudes. Only Tout 1 was found in native oocytes and its amplitude was much smaller than that seen in RNA-injected oocytes. Tout 1 was more sensitive than Tout 2 to changes in extracellular calcium but only Tout 2 was enhanced by exposing the oocytes to serotonin (0.5-2 nM) or phorbol myristate acetate (10 nM). Injecting the oocytes with barium (300-400 pmol) blocked Tout 2 but not Tout 1. It is concluded that the brain RNA induces a novel calcium-induced calcium release mechanism in oocytes which can be regulated by transmitter via the the phosphatidylinositol second messenger system.

SUPPORTED BY VA GRANT # 4125-020.

261.2

CHLORIDE CURRENTS GENERATED BY INJECTION OF ETHANOL INTO XENOPUS OOCYTES. K.A. Wafford* R.A. Harris and T.V. Dunwiddie, Dept. of Pharmacology, Univ. of Colo. Hlth. Sci. Cntr., and V.A. Medical Center, Denver, CO 80262

Xenopus oocytes possess a common receptor-channel coupling system for a number of different receptors. Native muscarinic receptors, and also those expressed from rat brain mRNA, including 5-HT, glutamate, noradrenalin and recently neurotensin and substance K, couple to this system (Parker et al., Proc. Roy. Soc. B, 231:37, 1987). These receptors activate calcium-dependent chloride channels by releasing calcium from intracellular stores via inositol phosphate formation (Nomura et al., Mol. Brain Res., 2:113, 1987). Injection of IP_3 also elicits a calcium-dependent chloride current (Parker & Miledi, Proc. Roy. Soc. B, 228:307, 1986). Ethanol has recently been shown to directly release calcium from intracellular stores in mouse brain synaptosomal preparations (Daniell et al., Mol. Pharm., 32:831, 1987). Ethanol and IP_3 also release microsomal calcium but from different pools (Daniell, pers. comm.). Defolliculated oocytes were perfused with modified Ringer's media (OR-2). Ethanol was pressure-injected into the oocytes, intracellular concentration determined by measuring the drop diameter with various injection times. Currents were recorded under voltage-clamp conditions and drugs were applied by perfusion. Injection of ethanol elicited a dose-dependent inward current consisting of a fast and slow component similar to that produced by IP_3. The EC_{50} of the slow response was 56 ± 10mM (n=4). The current reversed at approximately -20mV, close to the equilibrium potential for chloride in oocytes and this reversal potential shifted in a positive direction in the absence of extracellular chloride. The response was also reduced in the presence of the chloride ion transport inhibitors, SITS (1mM) and DIDS (500uM). Perfused ethanol gave similar responses. These results show that ethanol elicits a chloride current similar to that produced by IP_3 and many receptors expressed in the oocyte membrane. Previous work suggests that this may be due to ethanol-induced release of calcium from intracellular stores which in turn opens calcium-dependent chloride channels in the membrane.
Supported by the V.A. and grants AA06399.

261.3

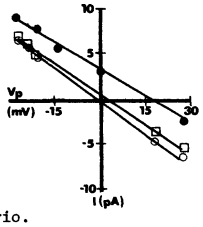
EFFECTS OF INHIBITORY AMINO ACIDS ON CHLORIDE INFLUX IN ADULT AND DEVELOPING MOUSE BRAIN. Pirjo Kontro, E.R. Korpi and S.S. Oja. Department of Biomedical Sciences, University of Tampere and Research Laboratories, State Alcohol Company (Alko Ltd.), Helsinki, Finland.

The effects of inhibitory amino acids on the chloride influx through the chloride channel associated with the GABA receptor were investigated with brain homogenates from adult and 3-day-old mice. The homogenates from different brain regions were incubated for 2 s at room temperature in Krebs-Ringer-Hepes-glucose medium, pH 7.5, with radioactive chloride. GABA increased the chloride influx dose-dependently, the stimulation being maximal, about 3-fold, at 0.1 mM GABA in both adult and developing cerebral cortex. The GABA stimulation was lowest, about 1.3-fold, in the adult brain stem. Taurine, β-alanine and hypotaurine also enhanced the chloride fluxes, but their effects were more pronounced only at millimolar concentrations in both adult and immature brain tissue. Glycine had no marked effects on the chloride fluxes in homogenates from either the brain stem or higher brain regions. Picrotoxin, bicuculline and strychnine did not affect the basal chloride flux but blocked the GABA-, taurine-, β-alanine- and hypotaurine-stimulated fluxes in all brain regions studied. Moreover, taurine at millimolar concentrations inhibited the GABA-stimulated fluxes. The results show that, besides GABA, also other inhibitory amino acids can interfere with the function of chloride channels.

261.4

EVIDENCE FOR A LARGE CONDUCTANCE CHLORIDE CHANNEL IN CULTURED GLOMUS CELLS OF THE RAT CAROTID BODY. A. Stea* and C.A. Nurse. Dept. of Biology, McMaster University, Hamilton, Ontario, L8S 4K1.

In order to understand chemosensory mechanisms in the carotid body we are studying the physiology of cultured (parenchymal) glomus cells using the patch clamp technique. We report on the presence of a large conductance chloride channel (ca. 300 pS) in inside-out patches of glomus cells. In symmetrical 140 mM NaCl solutions the single channel I-V relation reversed at 0 mV (see Fig. open circles) and was hardly affected when the bath contained 70 mM NaCl: 70 mM choline Cl (open squares). Replacing half of the Cl^- in the bath by the impermeant anion glutamate resulted in a shift in the reversal potential to ca. +18 mV (V_p; closed circles), the predicted value for E_{Cl}. This finding together with the demonstration that these cells contain carbonic anhydrase (see accompanying abstract, Nurse et al.) raises the possibility that intracellular and probably extracellular pH regulation may be an important function of glomus cells. Other ion channels in these cells are presently being characterized. Supported by the Heart and Stroke Foundation of Ontario.



261.5

DEVELOPMENT OF NEURONAL EXCITABILITY: A DECREASE IN CALCIUM-ACTIVATED CHLORIDE CONDUCTANCE OF EMBRYONIC SPINAL NEURONS IN CULTURE. N. Hussy*(SPON: G.L.Harris). Dept of Neurosci., UCSD, La Jolla.

Voltage-dependent calcium, sodium and potassium conductances have previously been shown to change as *Xenopus* spinal neurons differentiate *in vitro*. However intracellularly recorded action potentials are always long in duration and Ca-dependent in the absence of external Na and the presence of TEA, to block K channels. Their enhancement by the reduction of external chloride and the dependence of this effect upon external calcium indicates the presence of a calcium-activated chloride conductance. This conductance is found in 75% of the neurons.

At one day in culture replacement of 90% of the external Cl with methane sulfonate caused a nearly 5 fold increase in action potential duration without altering its amplitude. When external Ca was reduced from 10 to 1 mM, replacement of Cl now caused an increase in both amplitude and duration of the smaller action potential. In experiments performed on cells after 6 to 9 hrs in culture, Cl substitution induced a nearly 20 fold increase in action potential duration. Reduction of extracellular calcium resulted in greater increases in amplitude and duration of the impulse than observed at one day in culture. In all cases, removal of external calcium abolished these action potentials.

The Ca-activated Cl conductance appears to undergo at least a 2 fold decrease during the first day in culture, when the developmental increase in potassium conductance is taken into account. This conductance will normally act to repolarize cells. However since it activates only slowly, it may not be involved in the action potential but in modulation of electrical activity. The ionic dependence, kinetics and pharmacology of this conductance are presently being studied with whole cell patch clamp recordings. Supported by NIH NS15918.

261.6

CHARACTERIZATION OF PROTON CURRENTS IN PERFUSED SNAIL NEURONS Y. Suen* & L. Byerly (Spon: George J. Augustine). Neurobiology Section, USC, Los Angeles, CA 90089-0371.

Internal-perfusion voltage-clamp and patch clamp techniques were used to study proton currents in neurons of the snail Lymnaea stagnalis. In whole cells the voltage-activated outward H^+ current density was typically 15 nA/nfd (range: 4.2-29.0 nA/nfd) measured at +40 mV with $[K^+]_i$=0 and pH_i=5.9, while the prolonged, voltage-activated K^+ current was typically 38 nA/nfd (range: 9.5-61.9 nA/nfd) measured at +40 mV with $[K^+]_i$=74 mM and pH_i=7.3. In inside-out patch studies, these currents were found to be distributed differently. While all patches had K^+ currents (2-35 pA at +30 mV) only 3 out of 30 patches studied had H^+ currents. The patch H^+ currents recorded, 5-21 pA in amplitude, exhibited little noise, suggesting that the unitary H^+ current is very small. The relative ability of various extracellular divalent cations to block the H^+ current was found to be Zn=Cu>Ni>Cd>Co>Mn>Mg=Ca=Ba. Thus, 100 μM Zn^{2+} helps to isolate Ca^{2+} currents from H^+ current. The Q_{10} of the H^+ current magnitude was 2.1, while that of the prolonged K^+ current was 1.4. All of these results demonstrate that H^+ current flow through distinct channel from those of the prolonged K^+ currents, even though these currents have similar voltage-dependence and kinetics.

261.7

COMPUTER ANALYSIS OF ION-SELECTIVE MICROELECTRODE RESPONSES: CALIBRATION AND RECORDING. A.W. Barolet, R. Andrews* and M.E. Morris. Playfair Neuroscience Unit and Departments of Anaesthesia and Pharmacology, University of Toronto, Toronto M5S 1A1.

A user-logical command-driven program, written in 'C' has been developed for the rapid and accurate analysis of both on- and off-line calibration and experimental data recorded with ion-selective microelectrodes. Required hardware includes an IBM-PC or compatible system with hard disc and graphics adaptor, parallel printer or serial plotter for graphics. Special features are (i) non-linear least squares fit of curves to data, (ii) optional selection and use of activity coefficients, (iii) calculation of selectivity coefficients using fixed interference or separate solution methods, (iv) conversion of voltage or length data to ion activity/concentration and correction for drift. Electrodes are calibrated in a stationary position in the recording bath or with a simple polyethylene flow-through chamber. Solutions with various concentrations of the ion to be measured are selected manually or by computer from one of six reservoirs by means of a controller circuit with relays and voltage-activated valves. Gravity flow of $\leq$ 4 ml/min to the calibration chamber is via an 8-way connector and monitored with a flowmeter.

262.1

CHARACTERIZATION OF CALCIUM CURRENTS IN RELAY NEURONS ISOLATED FROM MAMMALIAN THALAMUS.

J.R. Huguenard, D.A. Coulter, and D.A. Prince. Department of Neurology, Stanford University Medical Center, Stanford, CA 94305.

The transfer properties of the thalamo-cortical relay neurons (RNs), which control the flow of information from the periphery to the neocortex, are critically dependent on a prominent low threshold calcium spike (LTS, Llinas & Jahnsen, *Nature* 297:406, 1982). We examined the Ca currents underlying the LTS in neurons isolated from the ventrobasal (VB) complex of the thalamus in guinea pig and rat. This is the first detailed study of the various components of Ca current in mammalian thalamic neurons.

VB neurons were isolated by the method of Kay and Wong (*J. Neurosci. Meth.* 16:227, 1986), and putative RNs (somatic diameter > 10 um) were selected for study. Ca currents were isolated with appropriate intracellular and extracellular solutions, and were recorded by whole cell clamp. RNs have 3 types of Ca currents, T, N, and L, all of which are similar to the Ca currents recorded in dorsal root ganglion (DRG) neurons (Nowycky, *et al.*, *Nature* 316:440, 1985). In contrast to DRG cells, RNs are characterized by a much larger transient (T) current (up to 1 nA) that was often larger than the maximal L current, while the N component was always either small or absent. The large proportion of T current in RNs compared to DRGs may account for some of the functional differences between these two neuronal types. The high threshold and low threshold components were selectively susceptible to blockade by Cd (20uM) and Ni (100 uM), respectively. The T component showed threshold activation around -70 mV, peak activation near -30 mV, and steady state inactivation was half complete near -90 mV. We conclude that the properties of T-current in RNs are perfectly suited to produce LTSs: T-current is large, transient, activated at relatively low threshold, steady state inactivated near rest, and inactivation is removed by hyperpolarization.

Supported by NIH grants NS06477 and NS 12151.

262.2

ANTICONVULSANTS DEPRESS CALCIUM SPIKES AND CALCIUM CURRENTS OF MAMMALIAN THALAMIC NEURONS IN VITRO.

D.A. Coulter, J.R. Huguenard, and D.A. Prince. Dept. of Neurology, Stanford University Medical Center, Stanford, CA 94305

Thalamic neurons are known to play a role in the generation of normal and abnormal thalamocortical rhythms, including spike-wave disharges characteristic of petit mal seizures. Low-threshold calcium spikes (LTS) in thalamic neurons are thought to be of primary importance in the generation of normal thalamocortical rhythms, such as sleep spindles. Therefore, we postulated that the thalamic LTS might also be important in the generation of abnormal thalamocortical rhythms, such as spike-wave discharges, and further that anti-petit mal agents might act, at least in part, to reduce this conductance as a mechanism of action.

Intracellular recordings from guinea-pig thalamic slices demonstrated that ethosuximide (ES) and dimethadione (DMD), anti-petit mal drugs, reduced LTSs in thalamic relay cells, resulting in changes in rhythmic firing properties. Whole-cell voltage-clamp recordings from dissociated guinea-pig or rat thalamic neurons revealed at least two distinct calcium currents, based on voltage-dependence and kinetics of activation, inactivation, and deinactivation (Huguenard et al, this volume). ES and DMD reversibly reduced the low- and high-threshold calcium currents (L- and HTCCs) in a dose-dependent manner, at concentrations equal to clinical free serum levels. ES reduced the LTCC to a greater extent than the HTCC, while DMD was less specific for the LTCC. Succinimide, the inactive structural analogue of ES, had no such effect at equivalent concentrations. Valproic acid had only small effects on the LTCC. Phenobarbital reduced the L- and HTCC, while carbamazepine and phenytoin had no effect on these currents at clinically relevant concentrations. These data suggest that reduction of calcium currents in thalamic neurons may be a potential mechanism of action of anticonvulsants effective in petit mal. Supported by NIH grants NS06477, NS12151 and NS07280.

262.3

PHORBOL ESTER INCREASES CA^{++} CURRENT IN APLYSIA SENSORY NEURONS. O. Braha, M. Klein* and E.R. Kandel, Ctr. for Neurobiol. & Behav., HHMI, Columbia Univ., New York, NY 10032

Phorbol dibutyrate (PDBu), an activator of protein kinase C (PKC), facilitates the connection between sensory and motor neurons in cultured neurons of Aplysia (Hochner et al., Soc. Neurosci. Abstr., 1986, 12:1340). We have examined the effects of PDBu on the Ca^{++} current of sensory neurons in the absence of external Na^{+}, and with K^{+} currents blocked by extracellular K^{+}-channel antagonists (tetraethylammonium, 3,4-diamino-pyridine) and by intracellular injection of Cs^{+}. Voltage-clamp steps from a holding potential of -50 mV to +10 mV elicit inward currents that are increased by 20-100 nM PDBu, while the alpha isomer of PDBu, which does not activate PKC, has no effect. The increase in current produced by PDBu occurs with either Ca^{++} or Ba^{++} as the charge carrier. It shows a bell-shaped voltage dependence with a peak around +10 mV, a shape that is characteristic of Ca^{++}-dependent currents, and is blocked by the Ca^{++} channel antagonist Cd^{++} (0.1 mM). The current increased by PDBu resembles the slowly inactivating, nifedipine-sensitive, Ca^{++} current described in these cells by Edmonds, Kandel and Klein (Soc. Neurosci. Abstr., 1987, 13:792). We do not yet know how this current is related to the Ca^{++} current induced by phorbol esters in bag cells of Aplysia (Strong et al., Nature, 1987, 325:714-717).

262.4

SOMATOSTATIN INHIBITS CALCIUM CURRENTS IN AN ENRICHED POPULATION OF NORMAL ANTERIOR PITUITARY CELLS. I.Nussinovitch* (SPON: M. Schramm) Department of Physiology, Hebrew University-Hadassah Medical School, P.O.Box 1172, Jerusalem 91010, Israel

Somatostatin is known as the physiological inhibitor of growth hormone secretion from the anterior pituitary gland. Recently, it has been reported that somatostatin inhibits a voltage-gated calcium current in a pituitary cell line (Luini et al, J.Neurosci., 6:3128, 1986). The present study shows that somatostatin inhibits a voltage-gated calcium current in rat somatotrophs (an enriched population). Calcium currents were recorded in the whole-cell configuration of the patch-clamp technique, usually in response to a depolarizing step from a holding potential of -80mV to 0mV. Bath solution contained: NaCl 130 mM, KCl 5 mM, $CaCl_2$ 10mM, $MgCl_2$ 1 mM, glucose 10 mM, Hepes 10 mM and TTX 3 µM. Intracellular solution contained: CsCl 140 mM, $MgCl_2$ 2mM, EGTA 11 mM, Hepes 10mM, ATP 2mM, GTP 80 µM. After a control period of recording, somatostatin (61 nM) was applied to the cell by diffusion from a pipette placed near the surface of the cell. In 7 out of the 8 cells exposed to somatostatin calcium currents were reduced by 30-60%. The onset of the inhibition exerted by somatostatin was rapid (seconds) and recovery was completed usually less than 60 seconds after removing the diffusion pipette from the cell. In 5 cells with similar results somatostatin was applied repeatedly (at least 3 times). This inhibitory effect of somatostatin may be the underlying mechanism for the inhibition of growth hormone secretion. This work was supported by a Kohn fellowship and by the U.S.-Israel Binational grant No. 86-00198.

262.5

SINGLE L-TYPE CALCIUM CHANNELS IN DORSAL ROOT GANGLION NEURONS CAN BE MODULATED BY NOREPINEPHRINE. C.S. Anderson* and K. Dunlap. Department of Physiology, Tufts Medical School, Boston, MA 02111.

Norepinephrine-induced modulation of macroscopic calcium current in embryonic chick dorsal root ganglion neurons has been well-described. Results of Nowycky et al. (Nature, 316:440,1985) illustrate that at least three types of voltage-dependent calcium current can be recorded from these cells and descriminated both at the macroscopic and single channel level. Which of these is (are) a target for modulation by the transmitters? On the basis of macroscopic currents alone, we have suggested that the L-type calcium channel is one target for modulation by norepinephrine, but definitive support for this idea requires single channel recordings. Here we report that, in cell-attached patch recordings, Bay K 8644-sensitive L-type channels (ca. 25 pS) could be recorded following depolarization and were subject to modulation by norepinephrine applied to the extrapatch membrane. The modulation was associated with an increase in the number of null sweeps and a decrease in the overall number of single channel events detected following stimulation. These results support the idea that L-type calcium channels are modulated by norepinephrine and underscore the notion that the mechanism of the modulation involves the activation of a second messenger pathway. Future experiments will address whether the other types of calcium channel recorded from these cells also undergo transmitter-induced modulation.

262.6

2-CHLOROADENOSINE SELECTIVELY REDUCES THE N-CALCIUM CURRENT OF MOUSE DORSAL ROOT GANGLION NEURONS IN A PERTUSSIS TOXIN-SENSITIVE MANNER. T. Ryan-Jastrow, R.A. Gross and R.L. Macdonald. Dept. of Neurology, Univ. Michigan, Ann Arbor, MI 48104

Adenosine and its analogues shorten calcium-dependent action potential (CAP) duration and reduce calcium (Ca) current in mouse dorsal root ganglion (DRG) neurons (Macdonald *et al.*, J Physiol 370:75, 1986). The effect of an adenosine analogue, 2-chloroadenosine (CADO), on the three types of Ca current (T, L, and N) in mouse DRG neurons was studied. The involvement of a G-protein in the regulation of the effect of CADO on CAP duration and Ca current was studied using pertussis toxin (PT).

Intracellular recordings were made with micropipettes filled with 3M KAc (CAP) or 3M CsCl (voltage clamp). Recording media (pH=7.35) contained (in mM): Tris, 13; NaCl (CAP) or choline-Cl, 67; KCl, 5; TEA, 5 (CAP) or 100; $CaCl_2$, 2.0; glucose, 5.6; $MgCl_2$, 0.8. A single electrode voltage clamp was used that switched at 8-12 kHz with a 70-30% cycle. Voltage steps were generated and currents stored using a microcomputer. CADO was applied by pressure ejection from blunt-tipped micropipettes. Three Ca current components were recorded, the transient low-threshold, transient high-threshold and slowly-inactivating high-threshold currents (T,N,L).

CADO reduced CAP duration (10-80 %) but did not affect isolated T- or L-Ca current. In combined N- and L-Ca current, CADO produced a 14-57 % reduction (n=22) in the peak current with little change in the late current, suggestive of a selective decrease in N-Ca current. Pretreatment of cultures with PT (100 ng/ml) for 16 hours blocked the reduction of CAP duration and N-Ca current by CADO.

Supported by NIH DA5345 to TRJ, NS01019 to RAG and NS19692 to RLM.

262.7

NEUROPEPTIDE Y AND PHORBOL ESTERS REDUCE THE N-TYPE CALCIUM CURRENT OF ADULT RAT NODOSE NEURONS BY DIFFERENT MECHANISMS. J.W.Wiley, R.A.Gross, N. Fox & R.L.Macdonald, Depts. Internal Medicine (JWW) and Neurology, Univ. Michigan, Ann Arbor, MI 48104, USA.

Neuropeptide Y (NPY) serves as an inhibitory neuroregulatory peptide in the central and peripheral nervous systems. We tested whether NPY reduces calcium (Ca) current via GTP binding (G) proteins. Ca currents were obtained from acutely dissociated adult rat nodose neurons using the single electrode voltage-clamp technique. Recording medium contained choline, and the recording micropipette contained cesium to permit recording of Ca currents. Three calcium current components were identified using 5mM Ca as the charge carrier. The transient low-threshold, transient high-threshold and slowly-inactivating high-threshold currents (T,N,L, respectively) differed in their voltage ranges of activation and inactivation, and their sensitivities to Ca-channel blockers. Drugs were applied from blunt-tipped micropipettes. NPY selectively reduced N-current 12 +/- 4 to 52 +/- 8% (1nM-1uM, respectively) in 47% (36/77) of neurons. The effect of NPY was voltage-independent and blocked by pertussis toxin (PTX), an inactivator of some G-proteins. Phorbol esters, activators of protein kinase C, have been shown to reduce Ca current in primary afferent neurons by a PTX-sensitive mechanism (Gross & Macdonald, Neurosci Lett., in press). Phorbol ester (PDBu-500 nM) selectively reduced the N-current 24 +/- 6% (n=6). This effect was present only at holding potentials positive to -70 mV and was blocked by pretreatment with 100 ng/ml PTX for 12-24 hr. Thus, the effects of both NPY and phorbol esters were blocked by PTX. The action of NPY was, however, voltage-independent while that of phorbol ester was voltage-dependent, suggesting different mechanisms of action on the N-channel. Supported by an AGA grant to JWW, NIH-NS01019 to RAG and NS19692 to RLM.

262.8

ACTIVATION OF A-KINASE REDUCES THE N-TYPE CALCIUM CURRENT IN MOUSE SENSORY NEURONS BY ENHANCING INACTIVATION. R.A. Gross and R. L. Macdonald, Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104, USA

Forskolin (FOR), 8-Br-cyclic AMP (8-Br), and cholera toxin (CTX) were used to study the effect of the cyclic AMP/A-kinase system on calcium currents in mouse dorsal root ganglion (DRG) neurons. Activation of A-kinase has been shown to increase Ca currents in invertebrate neurons and in vertebrate cardiac cells. In contrast, FOR and 8-Br reduced Ca current in mouse DRG neurons (Gross and Macdonald, Neurosci. Lett., in press).

Intracellular recordings were made with micropipettes filled with 3M CsCl (20-30 MOhm). Recording medium (pH=7.35) contained (in mM): Tris, 13; choline-Cl, 67; KCl, 5; TEA, 100; $CaCl_2$, 2.0; glucose, 5.6; $MgCl_2$, 0.8. A single electrode voltage clamp was used that switched at 8-12 kHz with a 70-30% cycle. Voltage steps were generated and currents were stored (digitized at 2-6 kHz) using a microcomputer. Drugs were applied by pressure ejection from blunt-tipped micropipettes. Three Ca current components were recorded, the transient low-threshold, transient high-threshold and slowly-inactivating high-threshold currents (T,N,L). They had different voltage ranges of activation and inactivation, and different sensitivities to Ca channel blockers.

None of the compounds affected the isolated T current. FOR, 8-Br and CTX reduced the peak but not the late current of combined N and L currents, but only when applied at holding potentials positive to -75 mV. Multi-exponential curve-fitting showed this to be a selective effect on the N current component. FOR also hastened current inactivation at depolarized potentials, and increased steady-state inactivation. These results suggest that activation of A-kinase selectively reduced N current by enhancing inactivation.

Supported by NIH NS01019 to RAG and NS19692 to RLM.

262.9

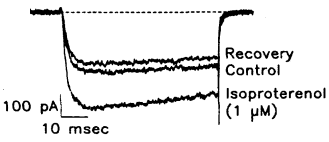
β-ADRENOCEPTOR MODULATION OF CALCIUM CHANNELS IN ACUTELY EXPOSED CA3 PYRAMIDAL NEURONS OF ADULT GUINEA PIG HIPPOCAMPUS. *R.E. Fisher*, R. Gray and D. Johnston* (SPON: H.F. Epstein). Prog. in Neurosci., Baylor College of Medicine, Houston, TX 77030

The induction of long-term potentiation (LTP) appears to require a post-synaptic calcium influx. Unlike at the Schaffer collateral to CA1 synapse, LTP at the hippocampal mossy fiber to CA3 synapse is not blocked by NMDA receptor antagonists. We are exploring the possibility that, in CA3 neurons, voltage-dependent calcium channels provide the necessary calcium influx. This is suggested by the findings that β-adrenoceptor agonists 1) enhance LTP at the mossy fiber synapse (Hopkins and Johnston, *J. Neurophysiol.* 59:667, 1988) and 2) enhance calcium channel activity in dentate granule cells (Gray and Johnston, *Nature* 327:620, 1987). In the present experiments, calcium channels in adult CA3 pyramidal cells were studied using patch clamp techniques applied to the acutely exposed hippocampal neuron preparation of Gray and Johnston (*J. Neurophysiol.* 54:134, 1985). Barium currents in cell attached patches revealed the presence of at least three distinct calcium channel conductances: 9, 14, and 26 pS. From a holding potential of -80 mV, the 14 pS channel showed significant activity during steps positive to -20 mV. The activity of this channel was enhanced by the β-adrenergic agonist isoproterenol, applied by pressure pipette (10 μM in pipette, avg 420% increase during steps from -80mV to either -10, 0, or +10 mV, n=7). Control applications of bath saline did not significantly affect channel activity (n=4). The 9 pS channel began to activate slightly during steps to -40 or -30 mV, and strongly during steps positive to -20 mV. It was largely inactivated by holding at -40 mV. Isoproterenol did not appear to affect the activity of this channel (n=2). The largest channel showed significant activation during steps from a holding potential of -40 mV to command potentials more positive than 0 mV. Its modulation has not yet been examined. These results support the hypothesis that at least one type of postsynaptic, voltage-dependent calcium channel may be involved in the neuromodulation and induction of LTP in CA3 neurons. (NIH grants NS11535 & HL31164 and AFOSR 85-0178).

262.10

REGULATION OF CALCIUM CURRENTS IN A SMOOTH MUSCLE DERIVED CELL LINE. T. N. Marks*, G.R. Dubyak*, and S. W. Jones. (Spon: E.F. Nemeth). Dept. of Physiol. and Biophys. Case Western Reserve Univ., Cleveland, OH 44106.

We have studied the regulation of calcium currents in the A7r5 cell line by applying techniques of electrophysiology, fluorometry, and radioimmunoassay (RIA). When cells were voltage clamped in the whole-cell configuration with near normal intra- and extracellular solutions, a current resembling the 'L' type Ca^{2+} current was dominant. With Ba^{2+} (10 mM) as the charge carrier, peak inward currents of -300 pA were typically recorded at test potentials between 0 and +20 mV. Forskolin (30 μM) or the β-adrenergic agonist isoproterenol (1 μM) increased peak currents an average of -x2. Confluent monolayers of cells loaded with the Ca^{2+} sensitive fluorescent indicator, fura-2, were apparently electrically coupled and showed spontaneous transient Ca^{2+} elevations. Bath application of isoproterenol or forskolin increased the frequency and magnitude of the transient elevations. Dihydropyridine Ca^{2+} channel agonists increased spike activity in a like manner, whereas antagonists abolished transient rises. Both isoproterenol and forskolin acted to raise cAMP in these cells as determined by RIA, suggesting that regulation of the Ca^{2+} current is mediated by cAMP dependent protein kinase.



262.11

SYNAPTICALLY RELEASED DOPAMINE INHIBITS CALCIUM CHANNELS. P.J. Williams, B.A. MacVicar and Q.J. Pittman, Neuroscience Research Group, University of Calgary, Calgary, Alberta T2N 4N1 Canada.

Dopamine (DA) inhibits spontaneous electrical activity and hormone release from melanotrophs of the pituitary intermediate lobe (IL). To test whether synaptically released DA directly inhibits Ca^{++} channels intracellular recordings were obtained from cells of the intact IL using electrodes containing K^+ channel blockers (.5MCs Acetate, 30mM TEA) and a sodium channel blocker (1mM QX-222). Voltage clamp studies showed 3 types of Ca^{++} current corresponding to T, N, & L types which were abolished by Co^{++} and Cd^{++}. Stimulation of the pituitary stalk (in the presence of Bicuculline (.1mM) to block GABAergic synapses) reduced all three currents (n=18) although the relative percent inhibition varied from cell to cell. These effects were mimicked by Quinpyrrole (50 μM,n=5) a DA D-2 agonist and were abolished by Domperidone (1 μM, n=3) a D-2 antagonist. Experiments were carried out to assess the role of G-proteins in this inhibition. In cells impaled with electrodes containing GTPγS, a Gi/Go activator, Ca^{++} currents were reduced and neither stalk stimulation nor Quinpyrrole caused a further reduction in current amplitude. We conclude that, in the cells of the IL, synaptic activation of the D2 receptor results in inhibition of Ca^{++} currents by a mechanism involving G-proteins in an intermediate step.

262.12

CHRONIC DEPOLARIZATION REDUCES Ca^{2+} CURRENT DENSITY IN CULTURED RAT MYENTERIC NEURONS. J.L. Franklin* and A.L. Willard. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599

We are interested in the role of Ca^{2+} influx through voltage-gated channels during neuronal development and have been investigating the influence of electrical activity on long term changes in Ca^{2+} currents.

Myenteric neurons dissociated from the small intestines of newborn rats were grown in culture medium containing either 5 or 25 mM K^+ (Nishi & Willard, 1985). The 25 mM K^+ causes a sustained 20-25 mV depolarization of the neurons. Whole cell patch clamp experiments were performed using 20 mM Ba^{2+} as the charge carrier. Current density was normalized to membrane capacitance. Na^+ and K^+ currents were eliminated by replacing them with impermeant ions.

A Ba^{2+} current with both rapidly (τ = 25-60 msec) and very slowly (τ > 450 msec) inactivating components was evoked by steps to potentials positive to -20 mV from holding potential negative to -70 mV. For a step to 0 mV from a holding potential of -90 mV, the mean density of the transient peak current was 76 ± 29 pA/pF for 17 neurons grown in 5 mM K^+. The mean density at 300 msec after the onset of the step was 57 ± 27 pA/pF. In 22 chronically depolarized neurons, the peak current was reduced 59% to 31 ± 20 pA/pF and the sustained portion of the current was reduced 54% to 26 ± 18 pA/pF (p .001 in each case). Work is in progress to determine if the decrease is due to a decreased number of Ca^{2+} channels. Support: NIH NS24362.

262.13

DIFFERENTIAL ROLE OF Ca++ CHANNELS IN THE RELEASE OF ^{3}H-ACh AND ITS MODULATION. D.B.Gray and G. Pilar. Dept. Physiol. & Neurobiol., Univ. Connecticut, 75 N. Eagleville Rd., Storrs, CT 06268.

We have shown that ^{3}H-ACh release can be evoked by 55mM KCl from intact ciliary ganglion (CG) nerve terminals on both isolated iris and choroid muscle in chick eye. We have also shown that somatostatin (SS) (Epstein, M. & J. Dahl, Soc. Neurosci. Abstr., 13: 683, 1987) is endogenous and inhibits this release in choroid but not in iris (Gray, D. & G. Pilar, Soc. Neurosci. Abstr., 13: 790, 1987). We are now interested in the nature of the voltage-sensitive Ca++ channels responsible for ACh release. Two types of channels, N and L, have been proposed for this function and can be differentiated with dihydropyridine (DHP) agents specific for L channels in either agonist or antagonist fashion. Nifedipine, a DHP Ca++ antagonist, even at 10μM, has no effect on 55mM KCl-evoked ^{3}H-ACh release from CG terminals in either iris or choroid. Similarly, Bay K 8644 a DHP agonist, cannot evoke ^{3}H-ACh release in normal Tyrodes from terminals in either iris or choroid, but inhibits the K+-evoked ^{3}H-ACh release by over 50% ($p < 0.5$) in the first 6 min. Adding Bay K 8644 to the iris preparation did not significantly affect K+-evoked ^{3}H-ACh release. These studies suggest that L channels do not directly regulate ACh release from terminals of CG neurons but may activate release of a neuromodulatory peptide such as SS from CG terminals in the choroid. Supported by NSF grant#BNS 8410581 and NIH grant#NS 10338

SENSORY SYSTEMS: AUDITORY SYSTEMS IV

263.1

THE EFFECTS OF NOISE ON TEMPORAL PARAMETERS IN AUDITORY NERVE FIBERS OF E. COQUI. R. Dunia and P.M. Narins. Dept. of Biology and Brain Research Institute, Univ. of Calif., Los Angeles, CA 90024.

E. coqui is an arboreal frog found in the rainforests of Puerto Rico. Behavioral studies indicate that E. coqui has adaptations to facilitate communication in its noisy environment (>95 dB SPL), and these adaptations suggest a high degree of temporal resolution.

We used two distinct paradigms to measure the minimum and maximum integration times (MinIT and MaxIT, respectively) of auditory nerve fibers. We measured MinIT by the synchrony of the fibers to SAM noise, and the effect of varying the noise spectrum level. Results from 113 fibers reveal that mid-frequency fibers (0.5<CF<1.8 kHz) have shorter MinIT than low-frequency fibers (CF<0.5 kHz) or high-frequency fibers (CF>1.8 kHz). MinIT decreased as noise level increased, consistent with decreased spectral resolution as noise levels increase.

We measured MaxIT by the shift in rate threshold with increased tone burst duration, and the effect of continuous noise (20-50 dB SPL) on MaxIT. Low-frequency fibers had longer MaxIT (270 ms) than high-frequency fibers (230 ms) or mid-frequency fibers (210 ms); continuous noise increased the MaxIT in 88% of the fibers tested. We conclude that much of the temporal integration in the anuran auditory system is already encoded in the fibers by the spike rate and does not require central processing. (Supported by NIH grant NS19725 to PMN).

263.2

RATE-INTENSITY RELATIONS IN SINGLE AUDITORY NERVE FIBERS OF THE MONGOLIAN GERBIL. K.K. Ohlemiller and S.M. Echteler. Auditory Physiology Laboratory and Department of Neurobiology and Physiology. Northwestern University, Evanston, IL 60208.

We have examined the relation of characteristic frequency (CF) and spontaneous firing rate (SR) to rate-intensity parameters of single auditory nerve fibers in the gerbil in order to permit comparison with other species.

Most of our data comprise steady-state firing rates (>20 msec after stimulus onset) in response to CF tonebursts. Sound levels ranging from -20 to 100 dB SPL are presented in pseudorandom order in 2 dB steps. The functions obtained are fit using a least-squares method to a variation of the model of Winslow and Sachs (J. Neurophysiol. 57:1002-21, 1987). Maximum firing rate (R_{max}) and slope at $R_{max}/2$ are derived from fitted curves.

Out of more than 100 rate-intensity curves obtained from fibers with CFs of .5 to 20 kHz, only 5 are nonmonotonic. The most prominent correlate of slope and R_{max} is CF; slope and R_{max} are greater for high CFs than for low. The distributions of these parameters show an abrupt rise near 4 kHz. Changing the stimulus frequency from CF to 1 kHz (a frequency where phase locking is pronounced) does not systematically alter slope (Schmiedt and Zwislocki, J. Neurophysiol. 43:1390-1405, 1980) or R_{max}. This suggests that the apparent CF-dependence is not related to neural phase locking. Slope is correlated secondarily with SR; low-SR fibers (SR<2 sp/sec) with thresholds above 20 dB (15 of 22 low-SR fibers) tend toward lower slopes than other fibers of similar CF (Ohlemiller and Echteler, Neurosci. Abstr., 1987). R_{max} values often could not be assigned to these fibers because most of them (11 of 15) are only partially saturated at 100 dB. We will also discuss the influence of data-collection paradigm on observed slope, R_{max}, and monotonicity.

(Supported by NIMH NRSA 5 F31 MH09565 to KKO and NS08635 from NINCDS.)

263.3

SINGLE-UNIT RESPONSES IN COCHLEAR NUCLEUS TO ELECTRICAL STIMULATION OF THE COCHLEA WITH BIPHASIC PULSES OF VARIABLE SHAPE. J.A. Wiler*, B.M. Clopton*, and M.A. Mikhail* (SPON: B. Pfingst). Kresge Hearing Research Institute, U. of Mich., Ann Arbor, MI 48109.

Electrical stimulation of cochlear axons and other sensory afferents with multiple electrodes is often requisite for prosthetic applications. Bipolar, pulsatile stimulation multiplexed across electrodes reduces crosstalk and provides many alternatives for encoding. Optimality for such pulses has not been established due to a lack of knowledge about their stimulation efficiency and its limitations on encoding strategies. We presented an extensive range of charge-balanced, square-wave pulse shapes to the guinea pig cochlea as constant-current waveforms. Their duration per phase was 20 to 900 usec and included initially positive and initially negative phases, some sets of stimulus pulses containing more than 180 pulse shapes. Spike counts for many single units in the ventral cochlear nucleus were increased by 100 times or more for specific pulse shapes as compared to less effective ones, and latencies, having minimal values of about 1.2 msec, also differed greatly. Usually a brief initial phase followed by a longer second phase produced the greatest spike counts, and in many instances more than one duration of the second phase was effective. The more effective polarity of the first phase was specific to a unit but about equally distributed between positive and negative for closely spaced bipolar electrodes. Minimal latencies were not necessarily associated with the pulse shapes producing maximal spike counts. Implications for encoding strategies follow from these results and reasonable assumptions about how information is represented in neuronal populations. (Supported by NIH Grant NS21440)

263.4

INTRACELLULAR RECORDINGS FROM DORSAL COCHLEAR NUCLEUS NEURONS IN VITRO. P.B. Manis* (SPON: P.F. Worley) Depts. of Oto.-HNS, Neuroscience, and Center for Hearing Sciences, Johns Hopkins Univ., Baltimore, MD 21205.

Neurons of the dorsal cochlear nucleus (DCN) respond to acoustic stimuli with discharge patterns significantly different than those of the auditory nerve fibers which innervate the nucleus. In order to clarify the physiological processes underlying these transformations, intracellular recordings were made in a brain slice preparation of the guinea pig DCN. Recordings were made from 36 cells; of these 16 meet the criteria (Vm<-50 mV, AP>45 mV) for acceptable recordings. These cells had a mean input resistance of 29.3 MΩ (N=12), and mean membrane time constant of 5.1 msec. Spike trains were regular (15/16 cells) one cell exhibited complex spikes. In 4 cells the membrane potential response to hyperpolarizing current pulses exhibited a sag suggestive of anomalous rectification. Seven of 8 cells injected with Lucifer Yellow were identified as pyramidal cells; the other cell was similar to the vertical cell type.

Paired shocks to the parallel fibers produce a burst of spikes from the cell in response to the second stimulus. Although the spike burst can be blocked by hyperpolarization of the cell, a small active potential remains in response to the second stimulus. This suggests the presence of a membrane conductance remote to the recording site which can facilitate the generation of spike bursts. (Supported by SNF BNS0867273 and NIH NS25065.)

263.5

RESPONSE PROPERTIES OF COCHLEAR NUCLEUS NEURONS IN YOUNG AND MIDDLE-AGED C57BL/6J MICE WITH PRESBYCUSIS. J.F. Willott, K. Parham, and K.P. Hunter*. Dept. Psychology, Northern Illinois Univ., DeKalb, IL 60115.

Extracellular recordings were obtained from the cochlear nucleus (CN) of anesthetized aging C57 mice using tone- and noise-burst stimuli. All typical CN response types (re: post-stimulus time histograms) were observed in middle-aged C57 mice (up to 15-mo.) with sensorineural impairment; however, there was a decline in the percentage of dorsal CN (DCN) units demonstrating inhibition and in ventral CN (VCN) units showing "chopper" or "on" responses.

Age-related decreases were observed in both the DCN and VCN for units' best frequencies (re: threshold or maximum discharge rates), upper frequency range, overall frequency range of response areas, sharpness of tuning, and threshold sensitivity for tones and noise. Changes in the latter three parameters were more pronounced in the VCN.

Supported by R01 AG03069, RR 07176, and RCDA AG00234.

263.6

Synaptic and Membrane Properties Shape Response Patterns of Neurons in the Gerbil Cochlear Nucleus. J. Feng*, E.-M. Ostapoff, S. Kuwada, and D.K. Morest. Dept. of Anatomy and Ctr. for Neurological Sciences, University of Connecticut Health Ctr., Farmington, CT 06032.

Neurons in the cochlear nucleus differ in their discharge pattern to tonal stimuli. Are these patterns shaped by different membrane properties or by synaptic circuitry? We made intracellular recordings from cochlear nucleus neurons and compared the responses to tones and to depolarizing current. Neurons that fired regularly to tones were of two types: sustained and transient choppers. Both types showed a sustained regular discharge to current, indicating that the regularity of firing for these neurons is an intrinsic property. Some transient choppers showed tone-evoked hyperpolarizations, which may have caused the transient nature of the chopping pattern. Neurons that fired irregularly to tones were of several types. Primary-like neurons fired irregularly to current, again suggesting intrinsic factors, albeit different from those seen in choppers. In contrast, other neurons that responded with an irregular discharge to tones fired regularly to current. A circuit with feedback inhibition is consistent with such a response profile. In sum, a neuron's response pattern may be determined by its membrane properties and its synaptic inputs.

Supported by NIH grants NS18027 and NS14347.

263.7

SEGREGATION OF SPONTANEOUS ACTIVITY IN AUDITORY NERVE INPUT TO THE COCHLEAR NUCLEUS: ENDBULBS OF HELD AND THEIR CONVERGENCE ONTO THE SAME SPHERICAL BUSHY CELL. S.Sento and D.K.Ryugo[1]. Dept. Public Health and Environ. Sci., Tokyo Med. and Dent. Univ., Tokyo, Japan and [1]Dept. Otolaryngol. and Neurosci., Johns Hopkins Univ., Sch. of Med., Baltimore, MD 21205.

Type I spiral ganglion neurons can be divided into two general groups based on their spontaneous activity (SR) and morphological properties (Liberman, Science, 216:1239, '82; Fekete et al., J.Comp.Neurolo.,229:432, '84). All axons of such neurons give rise to a terminal endbulb of Held, and when the form factor(FF), silhouette area divided by silhouette perimeter, is applied to an endbulb, a value is produced that reliably indicates the parent fiber's SR (Benson et al., Neurosci.Abst., 12:1266, '86). In this study, we applied the FF to endbulbs converging onto the same spherical bushy cell in order to investigate how segregation of SR might be expressed in the cochlear nucleus.

Eighteen pairs of endbulbs of Held from 4 cats were labeled following extracellular injections of horseradish peroxidase into the auditory nerve (AN). On the basis of intracellular labeling data, FFs greater or less than 0.52 indicated high (>18 s/s) or low (≤18 s/s) SR, respectively. Fourteen pairs had FFs typical of high SR fibers and 3 pairs had FFs typical of low SR fibers; only 1 pair straddled the line (0.50 and 0.53). The FF ratio of all pairs was close to 1 despite an individual FF range of 0.27-0.83. These data indicate that AN input remains segregated at the level of spherical bushy cells with respect to spontaneous activity.

263.8

ROC ANALYSIS OF AVERAGE FIRING RATES OF COCHLEAR NUCLEUS NEURONS IN THE GERBIL. W.P. Shofner and R.H. Dye*. Parmly Hearing Institute, Loyola Univ., Chicago, IL 60626.

The cochlear nucleus (CN) contains several subsystems where neural information in the auditory nerve can be degraded, preserved or enhanced. We present preliminary results from experiments designed to quantify the information in the average firing rates of CN units. Single unit responses to BF tones were recorded from the CN of anesthetized gerbils with 3M NaCl micropipettes. Based on interval histograms, units were classified as regular or irregular. Regular units were generally characterized as choppers; irregular units included primary-like, on-L and transient choppers. Receiver operating characteristic (ROC) curves were generated from empirical spike count distributions, and the area under the ROC curve (P(A)) was computed. For a given difference in the means of two spike count distributions, regular units typically give larger P(A) values than do some irregular units. Among irregular units, primary-like with a notch and on-L units give large P(A) values than do primary-like units. The results suggest that rate information may be enhanced in certain subsystems of the CN.

263.9

"FORWARD MASKING" OF SINGLE NEURONS IN THE COCHLEAR NUCLEUS: EFFECT OF MASKER FREQUENCY F.A. Boettcher*, R.J. Salvi, and S.S. Saunders*. Hearing Research Lab, SUNY-Buffalo, Buffalo, NY, 14214.

Forward masking is a psychophysical phenomenon in which the detectability of a brief probe signal is reduced by a stimulus which precedes the probe in time. The neural analogue of forward masking is the reduction in neural firing rate due to preceding stimulation. The purpose of this project was to determine how different types of neurons in the cochlear nucleus respond to a brief probe tone at the unit's characteristic frequency (CF) when the probe is preceded by a masking tone having a frequency above, below, or at the unit's CF. In general, if the masker excites the unit, neural firing to the probe is reduced compared to the unmasked condition. The degree of reduction varies with the firing rate of the unit to the masker. If the masker inhibits the unit, either enhanced or suppressed response to the probe is observed. The effect varies with the duration of the inhibition and the temporal separation of masker and probe. If the masker does not excite or inhibit the unit, no effect on the probe is observed.

263.10

RESPONSE LATENCIES OF FM UNITS IN THE INFERIOR COLLICULUS OF THE RAT. X.Y. Chen*, P.W.F. Poon*, Y.M. Cheung, and J.C. Hwang. Dept. Physiology, Univ. Hong Kong, Sassoon Road, Hong Kong

Knowledge of the response latency of the various types of frequency modulated (FM) cells is important for the understanding of feature extraction of complex sounds. Response latency to FM tones in 164 inferior collicular (IC) units of anaesthetised rats was estimated using linear regression methods based on PSTH to tones linearly modulated at different rates. Units were classified as either bi-directional (49%) or uni-directional (51%) based on their directional selectivity to tone sweeps. Results showed that uni-directional units respond to FM sounds with delays significantly longer than those of the bi-directional units (difference 3.1 ms, $p<0.01$).

We have provided evidence to support that in the IC of the rat, uni-directional cells, when compared with bi-directional cells, are activated by the FM tones through a longer synaptic linkage or via a slowly conducting pathway.

(supported by Hong Kong University Research Grant and Medical Faculty Research Grant)

263.11

INTERACTIONS OF AUDITORY NERVE FIBERS AND VENTRAL COCHLEAR NUCLEUS CELLS STUDIED WITH CROSS-CORRELATION. E.D. Young and M.B. Sachs. Center for Hearing Sciences, The Johns Hopkins University, Baltimore, MD 21209.

We have computed cross-correlograms (CCGs) between the spike trains of simultaneously-isolated auditory nerve (AN) fibers and cells in ventral cochlear nucleus (VCN). CCGs were computed from spontaneous activity or from steady activity driven by long-duration (50 s.) tones or noise. An excitatory peak (EP), consistent with a monosynaptic excitatory connection, is observed in the CCGs of 32/179 AN-VCN pairs examined with spontaneous activity or tone-driven activity. EPs are observed only when the best frequencies of the two units differ by less than 15%; BFs differ by less than 5% for 65% of correlated pairs. The EPs are small, indicating that 10% or fewer of the spikes in the VCN unit are attributable to spikes in the AN fiber. EPs are largest for spontaneous activity and for sound levels near threshold and decline monotonically as sound level increases. Surprisingly, results for primarylike units are essentially the same as for choppers. Large EPs appropriate for AN end-bulb terminals on bushy cells have not been observed.

EPs are observed in 36/125 pairs studied with noise stimuli. In 7 cases, EPs are seen with noise but not with tones or spontaneous activity, whereas EPs are always seen with noise stimuli if an EP is seen with tones or spontaneous activity (8 cases). The implications of these results for input/output processing in VCN will be discussed. (Supported by NIH grant NS12524).

263.12

EFFECTS OF OFF-BF TONES ON RATE AND REGULARITY OF COCHLEAR NUCLEUS CHOPPER UNITS. C.C. Blackburn* and M.B. Sachs, Dept. of Biomedical Engineering and Center for Hearing Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We are studying the effects of off-best frequency (off-BF) input on the responses of chopper units in the anteroventral cochlear nucleus (AVCN) of nembutal-anesthetized cats. Chopper units, recorded from stellate cells in the AVCN, fire in a regular manner in response to a BF tone. Responses of single units to tones and two-tone combinations were recorded, and the mean inter-spike interval and its regularity were computed as a function of time through the response (Young, E.D. et al. (1988) J. Neurophys. in press). The effects of off-BF input on the response patterns of choppers include rate suppression but reveal other changes which are more complex. Two generalizations may be made. First, off-BF inhibition (measured as rate suppression) tends to be weak in regular choppers (ChS) and stronger in irregular choppers (ChT). Second, the time course of the changes in rate and regularity that these units undergo can be dramatically affected by off-BF input. Changes in the temporal adaptation patterns of chopper unit responses, which have been recorded in both ChS and ChT units, cannot be accounted for on the basis of changes in sustained firing rate, suggesting that the mechanism is not simply two-tone suppression at the level of the auditory nerve fiber input. (Supported by NINCDS grants NS12112 and NS07283.)

263.13

ONTOGENY OF GABA ACTIONS WITHIN THE CAUDAL COCHLEAR NUCLEI OF KITTENS. E.J. Walsh, J. Fitzakerley and J. McGee*. (SPON: H. Konrad). Depts. of Surgery and Pharmacol., Southern Illinois Univ. Sch. of Med., Springfield, IL 62794-9230.

Responses to acoustic and microionophoretic GABA stimulation of neurons within the caudal cochlear nucleus were studied during postnatal development using extracellular recording techniques. GABA application reduced acoustically-evoked as well as spontaneous discharge rates in a dose-dependent manner at all ages studied, generally abolishing discharge activity at sufficiently high doses (currents). Dose-response curves generated during acoustic stimulation (roughly 20 dB sensation level, SL) were sigmoidal at all ages studied. Although discharge rates produced using equal sensation level stimuli increased with age, effective dose ranges (computed as the difference between currents required to produce 90% and 10% of the acoustically-evoked discharge rate) were smaller for neurons recorded from kittens younger than 10 days than those computed for adults. The application of GABA at doses producing a 50% reduction in the discharge rate evoked by acoustic stimulation at 20 dB SL, produced constant discharge rate decrements across much of the acoustic intensity range in most neurons recorded from animals older than 11 postnatal days. In younger kittens, GABA-induced effects on discharge rate were more pronounced, by as much as 5 times, at high sensation levels compared to low SLs (i.e., rate/intensity curves obtained in the presence of GABA were shallower than control curves and thresholds were higher during GABA application in young animals compared to adults). The influence of GABA on temporal response profiles and the time course over which GABA exerts its effects will also be considered for different cell types. (Supported by NIH grant NS21171).

263.14

SENSITIVITIES OF CELLS IN THE ANTEROVENTRAL COCHLEAR NUCLEUS OF CAT TO DISCHARGE PATTERNS ACROSS THE POPULATION OF AUDITORY NERVE FIBERS. L. H. Carney, Depts. of Neurophysiology and Electrical & Computer Engineering, University of Wisconsin-Madison, Madison, WI 53706.

Different types of cells in the anteroventral cochlear nucleus (AVCN) receive characteristically different forms of convergent input from the auditory nerve. The different forms of convergence should produce varying degrees of sensitivity to the temporal discharge patterns across the population of auditory nerve fibers in response to complex stimuli. This hypothesis can be tested by inserting a smooth transition into the phase spectrum of a complex stimulus, while leaving the magnitude spectrum unchanged, so that systematic time shifts are introduced between the responses of auditory nerve fibers with different characteristic frequencies (CFs). Responses to such stimuli were recorded from primary-like (PL) and primary-like-with-notch (PLn) cells, which probably receive from one or two to a few primary inputs with large somatic terminals, as well as choppers, which probably receive a large number of converging primary inputs ending in small terminals on both dendrites and soma. Responses of PLn cells are affected by the introduction of a phase transition; they show different sensitivities, as measured by threshold and rate, to stimuli with different phase transitions. This change in sensitivity suggests that PLn cells receive convergent input from fibers with different CFs and that the temporal pattern of discharges across these inputs is important in determining the responses of these cells. The change in sensitivity of PLn cells as a function of these systematic manipulations of the stimulus spectrum is consistent with a simple coincidence model, which is also compatible with several aspects of their responses to other stimuli, such as tones, clicks, and noise. Some PL cells and choppers also have responses which are affected by manipulations of the phase spectrum, suggesting some degree of sensitivity to the temporal pattern of their inputs.

263.15

ENHANCEMENT OF PHASE-LOCKING TO LOW FREQUENCY TONES IN SOME CELLS OF THE VENTRAL COCHLEAR NUCLEUS OF THE CAT. T.C.T. Yin, L.H. Carney, P.X. Joris*, and P.H. Smith. Dept. of Neurophysiology, Univ. of Wisconsin Medical School, Madison, WI 53706

It is generally believed that the synchronization of auditory nerve fibers to low frequency (<3-4 kHz) tones is relayed by the bushy cells in the ventral cochlear nucleus (VCN) with little or no change, i.e., that the synchronization coefficient of phase-locked responses in the VCN can be as high as in the nerve, but no higher. We report here evidence that a significant proportion of low-frequency (<1.2 kHz) VCN cells can have maximal sync coefficients that are between 0.9 and 1.0, greater than that ever seen in 8th nerve fibers of comparable characteristic frequencies (CFs). Recordings were made in the VCN or from axons of VCN cells in the trapezoid body of barbiturate anesthetized cats. We examined rate-level responses using short (25 msec) tone bursts at CF (as determined by a threshold tuning curve) over different SPLs. Sync coefficients were computed from period histograms contructed over the last 15 msec of the duration of the stimulus in order to avoid the large onset response that sometimes occurs out of phase with the sustained response. Such increases in the sync coefficient can be modeled by requiring coincidence and convergence of 8th nerve inputs on some VCN cells, for example, the small spherical and globular bushy cells.

263.16

RECEPTIVE FIELD PROPERTIES OF INTERNEURONS IN THE DORSAL COCHLEAR NUCLEUS. G.A. Spirou* and E.D. Young (SPON: David K. Ryugo). Dept. of Biomedical Engineering and Center for Hearing Sciences, Johns Hopkins University Baltimore, MD 21205.

We have measured the receptive fields of type II interneurons in the dorsal cochlear nucleus (DCN) of decerebrate cats. These units are responsible for a significant part of the inhibition of DCN principal cells. The interneurons have no spontaneous rate, so the effects of inhibitory sounds must be measured against a simultaneous excitatory sound. This two-tone paradigm reveals asymmetric sideband inhibition which is deepest adjacent to the excitatory tuning curve. Sideband inhibition is active in some units at levels as low as the excitatory threshold. This is consistent with the lack of response of these units to broadband stimuli even at very low levels. A reduction in rate is evident at levels as low as 3 dB SPL, indicating that inhibitory mechanisms, rather than 2-tone suppression, are the important determinants of this response at low sound levels. (Supported by NINDCS NS07283.)

263.17

EFFECTS OF INHIBITORY AMINO ACID NEUROTRANSMITTERS ON ONE POPULATION OF VENTRAL COCHLEAR NUCLEUS NEURONS.

D.M. Caspary, S.D. Yuscius*, B.A. Lawhorn*, and P.G. Finlayson.
Depts. of Pharmacology and Surgery, Southern Illinois University School of Medicine, Springfield, Il. 62794-9230.

Neurochemical, neuropharmacological, and morphological studies suggest roles for GABA and glycine in the processing of acoustic information in the ventral cochlear nucleus (VCN). VCN neurons have primarily been described as displaying excitation in response to tonal acoustic stimuli. However, inhibitory sidebands (Martin and Dixon, '83), near threshold suppression of spontaneous activity, and electrically evoked ipsp's have been described (Wu and Oertel, '86). This study examined VCN neurons displaying "phase-locked" temporal responses at CF and presumed to be bushy cells. Forty-four neurons displaying "phase-locked" temporal response patterns were localized to the anterior or interstitial VCN. When responses to CF tones were compared with responses to broadband noise, 14 neurons displayed rate-intensity functions with similar slopes while 30 neurons showed noise/tone slope differences. Twenty-six neurons displaying "phase-locked" responses were examined during iontophoretic application of GABA and glycine and the receptor antagonists bicuculline and strychnine. Both antagonists could selectively alter rate-intensity functions to broadband noise. Application of bicuculline and strychnine resulted in both near-CF and off-CF changes in response area. Efficacy of GABA and glycine were compared. These findings suggest that GABA and glycine receptors are present on VCN bushy cells and may function to shape selected portions of response areas. (Supported by NINCDS grant NS15640)

263.18

RESPONSE PROPERTIES OF SINGLE AUDITORY NEURONS IN THE DORSAL MEDULLARY NUCLEUS OF THE FROG.

J.C. Hall* and A.S. Feng. Dept. Physiol. & Biophys. Univ. of Illinois, Urbana, IL 61801.

The frog dorsal medullary nucleus (DMN), a homolog of the mammalian cochlear nucleus, is an obligatory synapse for auditory fibers of peripheral origin and the first site for the central processing of acoustic signals. We have studied the role of the DMN in the processing of complex sounds, particularly those serving a communicative function.

In this study, we characterized the discharge pattern of single neurons in the frog's DMN to "simple" stimuli consisting of tone bursts of different frequency and intensity. Neurons were further characterized on the basis of their spontaneous firing rate, best excitatory frequency, sharpness of tuning (Q_{10} dB), and firing threshold. Previously, we recognized two response types in the DMN, phasic and tonic. We now report a third type, pauser, so named because its discharge pattern resembled that of the pauser neurons found in the mammalian cochlear nucleus. We also identified four distinct classes of tonic units based upon differences in the shape of their post stimulus time and interspike interval histograms, average discharge rate to tone bursts, and tuning. The response properties of DMN neurons resembled those of VIIIth nerve fibers in many respects; but, several differences, particularly with respect to the diversity of response types, were noted. These differences provide an additional basis for our previous observation that DMN neurons are more selective for specific features of complex sounds than primary auditory nerve fibers.

263.19

RESPONSE CHARACTERISTICS OF FROG SUPERIOR OLIVARY NEURONS TO AMPLITUDE-MODULATED (AM) ACOUSTIC STIMULI.

S. Chang*, C. J. Condon*, and A. S. Feng. (SPON: T. G. Weyand). Department of Physiology and Biophysics and The Neural and Behavioral Biology Program, University of Illinois, Urbana, IL 61801.

We investigated the responses of single units in the superior olivary nucleus (SON) of the leopard frog to AM signals and determined their selectivity to various temporal parameters (AM rate, rise-fall time, duration). Pulsatile and sinusoidal amplitude modulated stimuli were presented at rates of 5-300 Hz in order to describe the modulation transfer function (MTF) of individual neurons. The MTFs were measured with respect to mean spike rate and the synchronization coefficient (SC) to the phase of the modulation cycle of the AM stimulus. The spike rate and synchronization MTFs were used to define temporal filtering characteristics; the later further indicated the ability of a unit to act as an envelope detector.

On the basis of synchronization MTFs, SON neurons displayed two filtering characteristics: low-pass and all-pass. All-pass units gave constant synchronization with wide ranges of SC, while low-pass units maintained high SCs at low modulation rates (<60 Hz) that decreased with higher rates of modulation. However, with respect to mean spike rate, the MTFs of SON neurons exhibited a greater variation in temporal selectivity. In addition to low- and all-pass transfer functions, high-pass, band-pass, and band-suppression response types were also observed. These functions were often related to response selectivity to other temporal parameters. These results suggest that SON neurons play an important role in auditory processing; they perform extensive computations on the rate of AM as well as other temporal parameters.

263.20

PROPERTIES OF THE AUDITORY PERIOLIVARY NUCLEI.

M.N. Semple*, L.M. Kitzes*, G.W. Huntley and W.B. Warr (SPON: R. Blanks). Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717, & Boys Town Institute for Communicative Disorders in Children, Omaha, NE 68131.

In the mongolian gerbil, *Meriones unguiculatus*, as in most species, the periolivary nuclei (PON) of the superior oilvary complex (SOC) are somewhat poorly defined histologically. However, the lateral (LNTB) and ventral (VNTB) nuclei of the trapezoid body, the dorsal periolivary nucleus (DMPO) and the superior parolivary nucleus (SPN) can be distinguished in nissl or protargol stained material. The gerbil appears intermediate between other rodents and the cat in having both a rodent-like SPN and a cat-like DMPO. Anterograde and retrograde tracing showed that the major afferent and efferent connections of gerbil SOC are comparable with those seen in cat.

Single-unit recording in the primary auditory nuclei of SOC revealed response properties consistent with previous studies in cat: units in the medial nucleus of the trapezoid body (MNTB) typically discharged in a primary-like fashion and often displayed a prominent pre-potential; chopper patterns were commonly observed in the lateral superior olive; units in all primary SOC nuclei had short initial discharge latencies. In PON, however, response properties were frequently very different. Some units, whose tuning curves were often broad, responded at long latencies — usually in a build-up fashion. Spontaneous activity of some units was inhibited during the stimulus and often the silent period was followed by rebound excitation after stimulus offset. A third class of response was characterized by a strong preference for broadband noise; discharging transiently to tonal stimuli, but in a secure, sustained manner to noise. In LNTB, units responding at long latency were found intermingled with units sharply-tuned to ipsilateral tones and responding with short-latency, primary-like patterns. Intra-axonal injection of HRP revealed the existence of some quite distinctive axon terminals in PON: in LNTB, for example, a calycine terminal clasping the soma, and a large *en passant* contact.

Supported by NINCDS grants NS17596 and NS23813.

SENSORY SYSTEMS: AUDITORY SYSTEMS V

264.1

RESPONSE SELECTIVITY OF NEURONS IN THE FROG TORUS SEMICIRCULARIS TO TEMPORAL COMPONENTS OF AMPLITUDE-MODULATED ACOUSTIC STIMULI.

D.M. Gooler, J.C. Hall* and A.S. Feng. Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

The responses of single units in the torus semicircularis (TS) of the leopard frog were examined for selectivity to the temporal parameters of acoustic stimuli associated with amplitude-modulated (AM) sounds. Sinusoidal, pulsatile, and square-wave AM stimuli were presented and the modulation transfer function (MTF) of individual neurons were calculated for AM rates of 5-400 Hz (or pulses per second). The MTFs were measured with respect to mean spike rate and the synchronization coefficient (SC) to the phase of the modulation cycle of the AM stimulus. The response functions to other temporal parameters (duration, rate of rise, duty cycle) were also derived in order to determine the response selectivity to these attributes.

The spike-rate response of single toral neurons to AM rate, signal duration and rate of amplitude change exhibited various filter functions: low, high, band or all pass. The response selectivity of neurons to these varying parameters was often tightly related to one another. For instance, the selectivity to the AM rate of sinusoidally AM signals could in some cases be attributed to a selectivity to the modulation rate itself, or the rate of rise, or duration. (Research supported by a NSF grant BNS 85-11055 and a NIH post-doctoral fellowship NS07939 to DMG)

264.2

ONTOGENY OF EXCITATORY AND INHIBITORY AMINO ACID LEVELS IN THE INFERIOR COLLICULUS OF RATS.

J. McGee*. (SPON: E.J. Walsh) Dept. of Pharmacol., Southern Illinois Univ. Sch. of Med., Springfield, IL 62794-9230.

Levels of excitatory (aspartic and glutamic acids and glutamine) and inhibitory (GABA, glycine and taurine) amino acids in the inferior colliculi (IC) of developing rats were determined using high performance liquid chromatography with electrochemical detection. Amino acid levels ranged between 14% and 32% of their adult values at birth, except for taurine which exceeded adult levels by 223% at birth. Generally, amino acid levels increased monotonically between birth and the end of the first postnatal month, exhibiting a 3- to 7-fold increase when levels were determined per colliculus. Aspartic acid levels increased beyond postnatal day 30, gradually acquiring adultlike values. Taurine levels, on the other hand, increased during the first 11 postnatal days and subsequently declined along an exponential time course achieving adultlike values by the end of the second postnatal month. Developmental trends were more complex when amino acid levels were considered in terms of tissue weight. Taurine levels remained constant during the first 11 postnatal days and declined to achieve adult values by the 35th day. Other amino acid levels increased sigmoidally to roughly the 18th postnatal day and declined thereafter to achieve adultlike values by the end of the first month. Aspartic acid levels displayed a second growth phase ending near the 60th postnatal day. Thus, the magnitude and rate of change in content of amino acids in the IC varied greatly over the course of postnatal development. These results will be considered in light of morphological and physiological aspects of maturation of the IC. (This investigation was supported in part by NIH grants NS15640, NS21171 and NSF grant BNS8646563).

264.3

COCHLEAR WHOLE-NERVE RESPONSES ALTERED BY ELECTRICAL STIMULATION OF THE INFERIOR COLLICULUS. David F. Dolan* and Alfred L. Nuttall. Kresge Hearing Research Institute, Univ. of Mich. Med. Sch., Ann Arbor, MI 48109.

The traditional method to achieve electrical activation of the efferent system to the cochlea is by stimulation of the crossed olivocochlear bundle (COCB) at the floor of the 4th ventrical. This is thought to excite fibers derived from cells which are located in and/or around the medial superior olivary complix (SOC). These cells are referred to as the "medial" portion of the efferent system and then send their axons primarily to the outer hair cells in the cochlea. There is currently little information about the descending neural input to these cells or to the cells that comprise the "lateral" portion of the efferent system which ends primarily on afferent dendrites from the inner hair cells.

We will show that electrical stimulation of the inferior colliculus produces similar, but not equivalent, effects on the whole-nerve response (CAP) as electrical stimulation of the COCB. CAP magnitude is reduced (equivalent to 10-20db attenuation) and latency increased most for low-level acoustic signals. The effects of electrical stimulation are abolished by the administration of strychnine.

The differences in the effects on the CAP between the two means of activation of the efferent system will be discussed in terms of the lateral and medial portions of the efferent system.

264.4

DYNAMIC PROPERTIES OF THE MAPS IN AUDITORY FOREBRAIN CENTERS OF A SONGBIRD. U. Haeusler*and H.J. Leppelsack. Inst.Zool.TU Muenchen 8046 Garching FRG

We introduce a new method of measuring functional maps in brain areas of awake animals. This method is based on multiunit recordings which are collected automatically under computer control. The response measure is the spike count as compared to spontaneous activity. Spatial resolution is determined by the chosen distance between recording points and the catchment area of the glass coated platinum electrodes. It is about 100 µm. Maps are plotted as iso-response lines or as dot density, and illustrate the distribution of response strength within a given time interval.Thus, the temporal development of a response cluster within a brain center can be investigated. Different stimuli can be used at the same recording point and maps of different parameter distributions may be compared within one individual brain.
Investigations of the tonotopicity of auditory centers in the caudal telencephalon of the starling confirm former results which showed that the tonotopic gradient is arranged dorsoventrally. The new results, however show an unexpected variety of functional subunits which change their shape during the course of constant stimulis. In addition, the centers of response can be observe to move along the tonotopic gradient. These changes can take place within only a few milliseconds.

264.5

DYNAMIC RANGE OF TONE LEVEL CODE IN RAT AUDITORY CORTEX D.P.Phillips and J.B.Kelly. Dept. Psychology, Carleton University, Ottawa, Ontario, Canada

The behavioral intensity dynamic range in hearing is about 100 dB wide. We investigated the fashion(s) in which the cortical neural representation of tone level exhibits a comparable dynamic range. We did so by obtaining spike rate-vs-tone level functions from single cortical neurons in anesthetized albino rats. Stimuli were 110-ms characteristic frequency tone pulses presented to the contralateral ear.

Most neurons had monotonic rate-level functions that tended towards firing rate saturation at high tone levels. The dynamic ranges of these functions were commonly 10 to 35 dB in breadth, though the distribution extended to at least 65 dB. Nonmonotonic rate responses were scarce, and were less well-developed than those seen in cats and primates. Neurons tuned to a single tone frequency in single cerebral hemispheres had thresholds that varied over a 50 dB range. The collective amplitude dynamic range expressed even by small such populations spanned 80 dB or more. These observations provide an estimate of the minimum population dynamic range of the rat's acoustic cortex. They suggest that this dynamic range may be comparable to the behavioral one, and that it resides in the firing rates of the relevant neurons.

264.6

CHOLINERGIC AGENTS MODIFY FREQUENCY RESPONSE FUNCTIONS OF SINGLE NEURONS IN AUDITORY CORTEX. T.M. McKenna, J.H. Ashe and N. M. Weinberger. Ctr. Neurobiology of Learning and Memory, Univ. California, Irvine, CA. 92717

Previously we have shown that cholinergic agonists modify spontaneous and evoked discharges to single tones in auditory cortex of unanesthetized cat (McKenna et al, Synapse, 1988, 2:54-68). Recent observations indicate that the cholinergic agonist methacholine (MCh,10-20 mM) and anticholinesterases eserine (0.2-0.4M) and soman (20mM) modify the frequency response function of these neurons. These agents, applied via multibarrel micropipettes (pressure ejection or iontophoresis) produce frequency specific modification in discharge rate of neurons tested with isointensity sequences of pure tone frequencies (1.0-30.0 kHz, 40-80db). These frequency specific changes were often accompanied by a shift in the frequency producing the maximum tone response and/or bandwidth changes. One aspect of shifts was decrease of response at the prior best frequency with increase at an adjacent frequency. As classical conditioning rapidly induces frequency-specific alterations of frequency response functions in auditory cortex (Diamond and Weinberger, Brain Res., 1986, 372:357-360), the current observations suggest that intrinsic cholinergic mechanisms may play a role in this phenomenon. Supported by DAMD 17-85-C-5072 to NMW.

264.7

ACETYLCHOLINE PRODUCES FREQUENCY-SPECIFIC RECEPTIVE FIELD ALTERATIONS IN CAT AUDITORY CORTEX THAT ARE BLOCKED BY ATROPINE. R. Metherate, K.C. Wong* and N.M. Weinberger; Center for the Neurobiology of Learning and Memory; University of California, Irvine

Cholinergic agents may affect auditory perception and sensory processing by modifying neuronal receptive fields. Previous work showed that single neuron responses to pure tones were modified by acetylcholine (ACh) administered iontophoretically in cat auditory cortex, and that this treatment could change a cell's frequency receptive field (FRF). The present study shows that the most common effect is a decrease in responsiveness that is restricted to the paired frequency.

Multibarrel micropipettes inserted into the auditory cortex of barbiturate anesthetized cats contained ACh chloride (1M), sodium glutamate (.5M), atropine sulphate (25mM) and NaCl (1M). A neuron's FRF was determined upon initial isolation, then ACh was paired with a repeated, non-best frequency tone. Following this, 45/90 neurons displayed altered receptive fields, and in 24 cases the FRF changes were highly specific to the paired frequency. Atropine selectively blocked the effects of ACh on single tone responses and frequency-specific FRF changes, suggesting that the effects of ACh are mediated via muscarinic ACh receptors.

Supported by DAMD 17-85C-5072 to NMW and NINCDS fellowship NS08001 to RM.

264.8

INHIBITORY EFFECTS OF ACETYLCHOLINE ON TONE ELICITED SINGLE UNIT RESPONSES IN RAT AUDITORY CORTEX. H. K. Rucker. Department of Physiology and the Neuroscience Group, Meharry Medical College, Nashville, TN 37208.

The effect of iontophoretically applied acetylcholine (ACh) and atropine on single unit responses to near best-frequency tone bursts was studied in the auditory cortex of urethane anesthetized rats. These studies were undertaken to ascertain the cholinergic effect on response gain in the rat auditory cortex. Neuronal activity was recorded with glass multi-pipettes and 500ms peri-stimulus time histograms (PSTH) constructed for isolated units in response to 50 tone bursts (100ms, threshold +10 dB). In a sample of 48 units, fifteen percent (n=7) exhibited response attenuation (compared to drug-free PSTH) by 20-38% during ACh application. Paradoxically, these units concurrently increased control period discharge rate and glutamate excitatory responses were also facilitated by ACh. These latter ACh effects were consistently antagonized by atropine whereas the response attenuation was generally enhanced. A presynaptic role relevant to ACh mediated inhibition is suggested. (Supported in part by NSF grants BNS 8617937 and RII 8704121)

264.9

TOPOGRAPHIC ORGANIZATION OF BINAURAL NEURONS IN THE PRIMARY AUDITORY CORTEX OF THE FERRET. P.W. Judge, and J.B. Kelly. (SPON: David Gardner). Laboratory for Sensory Neuroscience, Dept. of Psychology, Carleton University, Ottawa, Ontario, Canada K1S 5E6.

Microelectrodes were used to map binaural responses in the primary auditory cortex of nine barbiturate-anesthetized ferrets. Gated pure tones were delivered independently to the two ears through a sealed speaker system and sensitivity to binaural intensity was determined at various sound pressure levels. Units within AI exhibited binaural response properties which were similar in most respects to those found in the cat's primary auditory cortex. The binaural neurons tended to be grouped together on the basis of their binaural response properties. Along the length of each isofrequency contour, areas of binaural summation (EE/F) alternated systematically with areas of binaural suppression (EO/I). A third major binaural response type, first noted by Reale and Kettner (1986), combined the properties of EE/F and EO/I units. These cells exhibited summation when the contralateral intensity was near threshold and the inter-aural intensity was small, but were suppressed at higher ipsilateral intensities. Seventy-four percent of these 'mixed' units were located between areas of EE/F and EO/I cells. The mixed responses appeared to mark the transition between summative and suppressive responses both by virtue of the location, and the duality of binaural response properties. Monaural and binaural thresholds obtained from units located within narrow-bandwidth isofrequency contours were examined statistically. Preliminary evidence suggests that monaural thresholds vary as a function of distance along an isofrequency contour.

264.10

THE MEANING OF STIMULUS DEPENDENT CHANGES IN CROSS CORRELATION BETWEEN NEURONAL SPIKE TRAINS. P.H. Bedenbaugh*, G.L. Gerstein, K.H. Boven* and A.M.H.J. Aertsen* (SPON: M. Bloom). Depts. of Physiol. and Bioeng., U. of Penna., Philadelphia PA 19104 and MPI for Biol. Cybernetics, Tuebingen, West Germany.

Several labs have observed dynamic changes in raw and corrected cross correlograms of two simultaneously recorded spike trains in the presence of different stimuli, even when their pst histograms and spike counts are similar. This suggests that the apparent functional connection between two neurons can vary on the time scale of hundreds of milliseconds. We want to know how to interpret these results in terms of changes in neuronal organization: do the differences in cross correlation reflect changes in the synaptic strengths of connections between the cells or some emergent property of the assemblies to which they belong ? To answer this question we are simulating networks on the order of 100 neurons using a computer program based on SYSTM22 described by MacGregor (1987, Academic Press). This program simulates the neurons as four state variable non-linear systems whose properties are determined by specifying conductances, thresholds and time constants. Preliminary results for simulated neurons with fixed strength of synaptic connections indicate that if the spike counts elicited by different stimuli are close, then the stimulus modulation corrected cross correlogram is constant. When the spike counts are different, the corrected cross correlogram varies with stimulus, especially when the connectivity is highly convergent. Recent work offers a better correction for cross correlograms when stimulus related non-stationarities are present (Gerstein and Aertsen, this meeting). Supported by System Development Foundation SDF 0013 and by ONR N00014-K-0766 to GLG.

264.11

RAPID STIMULUS-MODULATION OF "EFFECTIVE CONNECTIVITY" BETWEEN TWO NEURONS. G.L. Gerstein, A. Aertsen, Dept. of Physiology, U. of Pennsylvania, Philadelphia PA 19104 and MPI for Biological Cybernetics, Tuebingen, West Germany.

Cross-correlation of two simultaneously recorded spike trains, with appropriate correction for the modulations of each firing rate by the stimulus, has been used to evaluate an "effective connectivity" between the two neurons. Does this quantity in turn show stimulus-locked modulation? We have developed new procedures to quantify and properly normalize the classical Joint Peri-Stimulus-Time Scatter Diagram (Gerstein and Perkel 1972). These allow separation of the "raw" correlation into time-varying components caused by direct stimulus modulations of the single neuron firing rates and those caused by various types of interaction between the two neurons. Application of the new procedures to spike trains from a neural simulator shows detection of fast stimulus-locked modulation of the "effective connectivity" even when this is masked by strong direct stimulus modulations of the individual firing rates. Application of the new procedures to real spike trains from several different preparations shows that fast stimulus-locked modulations of "effective connectivity" also occur for real neurons. This observation suggests that the organization among a group of neurons can be rapidly time-varying, on the scale of a stimulus presentation. Such changes may represent successive stages of the computation of stimulus information. Known mechanisms like pre-synaptic inhibition and/or interneurons can account for these modulations of interaction among neurons. However, it does seem that the usual concept of neurons with static interconnections of fixed or only very slowly changing value (during learning), is no longer appropriate. Supported by System Development Foundation SDF 0013 and ONR N00014-K-0766.

REGENERATION: GENERAL I

265.1

REGENERATIVE ORGANIZATION AFTER MANDIBULAR NERVE SECTION AND REPAIR. J. Zuniga*, A. Hegtvedt*, J. Pate*, W. Maixner (SPON: G. Essick). Dental Research Center, Univ. NC, Chapel Hill, NC 27599.

Two-stage double-label (DL) methods were used to determine whether the regeneration of adult trigeminal segments following mandibular nerve section and surgical repair is associated with regeneration or collateral sprouting of first-order neurons.

Twelve ganglia from 6 male adult Sprague-Dawley rats were analyzed following: 1) injection of 1 ul of 4% Fast Blue (FB) into the mandibular nerve; 2) transection and repair; 3) injection of 1 ul of 4% Diamidino Yellow (DY) distal to the repair 30, 60 and 90 days after repair. Following fixation, trigeminal ganglia were removed, cut serially (20 um) in longitudinal sections and examined using fluorescent microscopic techniques. The results were compared with data obtained from 12 ganglia of sham operated and positive (no repair) controls.

The total number (FB, DY & DL) of labeled neurons did not differ between groups. The DL neurons were confined to the mandibular segment and the number of DL neurons was the same for sham operated and operated groups at 90 days. The number of DY neurons (collaterals) was not different from sham control.

This study demonstrated that sectioning and immediate repair of the mandibular nerve results in the regeneration of fibers whose cell bodies are confined to the mandibular segment. There was no evidence of collateral sprouting at the level of first-order neurons. NIDR grant DE0827201A1.

265.2

NEONATAL TRANSECTION OF THE RAT'S INFRAORBITAL NERVE INCREASES THE PERCENTAGE OF SUBSTANCE P-POSITIVE GANGLION CELLS THAT REGENERATE AXONS INTO THIS TRIGEMINAL BRANCH. H.L. Enfiejian, N.L. Chiaia and R.W. Rhoades (SPON: R. Wazirí). Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Neonatal transection of the rat's infraorbital nerve (ION) results in an expansion of the laminar distribution of substance P-like immunoreactivity in the medullary dorsal horn by the time the animal reaches adulthood (Rhoades, R.W. et al. J. Neurosci., in press). In the present study, we combined retrograde tracing and immunocytochemistry to determine whether this change was associated with an increase in the percentage of substance P (SP) positive ganglion cells that projected into the regenerate ION. In the normal trigeminal (V) ganglion (N=8), 11.6 $\pm$ 3.2% of the cells retrogradely labelled by application of true blue to the ION were also immunoreactive for SP. In ganglia ipsilateral to the regenerate ION, this value was 18.5 $\pm$ 4.7% ($p<0.001$). We also compared the SP content of normal ganglia and ganglia ipsilateral to the regenerate ION by means of radioimmunoassay in 10 rats. The normal V ganglion contained an average of 3,454 pg SP/mg protein. In the ganglion ipsilateral to the lesion, this value was 5,469 pg/mg ($p<0.01$). Supported by BNS 85-17537 and DE 07734.

265.3

MORPHOLOGICAL RESPONSES OF DORSAL ROOT GANGLION NEURONS CONTRALATERAL TO CRUSH LESIONS OF THE SCIATIC NERVE. M.R. Wells and U. Vaidya. Neurochemistry Research, Veterans Admin. Hosp. Washington, D.C. 20422.

Responses of analogous neurons contralateral to those injured by a peripheral nerve lesion have been observed, but not well characterized. The present study examined the time course of morphological responses of L5 dorsal root ganglion neurons contralateral to a sciatic nerve crush. Male, Wistar-Furth rats (n=2/time) received a unilateral crush of the sciatic nerve at the level of the sciatic notch. At 1,2,3,4,5, 7,8,9, 11,14, and 30 days after injury, animals were perfused with fixative, both L5 ganglia embedded in plastic and sectioned. Camera lucida drawings were made of 70 neurons from each ganglia. Soma, nuclear and nucleolar area were measured and eccentricity of the nucleus examined. The response of the injured side has been described in prior studies. Compared to measurements obtained from normal animals, the uninjured side showed significant alterations characterized by a transient increase in most parameters from 3 to 7 days after surgery. Changes in nuclear and nucleolar area corresponded to increases in synthesis suggested by prior studies. A group of sham operated animals which had received a dorsal midline incision and retraction of musculature from the T2 spinal vertebrae also exhibited a response of L5 ganglion morphological parameters. A similar group of animals receiving anesthesia alone did not show a response. The experiments suggested that a significant contralateral alteration in morphological parameters occurs after unilateral sciatic nerve section. At least one component of this reaction appears to be a nonspecific response to surgical trauma. Supported by the Veterans Administration

265.4

REGENERATION OF SENSORY NEURONS AFTER SCIATIC NERVE TRANSECTION AND ENTUBULATION REPAIR. F. Langone*, J.C. Bittencourt* and C.F. Da Silva.
Department of Anatomy, Institute of Biomedical Science, University of São Paulo, São Paulo, Brazil.

A quantitative study on dorsal root ganglion (DRG) neuron number and sizes after peripheral nerve transection and tubulation repair is presented. Ten adult C57BL/6J mice received sciatic nerve transections at mid-thigh level and proximal and distal nerve stumps were sutured into a 6 mm polyethylene tube, to give a nerve gap distance of 4 mm. After 12 weeks, the distal nerve stump was cut and exposed to an HRP solution. After an additional 3 days, animals were perfused with fixative and the L3-L5 DRG were removed on the operated side. The DRG were then either serially frozen sectioned (4 animals) or processed as whole-mounts (6 animals) and reacted with TMB. The total number of labeled cells was counted using the serial sections and cell sizes (areas) were measured by selecting 200 cells/animal in the whole-mount preparations. Aditional 10 animals were used to estimate the number and sizes of DRG neurons of origin of mouse sciatic nerve. The results showed that the regenerating DRG cells represented only about 40% of normal DRG cell counts (1707±139 X 4211±96). On the other hand, the measurements showed that the total cell size range was similar for both operated and control animals, with no preferential loss of small neurons. One possible explanation for these findings is that peripheral nerve transection may result in a non-selective death of primary sensory neurons, decreasing the potential for sensory axon regeneration.
Supported by grants from FAPESP, CNPq and FUNCAMP.

265.5

DO LUMBAR DORSAL ROOT GANGLION CELLS SURVIVE NEONATAL DORSAL COLUMN AXOTOMY? S.P. Lahr* and D.J. Stelzner. Dept. of Anatomy & Cell Biol., SUNY Health Sci. Ctr., Syracuse, NY 13210.

Ascending dorsal column (DC) axons do not grow around a cervical spinal overhemisection (cutting the dorsal funiculus bilaterally) made in newborn rats (Lahr and Stelzner, Neurosci. Abst. 13:750). Since the DC projection can be labeled shortly after birth in its normal target, the gracile nucleus (Lahr and Stelzner, Anat. Rec. 220:56A), one possible reason for lack of DC growth after neonatal overhemisection is death of axotomized dorsal root ganglion (DRG) cells. To investigate this possibility Fast Blue (3%, 0.1 µl) was injected into the gracile nucleus of newborn rats. Two days later some of these rats received a cervical spinal overhemisection injury and were allowed to survive for postinjection periods of 10 (n=4) and 22 (n=4) days. Frozen sections from L4 and L5 DRG were examined and the percentage of labeled cells was determined and compared to unlesioned animals with postinjection periods of 3 (n=4), 10 (n=4), and 22 (n=4) days.

Cell counts reveal no sign of DRG cell loss after newborn DC axotomy. Approximately 20% of L4 and L5 DRG cells are labeled at 3 and 10 days postinjection with or without spinal injury. At 22 days postinjection a smaller percentage of cells is labeled but the percentage is the same for both lesioned and unlesioned groups. Supported by NIH Grant NS 14096 (D.J.S.).

265.6

CELLULAR DEBRIS STIMULATES CNS SCAR FORMATION. G. Rodziewicz*, J. Rudge*, G. Smith*, J. Silver (SPON: R. Hardy). Neuroscience Center, CWRU Medical School, Cleveland, Ohio 44106.

The role of cellular debris in scar formation was studied in a bilaterally symmetric model of minimal scar formation.

Adult rats had bilateral frontal craniectomies and bilateral implantation of 8 u pore-size nitrocellulose filters. On the experimental side, the implants were transplanted from neonatal rat forebrains to the adult rat brains under a variety of conditions. Contralaterally the cell-free controls were implanted. Fourteen days later, the rats were killed and the forebrains were prepared for TEM and immunohistochemistry (laminin and GFAP).

In many areas of the control implants, basement membrane and collagen were absent, and astrocyte processes grew into the pores of the filter. Myelinated axons ran in close proximity to these implants. Around the debris-coated experimental implants, there was uniform scar formation, with collagen and phagocytes present in basement membrane, surrounded by layers of astrocytes.

Persistent phagocytes marked the areas of scar formation. Our experiment suggests that cellular debris enhances scar formation in this type of CNS wound.

265.7

CHLOROQUIN INHIBITS REACTIVE GLIOSIS IN VIVO. G. Spurr and M. Politis,(R.Pellegrino, spon.) Orth. Surg., U.B.C., Vancouver, V5Z 4H4

A number of studies in recent years suggest a link between activation of the immune system and reactive gliosis. In the present study, chloroquin, an antimalarial agent with action that include diminishing interleukin 1 secretion, was tested for its ability to inhibit reactive glial changes in traumatized rat optic nerve.

Right optic nerves were crushed behind the eyeball and animals given daily i.p. injection of either drug (chloroquin at 6 or 20mg/kg BW supplimented with 0.01 mg/kg BW colchicine) or saline. Animals were sacrificed at 9 and 14 d.p.o. and post-traumatic changes assessed in 1 micron toluidine-blue-stained cross-sections obtained 2 mm distal to the crush site. The following were blindly quantitated: (a) number glial nuclei, (b) percent cross-sectional area occupied by glial cytoplasm, and (c) number of intact myelin sheaths.

The extent of glial hyperplasia and hypertrophy in high-dose drug-treated rats was less than 50% of that in saline-treated animals at both time points. A significant delay in clearance of myelin debris was also indicated. No inhibition of gliosis was seen in low-dose drug rats.

Results suggest feasibility of using chloroquin to decrease reactive gliosis after CNS trauma.

265.8

DETECTION OF GROWTH CONES FOLLOWING NERVE CRUSH WITH THE VIBRATING PROBE AND ELECTRON MICROSCOPY. J.M. Kerns, Anatomy Dept., Rush Medical College, Chicago, IL. 60612

Regenerating axons proceed as a front into the distal segment of a lesioned nerve. The vibrating probe of Freeman was tested as a detector of actively growing axonal sprouts at the lesion site and the distal front. Rat sciatic nerves were given crush lesions under Nembutal anesthesia and aseptic surgery. After 7 days the current densities were measured in situ with the probe and represented as vectors on a video image of the nerve. Peaks were found at the lesion site and 14mm distally. They were eliminated following exposure to verapamil, a calcium channel blocker. The nerves were then fixed for electron microscopy and examined at five selected regions. Growth cone profiles were present at the peaks, while the "silent" regions displayed the following morphology: proximal to the lesion site were normal axons; in the "valley" between the peaks were degenerated and regenerating axons associated with Schwann cells; in the segment beyond the distal peak were degenerated axons and empty Schwann cell tubes. This study demonstrates that axonal growth cones are associated with regions of a regenerating nerve that exhibit an endogenous Ca^{+2} current. Clinical application for human nerve injuries is suggested.
Supported by NIH NS19769 and BRSG SO7 RR05477 grants.

265.9

EARLY CHANGES IN THE DISTRIBUTION OF FREE CALCIUM IN TRANSECTED LAMPREY SPINAL AXONS. Alan F. Strautman* and Kenneth R. Robinson*, (SPON: J. Strong), Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.

An increase in intracellular free calcium concentration ($[Ca^{2+}]_i$) has been implicated in the degeneration of transected axons. We used fura-2 to measure the $[Ca^{2+}]_i$ in the last few mm of the proximal segments of transected axons in the larval sea lamprey spinal cord (Petromyzon marinus). There is a spatially graded increase in $[Ca^{2+}]_i$ that is complete within minutes; at 0.5 mm from the cut end the $[Ca^{2+}]_i$ is 14 times greater than basal levels and at two mm, it is three times greater. Superimposed on this initial change is a moving front of calcium that progresses up the axon, reaching 1.6 mm from the cut end in three hours. In thes regions, $[Ca^{2+}]_i$ exceeds what can be reliably measured with fura-2 (10 μM). One day after transection, $[Ca^{2+}]_i$ returns to precut levels except in the distal 300 μm indicating that the high levels of $[Ca^{2+}]_i$ do not result in the immediate destruction of the axon.

265.10

A PARTIAL CELL BODY REACTION TO AXOTOMY IN SYMPATHETIC B CELLS OF COLD-ACCLIMATIZED BULLFROGS. M.E.M. Kelly*, M.A. Bisby, A.G.M. Bulloch & K. Lukowiak. (Spon:R. Carlsen) Med. Physiology, Univ of Calgary, Calgary, Alta.

Axotomy of B-cells decreases amplitude and duration of action potential (AP) after-hyperpolarization (AHP), and increases AP duration. After regeneration, only AHP duration recovers (Kelly et al, J. Neurobiol. in press). We have now axotomized B-cells in frogs acclimatized to 15°C. At 15°C motor and sensory axons fail to regenerate, with an absence of most morphological and physical manifestations of the cell body response (CBR) (Carlsen et al, 1982, Brain Res. 234:11). The usual changes in AHP amplitude, AP duration, and sprouting from the initial segment occurred, albeit with a slower time-course. However, no significant changes occurred in AHP duration. Thus 15°C frogs respond to axotomy with only a partial CBR. To determine the site of the cold blockade of the CBR, we warmed the frogs to 21°C for one week following axotomy, before returning them to the cold. The usual reduction in AHP duration still occurred. We also took frogs from the cold 4 weeks after axotomy and placed them in the warm. One week later, the reduction in AHP duration had occurred. Thus, cold does not prevent cellular recognition of the axotomy signal or interfere with the expression of the response initiated in the warm, but interrupts a step between receipt of signal and expression of response, and can be reversed by rewarming.

265.11

PLASTICITY OF DORSAL ROOT GABA SENSITIVITY DURING PERIPHERAL AXON INJURY AND REGENERATION. R. B. Bhisitkul*, T. R. Gordon, J. D. Kocsis and S. G. Waxman. Dept. of Neurology, Yale Univ. Med. Sch., New Haven, CT 06510.

Axotomy of the sciatic nerve leads to a decrease in GABA sensitivity of L4 and L5 dorsal root axons. To examine the mechanisms of this alteration, unilateral tight ligatures were placed on sciatic nerves of adult Wistar rats for 4 weeks, then released to allow 3-4 weeks of regeneration. Isolated dorsal root segments excluding cell body and terminal regions were studied in a modified sucrose gap chamber to measure changes in membrane potential and input resistance. After 4 weeks ligation, the dorsal root depolarization elicited by GABA was reduced to approximately 60% of controls, and the effect of GABA on input resistance was diminished by about 40%. These decrements were reversed following release of the ligature; the GABA-induced depolarization was restored to approximately 75% of controls, and input resistance changes were about 10% less than controls. These effects were not accompanied by changes in compound action potential amplitude. The results suggest that dorsal root GABA receptor function is modulated by remote axon disruption, and that some component of the local regenerative environment may be involved in the restoration of axonal GABA sensitivity.

REGENERATION: FUNCTIONAL RECOVERY

266.1

DEVELOPMENT AND RECOVERY OF COMMAND FUNCTION BY THE PONTINE LOCOMOTOR REGION IN THE LAMPREY. L. Margolin* and J. Ayers. (*Spon: Norman Boissé*). Dept. of Biology and Marine Science Center, Northeastern University, East Point, Nahant, MA 01908.

Previous work has shown that swimming can be elicited by focal electrical stimulation of locomotor command systems in the rhombencephalon of larval lampreys that have behaviorally recovered from full spinal cord transection. This result had not been obtained in adults (Soc. Neurosci. Abstr.11:589). HRP studies (Swain and Ayers, this volume) have demonstrated that regeneration of a cell group *corresponding to the pontine locomotor region (PLR) (J. Neurophysiol.40:284)* was highly correlated with behavioral recovery. We stimulated the PLR in unoperated control and recovered larvae and adults and compared its effects with those evoked by rhombencephalic stimulation.

Two preparations were utilized: 1) *In situ*, the anterior quarter of the body was pinned down, allowing the caudal regions to behave freely and the brain exposed for electrical microstimulation. Movements were quantified by analysis of curvature (Science 221:1312). 2) The specimen was reduced to an isolated neuraxis for *in vitro* stimulation. Stimulus-evoked activity was recorded from ventral roots caudal to the lesion.

Focal stimulation of the PLR in larvae evoked swimming with normal timing and curvature in controls and normal curvature but slower timing in recovered specimens. In normal adults,the PLR evoked behavior was shower, less regular and of greatly reduced curvature when compared to the rhombencephalic effects. Behavior in PLR-stimulated recovered adults is similar to that found in recovered adults that were stimulated in the rhombencephalic locomotor command system. Rhythmic electrical activity was recorded in ventral roots several (~20) body segments posterior to the lesion upon focal stimulation of the PLR in recovered adults. We conclude that the PLR makes a major contribution to behavioral recovery in larvae but is only partially responsible in recovered adults.

Supported by NSF Grant BNS-8406880.

266.2

ROLE OF CORTICO- AND SUBCORTICOSPINAL PATHWAYS IN THE RECOVERY OF FORELIMB MOVEMENTS FOLLOWING NEONATAL SPINAL HEMISECTION IN MONKEYS. S.S. Cheema, M. Galea* and I. Darian-Smith. Dept. of Anatomy, Melbourne University, Victoria 3052, Australia.

Infant monkeys with hemisection of the upper cervical spinal cord recover from most but not all the deficits involving movements of the hand and arm (Caine et al., *Neurosci. Letts. Suppl.*, 30:S53, 1988). We have investigated the alterations in cortico- and *subcorticospinal pathways in juvenile monkeys, following* neonatal lesions of the cord, when recovery of movement is maximal.

WGA-HRP injections into the sensorimotor cortex of the normal newborn monkey showed strong anterograde labeling of axons and terminals in the thalamus but sparse labeling in the brainstem and cervical spinal cord. After injections of fluorescent dyes into the spinal dorsolateral funiculus, the pattern of retrogradely labeled somas in the normal sensorimotor cortex was almost adult-like. These findings suggest that in the normal neonate the corticospinal terminations are poorly developed although the axons are already present in the spinal dorsolateral funiculus (Kuypers, H.G.J.M., *Science*, 138:678, 1962). After the cord lesion, there is a uniform drastic reduction of retrogradely labeled neurons in the ipsi- and contralateral sensorimotor fields which contrasts with a claim that an increase in the ipsilateral corticospinal component occurs in adult monkeys after spinal cord hemisections (Aoki et al., *Neurosci. Res.*, 3:617, 1986).

The reduction in corticospinal connections which result after neonatal spinal hemisection suggests that subcorticospinal pathways may play an important role in the recovery of limb movements.

266.3

REGENERATION AND FUNCTIONAL RECOVERY FOLLOWING CERVICAL AND THORACIC TRANSECTION OF THE SPINAL CORD OF BULLFROG TADPOLES. P.R. BRENNER* and D.J. STEHOUWER. Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Previous studies suggest that there is greater behavioral recovery following cervical transection than following thoracic transection of the spinal cord of larval amphibians. The present study was conducted to determine whether this difference in behavioral recovery could be attributed to differences in anatomical restitution of the spinal cord. Bullfrog tadpoles (Rana catesbeiana) were transected either cervically or midthoracically and allowed to recover for five weeks. Horseradish peroxidase crystals were inserted into the spinal cord caudal to the transection site four weeks post operatively. Despite abortive regeneration exhibited by many tadpoles, others produced large numbers of axons that crossed the transection site. Comparison of cervically and thoracically transected tadpoles showed that the amount of growth across the transection site varied little with the level of transection. However, retrograde labelling revealed many cell bodies in the brainstem of cervically, but not thoracically, transected tadpoles. This difference in the source of axons is thought to underlie the greater behavioral recovery found in cervically transected tadpoles. Supported by PHS grant NS 24442 to DJS.

266.4

REGENERATED RETINO-COLLICULAR SYNAPSES EIGHTEEN MONTHS AFTER SUBSTITUTION OF THE OPTIC NERVE BY A PERIPHERAL NERVE GRAFT IN ADULT RATS. G.M. Bray, M. Vidal-Sanz*, and A.J. Aguayo. (SPON: M. Rasminsky). Neurosciences Unit, The Montreal General Hospital and McGill Unversity, Montréal, Québec, H3G 1A4, Canada.

When the optic nerve (ON) was transected and the eye and the midbrain were linked by peripheral nerve (PN) grafts for 2 months, retinal ganglion cell (RGC) axons regenerated along the grafts and formed synapses in the superior colliculus (SC) of adult Sprague-Dawley rats (Vidal-Sanz et al., J.Neurosci., 7: 2894, 1987). The aim of the present study was to determine if these regenerated synapses would persist for longer periods of time. Thus, 18 months after graft placement, ^{3}H-proline-leucine was injected into the PN-grafted eye to label the terminals of regenerated RGC axons by anterograde transport. Examined by light and EM autoradiography, two types of labelled profile were observed within the SC neuropil near the end of the PN grafts: 1) Axons, some of which were ensheathed by CNS myelin, indicating that certain oligodendroglial cells in the tectum retain their capacity to respond to the regenerating axons and reform myelin sheaths; 2) Axon terminals forming asymmetric (Gray type II) synaptic contacts with dendrites. These results suggest that regenerated RGC axons in adult rats may maintain synaptic contacts in the SC for long periods of time.

266.5

TERMINAL ARBORIZATION AND SYNAPSE FORMATION BY REGENERATING RETINAL AXONS DIRECTED ALONG PERIPHERAL NERVE GRAFTS TO THE SUPERIOR COLLICULUS OF ADULT HAMSTERS. D.A. Carter,* G.M. Bray, and A.J. Aguayo. Neurosciences Unit, The Montreal General Hospital and McGill University, Montréal, Québec, Canada, H3G 1A4.

The retina and the superior colliculus (SC) were joined by a peripheral nerve (PN) graft after optic nerve (ON) transection in adult hamsters. Two-4 months later, the terminals of axons regenerating from the retina were labelled by anterograde transport of HRP. The ultrastructural features of the regenerated RGC terminals were compared with the retino-collicular terminals of control animals. The regenerated RGC terminals, observed in the neuropil of the superficial SC within 0.5 mm of the ends of the grafts, showed the densely-packed, spherical synaptic vesicles and mitochondria typical of the terminals of intact RGCs. Although some regenerated RGC terminals were larger than normal, most resembled the HRP-labelled RGC terminals seen in control animals in terms of: the proportions that showed well-differentiated synaptic specializations; the predominance of synaptic specializations of asymmetric type; and the proportions of contacts with dendritic profiles that contained vesicles. These results suggest that most regenerating RGC axons that re-enter the SC of these adult hamsters undergo pre- and post-synaptic differentiations that resemble those of normal retino-collicular synapses.

266.6

DEVELOPMENT OF A CORTICAL MAP OF THE HAND AFTER NERVE INJURY AND REGENERATION IN NEONATAL MACAQUE MONKEYS. J.T. Wall, J.H. Kaas, C.G. Cusick, S.L. Florence, P.E. Garraghty, M.F. Huerta, and M.A. Sesma. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

Macaque monkeys were raised from the first 1-2 weeks after birth with an injured (transected and ligated) or regenerated (transected and repaired) median nerve to the hand. Maps of cutaneous tactile inputs in cortical area 3b of these monkeys were made with neurophysiological techniques 2-3 years after birth, and compared to maps from normal monkeys. Following injury, a persisting denervated zone is produced peripherally on the hand; however, an organized, but topographically abnormal representation of hand zones innervated by the 2 remaining nerves develops in cortex. Following regeneration, a map reflecting innervation by all 3 hand nerves is formed. Although indications of abnormal convergence/divergence are seen, normal topographical features (e.g. receptive fields, digit representation locations) are also apparent. Primate sensory systems undergo developmental changes during the first weeks and months after birth; however, it is not known whether a combination of peripheral regeneration and neonatal mechanisms of central change (e.g. exuberance, competition, segregation, etc.) can lead to postnatal formation of a normal map. The current findings suggest neonatal regeneration and developmental mechanisms are sufficient for at least a partial recovery of normal cortical features. (Grants NS21105 and NS16446).

266.7

ANATOMICAL AND FUNCTIONAL REGENERATION OF FIBER TRACTS IN THE NEONATAL RAT SPINAL CORD. A.A. Alfaro* and V.R. Holets (SPON: Å. Seiger). Dept. of Neurological Surgery, Univ. of Miami, Miami, FL 33136.

The neonatal rat spinal cord is immature at birth, with descending pathways developing during the first 2 post- natal weeks. Complete transection of the spinal cord at the T10 spinal cord level was done in 2-, 4-, 5-, 6-, 8-, 10-, 12-, 14- and 21-day old rats. The cut ends of the spinal cord remained opposed following the transection. Age-matched, sham-operated animals received a laminectomy, but no spinal cord lesion.

Animals lesioned at 2 to 21 postnatal days exhibited a >90% functional deficit during the first 2 weeks post-lesion using a modification of the system of Gale et al. (Exp. Neurol. 88:123-134,1985). Significant recovery was observed in the rats lesioned at 2 to 6 postnatal days from 2 weeks to 60 days post-lesion, with a recovery to <30% functional deficit. Neonates lesioned at 8 to 21 postnatal days did not show any functional recovery during this time. Immunohistochemical and retrograde axonal studies revealed significant growth of monoamine and peptidergic pathways from brainstem and hypothalamic nuclei, and from the red nucleus and the cerebral cortex through the lesion site and into the distal spinal cord segments. These results suggest that the neonatal spinal cord allows for regeneration of descending pathways during a narrow window of time during the first postnatal week. Funded by The Miami Project.

266.8

FUNCTIONAL RECOVERY AFTER LINGUAL NERVE INJURIES IN CATS. P.P. Robinson* (SPON: J. Stephens). Depts. of Physiology & Oral Surgery, Birmingham Univ. Med. School, B15 2TJ, U.K.

The recovery of gustatory, thermosensitive, mechanosensitive, vasomotor and secretomotor fibres in the combined trunk of the chorda tympani and lingual nerves has been investigated following nerve injury. Under anaesthesia the nerve was either crushed (4 cats) or sectioned (3 cats) unilaterally and recovery allowed for 12 weeks. Recordings made from 52 single units in the chorda tympani after nerve crush revealed that the proportions of gustatory, thermosensitive and mechanosensitive units were similar to those of controls but the units had slower conduction velocities, responded less vigorously and to a narrower range of stimuli. Recordings made from 46 units in the chorda tympani after nerve section revealed very few gustatory or thermosensitive units, the majority being purely mechanosensitive. The decrease in conduction velocity was greater than after nerve crush. Electrical stimulation of efferent vasodilator fibres in both the chorda tympani and lingual nerves, evoked a temperature rise on the dorsal surface of the tongue. This effect was completely restored after nerve crush but was significantly smaller after nerve section. The flow rate of saliva from the submandibular salivary gland was not significantly changed after nerve crush but was significantly smaller after nerve section. There was no evidence for functional reinnervation by inappropriate fibre types. Supported by the MRC (U.K.).

267.1

SYNAPTIC BOUTON CHANGES FOLLOWING CRUSH INJURY OF THE FACIAL NERVE. S.K. Jacob and T.E. Durica, Anatomy Dept., Rush Medical College, Chicago, IL 60612.

This study examines the short-term changes in axosomatic synaptic bouton contacts on facial motor neurons following crush injury of the facial nerve. Comparisons are made to results following axotomy (cut and removal of a segment) of the facial nerve. Adult hamsters underwent a crush of the right facial nerve; brains were processed for routine ultrastructural examination at 5 days postoperative (dpo). Following either injury the neurons exhibited a morphology typical of the chromatolytic response. At the perimeter of some injured neurons were many thin astrocytic processes. A quantitative study assayed the percent coverage of somal profile by synaptic boutons in normal, 5dpo crush, and 5dpo axotomy. Normal facial motoneurons have 30% of the soma contacted by synaptic boutons. Both injuries result in significant decreases in bouton contacts: 5dpo crush = 13% coverage and 5dpo axotomy = 8% coverage. The mean number of synaptic boutons/neuronal profile was compared: normal= 15 contacts, 5dpo crush = 9 contacts and 5dpo axotomy = 6 contacts. The less severe response seen after crush injury might reflect the fact that these neurons can regrow axons and return to normal function; while axotomy, which prevents axon regrowth, causes a greater response.

267.2

CHANGES IN SYNAPTIC BOUTONS AFTER AXOTOMY OF THE VAGUS AND HYPOGLOSSAL NERVES IN THE ADULT HAMSTER. T.E. Durica and S.K. Jacob, Anatomy Department, Rush Medical College, Chicago, IL 60612.

This study examined the short term changes in axosomatic synaptic bouton contacts on vagal and hypoglossal motoneurons after axotomy. The right vagus and hypoglossal nerves of adult hamsters were cut and at 5 days postoperative (dpo) the brainstems were processed for routine ultrastructural examination. These motoneurons exhibited a typical chromatolytic response to axotomy. The Bioquant System IV was used for measurement and data analysis. A quantitative study assayed the percent coverage of somal profiles by synaptic boutons on normal and axotomized neurons. After axotomy for both neuronal populations there is a decrease in percent coverage. For vagal neurons: normal = 14%; axotomy = 3%; for hypoglossal neurons: normal = 30%; axotomy = 18%. There was also an accompanying decrease in the mean number of synaptic boutons per neuronal profile. For vagal neurons: normal = 6; axotomy = 1; for hypoglossal neurons: normal = 18; axotomy = 14. These changes which are most dramatic for vagal neurons might be expected because long term studies have shown that these neurons do not survive axotomy.

267.3

DEVELOPMENTAL PLASTICITY OF THE OPOSSUM'S RUBROSPINAL TRACT-REGENERATION OR NEW GROWTH? X.M. Xu, R.R. Pindzola and G.F. Martin. Department of Anatomy and Neuroscience Program, The Ohio State University College of Medicine, Columbus, Ohio 43210.

We have shown previously that rubral axons can grow around a lesion of their spinal pathway in the developing opossum, but it is not clear whether they are the ones originally cut or later growing ones. In an initial attempt to address that issue, we tried to determine whether any rubral neurons survive axotomy during the early part of their critical period for plasticity. If not, rubrospinal plasticity must be a result of new growth, not regeneration of cut axons. In these experiments pouch-young opossums of the appropriate age were anesthetized so that Fast-Blue (FB) could be injected into the thoracic cord. Three to four days later they were reanesthetized and the rubrospinal tract was cut 3 segments rostral to the injection. The animals were retained for approximately 1 month after the lesion before being anesthetized and sacrificed by perfusion. In all cases, rubral neurons were labeled contralateral to the lesion but their number varied depending on the size of the injection. Our results indicate that at least some rubrospinal neurons survive axotomy suggesting that true regeneration may have occured. It is possible, of course, that the labeled neurons survived the lesion, but their axons did not grow around it. Additional experiments will have to address that issue. (Supported by NS-25095).

267.4

EFFECTS OF CLAW REMOVAL AND REGENERATION ON THE GANGLIONIC NERVE AND NEUROPIL OF MALE FIDDLER CRABS. J.I. Mayes* & C.K. Govind, Scarborough College, University of Toronto, Scarborough, Ont. M1C 1A4, Canada.

The paired dimorphic, major and minor, claws in male fiddler crabs, Uca pugnax, have a corresponding asymmetric nervous system. In a crab with pristine claws, the number of sensory axons in the nerves of the major side of the claw ganglion was 3x greater than on the minor side. The neuropil, however, had a higher density of nerve terminals (1.4x) and synaptic contacts (1.8x) on the minor side compared to the major side. In a crab with a newly regenerated major claw, the number of axons in the major claw nerves was only 1.8x greater than in the minor side. Since this ratio is smaller than that seen in a crab with pristine claws, it suggests that the regenerated claw has fewer axons. In this same animal, the density of terminals and synapses is higher (1.2x) in the minor side neuropil compared to the major side, although the difference is not as great as in the pristine condition, suggesting a slight increase in the number of these elements, or a decrease in the size of terminals, on the major side. The major side also shows degenerating profiles in the nerves and neuropil soon after the claw has been removed and even after it has regenerated, indicating a remodeling of the ganglion each time a claw is lost and regenerated. Research supported by NSERC and MDAC.

267.5

GROWTH AND ELECTRICAL COUPLING OF REGENERATING NEURITES FROM SEVERED AND ROTATED GIANT FIBERS IN THE EARTHWORM. A.W. Lyckman and G.D. Bittner. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

The ventral nerve cord (VNC) of the earthworm contains two lateral giant fibers (LGFs) and one median giant fiber (MGF). Lucifer yellow injections show that severed giant fibers grow neurites across the lesion site within several weeks after transection. In addition, if a segment of the VNC is doubly cut, removed and replaced after rotating 0-180° in the dorsoventral plane (DV-rotation), neurites regenerate across the lesion sites.

At all post-operative periods from 2 weeks to 9 months in both VNC-transections and DV-rotations, conduction of action potentials across the lesion site correctly restores the normal functional connections between severed giant fibers, *i.e.*, severed MGFs and LGFs reconnect appropriately. However, conduction of action potentials across the lesion site is much more easily fatigued by high frequency stimulation than action potential conduction in the unoperated VNC. Through-conducting MGFs or LGFs are electrically coupled with coupling ratios up to 0.5, a lower coupling ratio than is found in intact giant fibers (1.0).

Data from these and other studies is being used to better understand cellular mechanisms which contribute to the regeneration of specific and appropriate connections between these giant interneurons.

Supported by TATP grant from Texas.

267.6

TEMPORAL PARAMETERS OF OLFACTORY NEURONS DEGENERATION AND REGENERATION. L.M. Alves*, P.P.C. Graziadei and A.G. Monti Graziadei (Spon. J.S. Elam). Department of Biological Sciences, Florida State Univ., Tallahassee, FL 32306-3050.

Axotomy of the fila olfactoria in adult rats results in degeneration followed by regeneration of the olfactory sensory neurons with functional recovery. In this study we wanted to investigate the temporal parameters of this phenomenon using the olfactory marker protein (OMP) as a marker. Axotomy was performed at the intracranial level of the lamina cribrosa. Survival times ranged from 1 to 45 days. The rats were anesthetized, perfused with Bouin and embedded in paraffin. Serial sections were cut in the horizontal plane and stained with hematoxylin and immunohistochemically for OMP. Two days after surgery all the OMP-positive perikarya had disappeared from the olfactory neuroepithelium. Their reappearance began after twelve days and from the 35th day the number of perikarya in the epithelium was comparable to control values. In the olfactory bulb, OMP was observed in the glomeruli up to eight days after surgery. After this time, the glomeruli remained negative up to 20-23 days postoperative survival time, when few isolated OMP-positive axons started to enter into the glomeruli. Control level of OMP-positivity in the olfactory bulb was reached after 45-50 days. The disappearance of OMP from the epithelium and from the olfactory bulb did not parallel the morphological changes previously described and the significance of these results will be discussed. (Supported by NIH grant NS 20699).

267.7

AN AUTORADIOGRAPHIC TIME STUDY OF HEALING AND REGENERATION IN FULLY DIFFERENTIATED XENOPUS RETINAS. L. Wunsh* and C.F. Ide. Dept. of Biology, Tulane University, New Orleans, LA 70118.

In previous studies, removal of the temporal 2/3 of the fully differentiated Xenopus eye (stages 38-48) resulted in restoration of an intact eye. In addition, these eyes formed pattern duplicated projections to the midbrain optic tectum. To determine the role of healing and cell division during regeneration in these progressively older larval animals, tritiated thymidine labeling patterns were analyzed. Nasal one-third sized eye fragments made at stages 46 through 48 were injected either one day, one week, or one month post-surgery. All animals were fixed one day post-injection. At all stages, healing involved dedifferentiation of pigmented retinal epithelial cells stretching across the lens and closing the wound. Heavy label was localized in the ventral retina in a neuroepithelium internal to the extended pigmented retinal epithelium. These results correspond to previous label pattern studies done on undifferentiated stage 32 eye fragments. Thus, in both undifferentiated and differentiated retinal fragments, movements from the eye's ventral region and associated local cell division correlate with pattern duplication of the retino-tectal projection. **Supported by NSF Grant PCM-8316142.**

267.8

HAIR CELL REGENERATION FOLLOWING EXPERIMENTALLY INDUCED HAIR CELL DEATH IN THE LATERAL LINE SYSTEM OF THE AXOLOTL SALAMANDER (AMBYSTOMA MEXICANUM). K.J. Balak* and J.T. Corwin. Békésy Lab. of Neurobiology, Univ. of Hawaii, Honolulu, HI 96822.

The sensory hair cells of the lateral line system of the axolotl were specifically stained with the fluorescent mitochondrial dye 2-(4-dimethylaminostyryl)-N-ethyl pyridinium iodide (DASPEI). We have used this cell-type specific staining to kill the sensory hair cells of an individual neuromast organ by exposing the stained hair cells to intense UV irradiation for 1-2 hours. After UV irradiation the neuromast organ was devoid of sensory hair cells and consisted of a single layer of supporting cells as observed by differential interference contrast microscopy and fluorescence microscopy of DASPEI stained cells. Within 2 to 5 days of hair cell depletion new hair cells were observed by DIC and uptake of DASPEI. The tritiated thymidine technique was used to determine if the new hair cells arose from the progeny of proliferating supporting cells. A neuromast organ was depleted of hair cells using DASPEI and UV irradiation. The axolotl was given tritiated thymidine intraperitoneally and periodically examined for the appearance of new hair cells. The tissue was processed for autoradiography. The regenerated sensory hair cells were labeled with tritiated thymidine indicating that these cells arose through cell division from the remaining supporting cell population in the absence of differentiated hair cells. We conclude that the sensory hair cells arose from supporting cell progeny following hair cell death. (Supported by a Deafness Research Foundation Grant to KJB and grants from NINCDS to JTC.)

267.9

CELL BEHAVIOR DURING HEALING OF HALF-EYE FRAGMENTS OF THE EMBRYONIC MOUSE. C. D. Claypool*, C. F. Ide, and K. Muneoka*. Dept. of Biology, Tulane University, New Orleans, LA 70118.

We have begun to investigate the effects of surgical ablations of the neural retina prior to differentiation during embryonic stages of the mouse. Developing neural retina tissue was removed from mouse embryos on embryonic day (E) 14 and 15, using *ex utero* surgery techniques (Muneoka, et al., 1986, J. Exp. Zool., 239: 289-293). Approximately one-half of the developing eye was removed without disturbing the lens. No attempt was made to surgically close the wound. The mice were allowed to develop to E19 (term), and were then analyzed histologically.

The developing mouse retina at the time of surgery is a thin, undifferentiated neuroepithelium. An optic stalk and forming cornea and lens are present. Half-eye ablate embryos fixed immediately post-surgery showed the cornea pulled back almost to the eyelid flexure, with a gaping opening between the cut edges of neuroepithelium. By E19, the unoperated eye showed a thick neuroepithelium with a definite ganglion cell layer, an optic nerve head, and differentiated cornea, whereas operated eyes left to heal until day 19 showed a neuroepithelium somewhat reduced in thickness, with no evidence of differentiated ganglion cells. Healing involved the formation of a thin layer of cells emanating from the cut edge and stretching around the lens. The ciliary margin in the undamaged region of the fragment appeared intact.

Healing modes appeared similar to those seen in late stage amphibian embryos in which similar cell behavior correlates with extra cell division and, over time, restoration of a full sized eye. **Supported by NSF PCM-8316142, HD 23921, HD 20662, and a gift from the Monsanto Company.**

267.10

UNEQUAL PROBABILITIES OF REGENERATION AMONG LAMPREY RETICULOSPINAL NEURONS. R.S. Croop*, J.A. Snedeker* and M.E. Selzer. (Spon: S. Shuman). Dept. Neurology and David Mahoney Inst. of Neurol. Sci., Univ. of Pa. Sch. of Med., Phila., PA. 19104-4283.

Regeneration of reticulospinal axons in the sea lamprey (P. marinus) shows an increase in latency as the transection site is moved more distal to the cell body. Half of all axotomized neurons regenerate a neurite that extends at least 2.5 mm past the scar. In order to determine whether different cell groups have different regenerative abilities, the reticulospinal cells were subdivided into 13 groups and the regeneration of each determined by retrograde transport of HRP across a previous transection. Analysis of variance demonstrated that two of the groups, one of which contained the Mauthner and the bulbar Muller cells, had significantly greater probability of neuritic regeneration than the rest.

For transections at the level of the 7th gill, maximal probability of regeneration was seen at 95 days, declining thereafter in all cell groups. More caudal lesions were associated with longer delays for peak regeneration.

(NIH grants NS14837 and NS25581).

267.11

AXONS IN REGENERATED LIZARD TAIL ARE PRIMARILY OF LOCAL ORIGIN. M.T. Duffy*, B.M. Davis and S.B. Simpson Jr. Dept. of Biological Sciences, Univ. of Illinois at Chicago, Chicago, Il., 60680.

Transection or autotomy of the lizard (*Anolis carolinensis*) tail results in regeneration of a new tail with concomitant regeneration of CNS axons. Electron microscopic analysis shows the regenerate spinal cord white matter consists of an average of 2,000 axons (R = 1,720 - 2,346 ; n = 6).

HRP labeling of a normal midback spinal cord shows lumbar projections from many brainstem nuclei (Rub, Rs, Ras, Rm, Vest, Rai and Ri; X=1,384; n=5). HRP labeling of axons from normal tail cord(X=388 cells) shows the same brainstem distribution while HRP labeling in mature regenerate tail cord(X=49; n=19) shows fewer cells but a similar distribution. Interestingly, there is an absence of a rubrospinal projection to the regenerate tail. If, however, HRP is placed in the normal cord 1-2 mm rostral to the regenerate cord the distribution and number of cells is identical to normal tail. This suggests that the paucity of regenerated bulbospinal projections (including the absence of rubrospinal) is not due to retraction and/or degeneration.

Since the number of regenerating bulbospinal axons cannot account for the number of axons seen in the regenerate tail cord, the majority of their origins must be elsewhere. EM analysis following transection of the spinal cord at all levels more than 1 segment rostral to the regenerate cord revealed no reduction in number of regenerated axons. Transection of the spinal cord one segment rostral to the regenerate produced a significant reduction (>50%) in the number of regenerated axons. This suggests that the majority of regenerated fibers have their origin within one segment of the regenerate cord. These origins must be either local circuit spinal neurons or primary sensory cells of the dorsal root ganglia. (Supported by NS24162 to SBS).

267.12

TIME COURSE OF SALAMANDER SPINAL CORD REGENERATION: BEHAVIORAL AND ANATOMICAL ANALYSIS. B.M. Davis, J. Ayers, L. Koran*, J. Carlson*, and S.B. Simpson, JR. Dept. of Biological Science, Univ. of Illinois at Chicago, Chicago, IL 60680.

Five groups (10 animals/group) of salamanders received complete low thoracic transections. All animals received a second transection 10mm caudal to the first transection 2,4,6,8 or 12 wks following the initial lesion. A pledget of HRP was inserted at the site of the second transection to labeled any descending axons which had grown10m past the first transection. Just prior to the second transection each animal was filmed to record the animals abiltiy to walk and swim. In addition, the animals in the 12wk group were filmed every 2wks following the initial lesion. These films were used to score the recovery of function and for future kinematic analysis of ambulatory and swimming behaviors in regenerated salamanders. The earliest return of coordinated swimming and walking behavior was seen 4 weeks (2/20 animals) following transection but most animals (13/27) did not demonstrate coordinated behavior until week 6-8 weeks following transection. Examination of the site of the intial lesion and analysis of the films suggests that animals that did not demonstrate behavioral recovery by week 6-8 had ceased to regenerate. This suggests that there is a critical period during which regeneration must take place if it is to be successful. All animals which exhibited spontaneous coordinated locomotion contained HRP labeled neurons in the medullary reticular formation. Other spinally projecting nuclei (e.g., red nucleus, vestibular nuclei) were not consistently labeled in animals exhibiting coordinated locomotion. (Supported by NS 24162 to SBS).

267.13

REGENERATING CGRP-IMMUNOREACTIVE DORSAL ROOT GANGLION (DRG) AXONS FORM TERMINALS IN TRANSPLANTS OF FETAL SPINAL CORD. Y. Itoh* and A.Tessler. VA Medical Center and Medical College of Pennsylvania, Philadelphia, PA 19129.

We have previously shown that the cut central axons of adult DRG neurons regenerate into transplants of embryonic spinal cord and that some of these elongating axons are immunoreactive for calcitonin gene-related peptide (CGRP). In the present study we used electron microscopic (EM) immunocytochemistry to compare the morphology of CGRP-immunoreactive axons regenerating into transplants with CGRP-labeled axons in normal dorsal horn.

Adult Sprague-Dawley rats received transplants of E14 or E15 spinal cord at the lumbar enlargement, and adjacent dorsal roots were cut and juxtaposed to the grafts. After 6 weeks to 3 months CGRP immunoreactivity was visualized both at the LM and EM levels. CGRP-labeled fibers arborized extensively within the transplants, and CGRP-immunoreactive axon terminals were seen. Most of these axons were unmyelinated and therefore resembled CGRP-labeled axons in normal dorsal horn. These results indicate that the axons of CGRP-containing DRG neurons which regenerate into transplants resemble those found in normal dorsal horn and form terminals within the transplants. Supported by the VA Medical Research Service, NIH grant NS 24707, and USAMRDC grant 51930002.

267.14

PERIKARYAL CHANGES IN SPINAL MOTOR NEURONS GIVING RISE TO LONG-TERM REGENERATED AXONS. C.M. Bowe and C.Y. Yu*, Section of Neurobiology, Brown University, Providence, RI 02912

Morphological and physiological abnormalities are reported in regenerated mammalian axons examined one year following sciatic nerve crush injury. In the present study, histological properties of rat motor neurons giving rise to regenerated axons were examined 14-16 months following unilateral sciatic nerve crush at 1-3 months of age and were compared to those of cells contralateral to the crush or in comparable cord segments from age-matched control rats. Regenerated and control sciatic motor neurons were identified by retrograde labelling with horseradish peroxidase applied to sciatic nerves distal to the site of crush. Many of the experimental motor neurons were enlarged and had thickened dendritic processes compared to control cells. Mean cell area ipsilateral to the crush lesions was larger than that determined for control cells (p-value <.001) despite representation of all control cell areas in the regenerated population. These data indicate that persistent or progressive morphological changes occur in regenerated mammalian motor neurons following simple crush injury.

267.15

REGENERATION OF DESCENDING PATHWAYS AFTER SPINAL CORD TRAUMA IN THE RED-EARED TURTLE. D. Santini* and R.H. Browner (SPON: D. Marbey). Dept. of Anatomy, NY Med. Coll., Valhalla, NY 10595.

Turtles were anesthetized with Brevital Sodium and maintained with oxygen, halothane, and nitrous oxide. The spinal cord was exposed at the caudal end of the first vertebral scute, the dura mater incised, and the spinal cord cut or crushed with a microscissor. The wound was rinsed and dental cement closed the bony opening. Turtles survived for the 15-, 30-, 45-, and 60-days with at least 2 animals/group. Four to 6 days before final survival time the spinal cord was exposed and cut at the caudal end of the 2nd vertebral scute. A gelfoam plug of HRP type VI (30%) was placed in the cut. The wound was washed and closed. At the survival times turtles were killed with Nembutal and transcardiaclly perfused with Reptilian Ringers, fixed and processed for HRP. Transverse sagittal frozen sections (45 µm) were made through the brains spinal cords, respectively. Alternate sections were stained with Cresyl Violet. The 30-, 45-, and 60-day survivors showed HRP product in the upper cervical spinal cord, nucleus raphe inferior, vestibular nuclei, and reticular nuclei. Supported by the Culpeper Found and NYMC Biomed Grant.

267.16

Regeneration of the Pontine Locomotor Region in the Sea Lamprey G. P. Swain* and J. Ayers.. Dept. of Biology and Marine Science Center, Northeastern University, East Point, Nahant, MA 01908.

The descending reticulospinal system in ammocoete, metamorphosing, and transformed sea lampreys was described using the HRP backfill technique (Soc. Neurosci. Abstr.12:318). The spinal cords of larvae and adults were completely transected at 25% of body length and in control specimens HRP-soaked GelFoam pledgets were inserted into the lesions. The HRP- implanted specimens were maintained ten to fifteen days for HRP transport. The HRP was developed using the Hanker-Yates method and whole mounts of the brains were made on glass slides. The HRP filled cells were segmented into 13 bilateral regions based on natural clusterings and quantified. A sample of HRP- filled normal brains (n = 60) serve as the control population for our regeneration experiments.

Regenerating specimens were allowed to recover to behavioral criteria. The recovery of swimming behavior may occur in as little as 50 days, depending upon the temperature at which the animals are held (Soc. Neurosci. Abstr.12:1574). At various times after the animals had recovered function a second transection was made distal to the initial regenerated lesion and an HRP-soaked pledget was inserted and processed as described above.

The extent of regeneration of particular cells or cell groups is quite variable with two exceptions: at least some cells in the anterior ischmic region (which probably correspond to the pontine locomotor region (PLR) cells described in cats, J. Neurophysiol 40:284) and the posterior cells of the bulbar region have regenerated in all recovered animals. Examination of regenerated cords of different recovery times indicates that regeneration of these neurons occurs continuously for periods of up to 15 months. As the process continues the proportion of cells which regenerate to the second lesion increasingly resembles that of the controls.

The majority of experimental animals (n=60) were backfilled from a second lesion made approximately 5 mm distal to the regenerated lesion. A number of animals (n=15) were backfilled from 3.0 cm distal to the lesion. These animals demonstrate that the same principle operates for long-distance regenerated axons: the longer the animal is allowed to recover, the more normal the descending projections become.

Supported by NSF Grant BNS-8406880.

267.17

ONE CLASS OF CENTRIFUGAL FIBERS TO THE RETINA IN CICHLID FISH DOES NOT REGENERATE. Anne C. Rusoff. Dept. of Biology, Montana State Univ., Bozeman, MT 59717.

The fish optic nerve is known for its ability to regenerate. One factor often implicated in this ability is the continued addition of ganglion cells to the retina into adulthood. The presence of factors which enable precursor cells to continue dividing into adulthood may facilitate regeneration, or it may be permitted because the system retains specific developmental mechanisms to enable new axons to find the tectum.

The fish optic nerve also contains centrifugal axons. In the cichlid fish Herotilapia multispinosa axons from the nucleus olfactoretinalis (NOR) project to the retina (as do axons from some diencephalic nuclei). Unlike the ganglion cells, all the centrifugally projecting cells in the NOR are born early. I have used this system to examine the requirements for regeneration.

One optic nerve of 6 month old fish was crushed. The presence of centrifugal fibers to the retina was assayed using an antibody to FMRFamide. (Some of the centrifugally projecting cells in the NOR stain with this antibody.) The NOR on both the normal and experimental sides of the brain stained intensely. Axons were visible entering both optic tracts. However, no FMRFamide + axons were visible entering the retina or in the normal terminal zone of efferents in the inner plexiform layer. This suggests that regeneration in the optic nerve is limited to axons from cell populations which are dividing. (EY06495.)

267.18

AXOTOMY OF RETINAL AXONS CAUSES GANGLION CELL DEATH IN YOUNG LARVAE OF ZEBRAFISH

C. Kuschel and C. A. O. Stuermer, Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Tübingen, FRG

Ganglion cells in the visual system of adult fish regenerate their axons after axotomy and reestablish appropriate connections with the tectum. Whether ganglion cells possess this ability at all developmental stages was investigated using zebrafish of various ages.

In zebrafish embryos retinal axons have formed connections with their retinotopic target sites in the tectum at 72 h post fertilization (Stuermer 1988). The optic nerves were sectioned (ONS) in fish of 7 d and 1, 2 and 6 months of age. 10 to 20 d after ONS HRP was applied to the retina to label the regenerating axons in the optic nerve and tectum.

The nerves and tecta of the fish with nerve cuts at 1, 2 and 6 months exhibited HRP labeled regenerated axons in high density. However, labeled axons were absent from the nerves and tecta of fish with nerve cuts at 7 d, even after survival periods of 20 d.

Examinations of serial transverse sections (4 µm) of the fish's head revealed that most of the ganglion cells of the 7 d ONS larvae had degenerated except for those at the peripheral margin. At the periphery cells are added continuously during the ongoing growth of the fish's eye. In the 1, 2 and 6 months ONS fish all ganglion cells were well preserved. As typical for ganglion cells with regenerating axons, they were larger than controls.

Thus, in contrast to juvenile and adult fish, retinal ganglion cells in larval fish die after injury of their axons. This suggests that ganglion cells change their properties with progress in development.

267.19

DEGENERATION AND REGENERATION OF RAT OPTIC NERVE FIBERS FOLLOWING AXOTOMY: EFFECTS OF NGF. C. Comelli*[1], G. Carmignoto*[1] and L. Maffei*[2] (SPON: G. Calderini). [1]Fidia Neurobiological Research Laboratories, Abano Terme, Italy and [2]Institute of Neurophysiology, CNR, Pisa, Italy.

Intraocular repetitive injections of NGF are effective on the survival of rat retinal ganglion cells after section of the optic nerve. Here we report that NGF has also an influence on the processes of degeneration and regeneration at the level of optic nerve fibers. Experiments were performed in adult rats 7 weeks after intracranial bilateral transection of the optic nerves. Animals received intraocular repetitive injections of NGF (3 μg/injection) into the right eye and cytochrome c into the left eye. Ultrathin sections were collected from both optic nerves every 500 μm starting from the site of the transection. Results show that a) the NGF treated optic nerves have much more numerous myelinated fibers with respect to controls; b) the NGF treated optic nerves show different stages of degeneration and regeneration at any distance from the globe. The degeneration in the NGF treated eye seems slowed down and regeneration, as evaluated by the presence of unmyelinated fibers, accelerated. The bundles of unmyelinated fibers show features similar to those observed in the regenerating optic nerve of lower vertebrates. Myelinated fibers are found in sections as distant as 5.0; 5.5; 6.0; 6.5 mm from the globe. At these levels no fibers were detected in the controls.

DRUGS OF ABUSE II

268.1

IN VIVO MICRODIALYSIS STUDY OF DA FUNCTION IN THE RAT NUCLEUS ACCUMBENS DURING ACUTE AND REPEATED COCAINE SELF-ADMINISTRATION. F. Weiss, Y.L. Hurd*, G.F. Koob, N.-E. Anden* and U. Ungerstedt*. Res. Inst. of Scripps Clinic, La Jolla, CA 92037, Dept. of Pharmacology Karolinska Institute, Box 60 400, S104 01, Stockholm, Sweden.

Previous research suggests that dopamine (DA) in the nucleus accumbens (NAS) is critical in both cocaine (COC) reward and dependence. We sought to further examine the role of mesolimbic DA in COC reward using the in vivo microdialysis method in awake, drug self-administering rats. Extracellular levels of DA and its metabolites were monitored in dialysate collected from the NAS during 3 h self-administration (SA) sessions in "acute" (first-) or "subchronic" (10th SA session) drug conditions. After completion of microdialysis, COC effects on DA synthesis (DOPA accumulation) were estimated from tissue homogenates of NAS, anterior (AS) and posterior striatum (PS). DA and metabolites in the dialysate of "acute" rats were markedly elevated and rose to 250% of control levels by the end of the SA session. In contrast, DA and metabolite levels in "subchronic" rats were indistinguishable from those of naive, saline-treated rats. COC treatments did not alter DOPA accumulation in any of the brain regions sampled. DA tissue levels in the AS of "subchronic" rats were increased 50% over those of "acute" animals; no such differences were found in the NAS or PS. These results suggest that extracellular DA levels in the NAS decline with repeated COC administration in the absence of changes in DA synthesis or reductions in intracellular DA concentrations. The results may also provide a neurochemical basis for anecdotal reports of profound tolerance to COC's euphoric effects after one exposure in man.

268.2

COCAINE: AN IN VIVO MICRODIALYSIS STUDY OF ITS PHARMACOKINETIC PROPERTY IN AWAKE SELF-ADMINISTERING AND ANAESTHESIZED RATS. Y.L. Hurd*, J. Kehr*, F. Weiss, G.F. Koob, and U. Ungerstedt* (SPON: E. Stein). Karolinska Institute, Pharmacology Dept., Box 60 400, S-104 01, Stockholm, Sweden.

Cocaine (COC), a highly addictive agent with major social problems, has been widely investigated and suggested to have potent action on the dopaminergic system in the nucleus accumbens (NAS) and striatum (STR) In this study the pharmacokinetic property of COC was tested by in vivo microdialysis in halothane-anesthetized and in freely moving rats during self-administration (SA). Microdialysis not only allows measurement of endogenous neurochemicals in the extracellular space of discrete brain regions, but also exogenous compounds such as COC. Following an acute intravenous (iv) injection, COC was detected within the first 10 min perfusate in the NAS, STR and blood. The response pattern of the actual level of COC in the NAS and striatum after this injection was consistent with the characteristic DA response pattern induced by this drug. Since in vivo microdialysis makes it possible to monitor the neurochemical consequence during SA of drugs, the extracellular level of cocaine in the NAS of rats during 3h "acute" (first) and "subchronic" (10th SA) COC SA sessions was also analyzed. The role of excitatory and inhibitory amino acids on neurotransmission after COC SA was also studied.

268.3

MONOAMINE INCREASE BY COCAINE AND PROCAINE IN THE NUCLEUS ACCUMBENS: A MICRODIALYSIS STUDY. L. Hernandez and B. G. Hoebel, Dept. Psychol. Princeton Univ., Princeton, NJ 08544

We showed previously that cocaine delivered locally into the nucleus accumbens can increase extracellular dopamine [1]. Like cocaine, procaine can induce mood changes in humans and is self-administered by monkeys. It has a molecular structure similar to cocaine, and both are local anesthetics. Therefore, cocaine and procaine were infused through a microdialysis probe in the nucleus accumbens to compare their actions. In Exp. 1, cocaine microinjections (20 ug/0.5 ul) significantly increased extracellular dopamine, norepinephrine and serotonin. The effect on dopamine and serotonin was seven times greater than on norepinephrine. DOPAC decreased while 5-HIAA and HVA were not affected. In Exp. 2, equimolar solutions of cocaine and procaine were compared; but to avoid local mechanical trauma, they were infused by reverse microdialysis. The infusion was 50 ug of drug in 20 ul of perfusate to produce an estimated 5 ug diffusing into accumbens tissue during 20 min. Both drugs increased dopamine significantly, but cocaine was 2.4 times more potent than procaine. These results suggest (1) that the actions of cocaine in the nucleus accumbens might be mainly due to dopamine and serotonin increase, (2) that cocaine and procaine act directly on monoaminergic terminals in the nucleus accumbens to increase extracellular dopamine, and (3) that mood changes and addiction with procaine are partly due to its cocaine-like effect on the dopamine terminals in the nucleus accumbens.

1. Hernandez, L. & Hoebel, B. G. Life Sci., 1988, 42, 1713-1723.

268.4

COCAINE STUDIES ON DOPAMINE RELEASE IN NUCLEUS ACCUMBENS WITH IN VIVO ELECTROCHEMISTRY. II. SEROTONIN RELEASE STUDIES. F.T. Phelan* and P.A. Broderick. Dept. of Pharmacology, City Univ. of New York Med. Sch., and Depts. of Biol. & Psych., CCNY, New York, NY 10031. (Rm. J-910)

Preclinical studies indicate that cocaine euphoria may be mediated by mesolimbic dopaminergic pathways. Yet, euphoria is not blocked in cocaine abusers treated with neuroleptics (Gawin, F.H. Psychopharmacol. 90:142, 1986). To understand this phenomenon, in vivo electrochemical studies of dopamine release in nucleus accumbens were carried out before and after administration of cocaine to chloral hydrate anesthetized male, Sprague-Dawley rats (250-350g). Stearate indicator electrodes were stereotaxically implanted in nucleus accumbens; Ag/AgCl reference electrodes and stainless steel auxiliary electrodes were placed in contact with dura. Careful attention was paid to the animals' well being. Cocaine hydrochloride (20 mg/kg SC and IP) decreased accumbens dopamine release. The alterations in brain reward dopaminergic mesolimbic neuronal circuitry suggest that directionality of released dopamine may not be the critical step linking neurophysiology with euphoric behavior. Preliminary data show that serotonin release may be simultaneously decreased. This is important because terminal serotonergic neurons are destroyed by designer drugs of abuse. Sponsored by DHHS, PHS Grant #2-SO7-RR07132, PSC/CUNY Award #667234 and Upjohn Award #775755 to P.A. Broderick.

268.5

IN VIVO ELECTROCHEMICAL STUDIES OF MESOLIMBIC DOPAMINE RELEASE AFTER MORPHINE IN THE ANESTHETIZED AND FREELY MOVING RAT. P.A. Broderick. (SPON: L. Mitchell) Dept. of Pharmacology, City Univ. of New York Med. Sch., and Depts. of Biol. & Psych. CCNY, New York, NY 10031. J-910

The effect of morphine on dopamine release from nucleus accumbens was studied in chloral hydrate anesthetized and freely moving male, Sprague-Dawley rats (250-350g) in order to elucidate brain reward mechanisms in mesolimbic neuronal circuitry. In vivo electrochemical studies of nucleus accumbens dopamine release were carried out before and after the administration of morphine (5mg/kg SC and IP). Stearate indicator electrodes were stereotaxically implanted in nucleus accumbens; Ag/AgCl reference and stainless steel auxiliary electrodes were placed in contact with dura. Careful attention was paid to the animals' well being. A slow onset decrease in accumbens dopamine release was seen after morphine was administrated to the freely moving rat; no change occurred in the anesthetized rat. Homovanillic acid increased and serotonin virtually remained unchanged in the freely moving rat after morphine administration. The data contrasts opioid effects on brain reward circuitry in the anesthetized vis-á-vis the freely moving rat and provides insight into release mechanisms of addicting drugs. Sponsored by DHHS, PHS Grant #2-SO7-RR07132, PSC/CUNY Award #667234 and Upjohn Award #775755.

268.6

THE DOPAMINERGIC THRESHOLD OF COCAINE REWARD MEASURED BY IN VIVO MICRODIALYSIS. H.O. Pettit, L.C. Nicolaysen* and J. B. Justice Jr.; Department of Chemistry; Emory University; Atlanta, Georgia 30322

Extracellular dopamine (DA) concentrations in the nucleus accumbens were measured following intravenous administration of cocaine. Results from anesthetized animals indicate that the DA concentration increases in a dose dependent manner following a single infusion of cocaine. Data obtained from both anesthetized and behaving animals indicate that DA concentrations can increase and stabilize in a schedule dependent manner when cocaine is administered repeatedly in 15, 5 and 2.5 minute intervals. When animals self-administer cocaine or when cocaine is administered by an experimenter in regular intervals, DA concentrations stabilize at a level substantially above the basal level. The data and pharmacokinetic calculations indicate that the DA concentration increases for a short period following each infusion. It then falls to a "reward threshold" at which point the animal again self-administers the drug. Animals may regulate self-administration responding for cocaine in order to maintain DA levels above this threshold, yet titrate responding so that levels are maintained below a higher threshold. Results indicate a dopaminergic window of reward in the nucleus accumbens.

268.7

GENDER AND ESTROUS CYCLE AFFECT COCAINE SELF-ADMINISTRATION ON A PROGRESSIVE RATIO SCHEDULE. S.A.L. Bennett*, D.C.S. Roberts and G.J. Vickers*. Department of Psychology, Carleton University, Ottawa, Canada, K1S 5B6

Although it has been demonstrated that many of the behavioural responses to psychomotor stimulants are sex dependent and hormonally sensitive, no studies have examined the possibility that gender and the estrous cycle interact with drug reinforcement. The present experiment assessed the effect of these variables on two aspects of cocaine self-administration behaviour in the Wistar rat: the rate of cocaine intake on a fixed ratio one (FR1) schedule and the "breakpoint" on a progressive ratio (PR) schedule.

On a FR1 schedule, the lever-press response in rats permanently implanted with jugular cannulae resulted in a single intravenous injection of cocaine HCL (0.5mg/0.1ml/4s delivery). On a PR schedule the response requirements for the reception of a single injection of cocaine escalated logarithmically. Behavioural extinction was defined as the failure to earn an injection over an one hour period. The "breakpoint" was defined as the final ratio the rat completed prior to behavioural extinction.

On a PR schedule, the "breakpoints" of female rats were significantly elevated during estrus compared to other stages of the cycle. The effect of gender was evident in the finding that female rats displayed higher "breakpoints" than their male counterparts Measuring the rate of drug intake on a FR1 schedule failed to detect the influence of gender and the estrous cycle on cocaine self-administration. The results reveal that the PR method is capable of detecting the profound effect of gender and the estrous cycle on cocaine self-administration.

268.8

INCREASED MOTIVATION TO SELF-ADMINISTER INTRAVENOUS COCAINE FOLLOWING 5,7-DYHYDROXYTRYPTAMINE LESIONS OF THE MEDIAL FOREBRAIN BUNDLE IN THE RAT. E.A. Loh and D.C.S. Roberts, Dept. of Psych., Carleton Univ., Ottawa, Canada, K1S 5B6.

Both lesion and pharmacological evidence suggests that cocaine achieves its reinforcing action by inhibiting the reuptake of dopamine into terminals within the mesolimbic system. While cocaine also has an action at the serotonin reuptake site, it is not clear whether this property is directly involved in cocaine reward. The present study was designed to evalute whether depletions of forebrain serotonin would affect intravenous cocaine self-administration behavior in rats. Male rats were trained to self-administer cocaine on a progressive ratio schedule in which the first lever response of the daily session resulted in a drug infusion (0.5 mg/kg) while each subsequent injection required progressively more responses. The final ratio of responses per infusion at which self-administration behavior extinguished was defined as the "break-point". After baseline breakpoints were established, rats were injectioned stereotaxically with 5,7-DHT into the MFB (following desipramine pretreatment), then tested daily for 2-3 weeks. Control animals received vehicle injections. Animals in the 5,7-DHT group showed a marked increase in breakpoints. This suggests that the action of cocaine on serotonin terminals may be aversive, and that the depletion of forebrain serotonin results in a significant increase in the rewarding action of cocaine.

268.9

DELAYED INCREASE IN AMPHETAMINE-INDUCED RELEASE OF DOPAMINE FROM STRIATUM FOLLOWING REPEATED COCAINE ADMINISTRATION. N.R. Zahniser, J. Peris, P. Curella and L.P. Dwoskin, Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Persistent behavioral sensitization involving the nigrostriatal dopaminergic pathway can be observed following a single dose of cocaine and is augmented following repeated administration. Another persistent change we have observed following treatment of rats with a single injection of cocaine (10 mg/kg; i.p.) is increased amphetamine-stimulated release of [^{3}H]dopamine ([^{3}H]DA) from striatal slices (Peris and Zahniser, Pharmacol. Biochem. Behav. 27:533-535, 1987). This 30-50% increase lasts at least two weeks. To test whether changes in striatal amphetamine-induced [^{3}H]DA release parallel behavioral changes and are augmented following repeated administration of cocaine, groups of male rats were injected once daily for 8 days with either saline or cocaine (10 mg/kg, i.p.). Cocaine-induced sensitization was confirmed by comparing the stereotypic behavior on the first and eighth day of treatment. Release from striatal slices preloaded with [^{3}H]DA was stimulated with amphetamine (2, 6 or 20 μM; 2.5 min) or 300 electrical pulses (5 Hz) and was measured 1, 3 or 7 days after the last injection. Surprisingly, the dose-response curves for amphetamine-induced release from the rats withdrawn from cocaine for 1 or 3 days were identical to those from the saline-treated rats. However, following the 7 days of withdrawal, a significant 20-30% increase in [^{3}H]DA release was observed at each dose of amphetamine tested. These results were similar to those previously observed 1-14 days after a single injection of cocaine. Release evoked by electrical stimulation was similar for control and treatment groups (4.5-5% total tissue tritium). These results demonstrate that at relatively short times after the cessation of repeated cocaine administration, there are no observable changes in amphetamine-induced [^{3}H]DA release; we hypothesize that short-term changes in DA autoreceptors obscure the augmentation of release. A longer withdrawal period is needed in order to observe the augmentation. Like sensitization, the lasting increase in amphetamine-induced [^{3}H]DA release is associated with increased dopaminergic transmission. Supported by USPHS DA04216.

268.10

COCAINE: BEHAVIORAL SENSITIZATION AND THE SEROTONIN SYNDROME. K. A. Cunningham. Dept Pharmacology/Toxicology, Univ Texas Medical Branch, Galveston, Tx 77550

Repeated cocaine treatment results in a progressive enhancement of cocaine-induced behaviors and increases the sensitivity of 5-HT neurons to cocaine (Cunningham et al., S N Abst 13: 1651, 1987). The aim of the present study is to analyze whether the 5-HT syndrome induced by 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) is altered in cocaine-sensitized rats; the "5-HT syndrome" is thought to be a result of direct or indirect stimulation of 5-HT-1 receptors. To this end, adult male rats were treated (ip) with cocaine (15 mg/kg) or saline (1 ml/kg) 2X/day for 7 days. On the last injection, behavioral sensitization was expressed in cocaine- (6.8 $\pm$ .3) but not saline-treated rats (2.4 $\pm$ .3; $p<.01$; Kilbey-Ellinwood scale). Twenty-four hours after the last injection, the behavioral response to 5-MeODMT (1-4 mg/kg) was scored every 5 min for 30 min. In preliminary experiments, the dose of 5-MeODMT which produced the 5-HT syndrome in 50% of rats (ED50) was reduced in cocaine- (1.3 mg/kg) as compared to saline-treated rats (2.3 mg/kg), although the duration of the 5-HT syndrome appeared unchanged (5-12 rats/group). These studies suggest that cocaine treatment which results in behavioral sensitization is possibly associated with modifications of receptors which mediate the 5-HT syndrome. Studies are underway to replicate and extend these initial findings. Supported by DHHS RR07205.

268.11

COMPARISON AND CONTRAST OF ADDICTIVE AGENTS ON CNS STRUCTURES. I: COCAINE AND MORPHINE EFFECTS FOLLOWING MICROIONTOPHORESIS INTO CAUDATE, NUCLEUS ACCUMBENS AND MEDIAL PREFRONTAL CORTEX. J.-T. Qiao, R.C. Wiggins, P.M. Dougherty* and N. Dafny (SPON: A.H. Lockwood). Dept. of Neurobiology and Anatomy, The Univ. of Texas Medical School at Houston, 77225.

Cocaine and morphine have greatly different acute effects upon behavior. However, these agents both share the characteristic of rapidly inducing a state of dependence. The objective of this study was to investigate the effects of both these agents following microiontophoretic application upon the single neurons of brain regions implicated in the establishment of this phenomenon. Urethane-anesthetized male Sprague-Dawley rats were used. An array of 5 micropipettes were used for drug injections. A total of 50 neurons were studied from these brain areas. In the majority of the cells, cocaine depressed neuronal activity, while morphine elicited excitation from the same cells. Three receptor antagonists (sulpiride, naloxone and methysergide) demonstrated that cocaine effects were reversed only by sulpiride, and those of morphine were reversed only by naloxone. Thus, for these brain regions, these two agents appear to have distinct mechanisms of action.

268.12

COMPARISON AND CONTRAST OF ADDICTIVE AGENTS ON CNS STRUCTURES. II: COCAINE AND MORPHINE EFFECTS ON HABENULA AND THE PARAFASICULUS THALAMI. R.C. Wiggins, J.-T. Qiao, P.M. Dougherty* and N. Dafny Dept. Neurobiol. & Anat., The Univ. of Texas Medical School at Houston, 77225.

Cocaine has quickly become the most abused drug in the USA, replacing opiates such as morphine. As a part of our effort to determine if cocaine and opiates induce a state of dependence through similar means, we have undertaken an investigation of the effects of these agents in several brain regions following microiontophoretic application. Sprague-Dawley rats anesthetized with urethane and mounted in a stereotaxic frame were used. An array of 5 micropipettes were used for drug injection; 63 units were recorded. The agents were most often found to have opposing effects in habenula and parafasciculus regions, as cocaine most often induced a suppression, and morphine induced excitation of single unit activity. Moreover, the cocaine antagonist desipramine, and the opiate antagonist naloxone, did not show interactive effects upon these agents. These results further underscored the conclusions that cocaine and morphine induce states of dependence that are distinct in both behavioral as well as neuronal characteristics.

268.13

COCAINE WITHDRAWAL IN RATS: INCREASE IN INTRACRANIAL SELF-STIMULATION THRESHOLDS AFTER CHRONIC COCAINE SELF-ADMINISTRATION. A. Markou, C.B. Hubner and G.F. Koob. Dept. of Psychology, Univ. of California, San Diego, CA 92093 and Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, BCR 1, La Jolla, CA 92037

Cocaine use frequently occurs in episodic, prolonged binges. Immediately following a period of cocaine use, the person suffers from severe depressive symptoms mixed with irritability and anxiety (post-cocaine "crash") which constitute cocaine withdrawal. The present study is an attempt to develop an animal model of post-cocaine depression or withdrawal.

Male Wistar rats were implanted with self-stimulating electrodes in the lateral hypothalamus and intravenous self-administration catheters in the jugular vein. At several time points after prolonged periods (6, 12, 24 and 48 hours) of cocaine self-administration, the animals' self-stimulating current thresholds are assessed. Current self-stimulation thresholds are assessed with a discrete trial procedure which is a modification of Kornetsky and Esposito's procedure (Federation Proceedings, 38, 2473-2476, 1979). It is presumed that self-stimulation thresholds are a measure of an animal's "hedonic-anhedonic" state.

Control animals that have never self-administered cocaine tend to have stable baseline self-stimulating thresholds over time. Preliminary data indicate that prolonged periods of cocaine self-administration lead to an elevation of self-stimulating thresholds above pre-drug baseline levels. Hence, termination of chronic cocaine self-administration may result in an anhedonic state.

268.14

LESIONS OF THE DORSOMEDIAL NUCLEUS OF THE THALAMUS PRODUCE DECREASES IN COCAINE SELF-ADMINISTRATION IN THE RAT. C.B. Hubner and G.F. Koob. Scripps Clinic and Research Foundation, La Jolla, CA. 92037.

Previous studies from our laboratory have demonstrated that 1) presynaptic dopamine terminals within the region of the nucleus accumbens (N.acc.), and 2) the ventral pallidum (V.Pall.), a major recipient of N.acc. efferent fibers, are critical in mediating cocaine's reinforcing effects. Efferent projections from the V.Pall. to the dorsomedial nucleus of the thalamus (DMT) have been described and the purpose of this study was to assess the importance of this second-order efferent projection from the N.acc. in maintaining cocaine self-administration.

Rats trained to self-administer 0.75 mg/kg/inj cocaine on an FR 5 schedule in 3 hour daily sessions received either bilateral injections of vehicle (n=8) or ibotenic acid (n=6) into the DMT. When tested 5 days post-lesion, subjects in the lesion group showed a significant decrease in cocaine self-administration when compared to vehicle control animals. In contrast, similar lesions (n=5) of the hippocampus (HPC) did not produce a significant effect.

These results demonstrate that V.Pall. efferent fibers to the DMT play an influential role in cocaine self-administration. Further, the HPC does not appear to be a substrate for psychomotor stimulant reinforcement.

268.15

24 HOUR EFFECTS FOR A SINGLE LOW DOSE OF BROMOCRIPTINE. I. Extein,* M.S. Gold, D.R. Sweeney. Fair Oaks Hospital, Summit, NJ 07901.

We have recently reported in a double-blind, random assignment, crossover study superiority for bromocriptine over DMI in the acute treatment of cocaine withdrawal. To further evaluate the efficacy of the dopaminergic agonist bromocriptine in cocaine abstinence, we studied within 72 hrs. of hospital admission 12 young adults (3 women, 9 men) with DSM-III-R cocaine dependence and clinically significant cocaine withdrawal symptoms. Patients gave written informed consent for oral administration of a single low-dose of bromocriptine (1.25 mgs) utilizing a single-blind design. Craving, mood, and energy were self-rated before and 1,3,5,8,12 and 24 hrs. after bromocriptine on 100 mm lines. From pre-treatment baseline ratings bromocriptine's anti-withdrawal effects were noted at 1 hour. Peak medication effects were demonstrated at 3-6 hours. Bromocriptine reduced mean craving 48% ($p<.05$ paired t-test), improved depressed mood 38% ($p<.10$). At 24 hrs. bromocriptine effect was marked and significant mean ($p<.05$) with craving remaining at 50% of baseline. The higher baseline symptomatology the greater the reduced craving ($p<.01$) and improved depressed mood and energy ($p<.05$). A single low-dose of bromocriptine has acute effects beginning one hour after administration, peaking at 3-6 hrs. but continuing for at least 24 hrs. This response suggests DA supersensitivity and supports the DA hypothesis for cocaine withdrawal symptoms.

268.16

AN OPEN TRIAL OF MAZINDOL TO TREAT COCAINE CRAVING IN A METHADONE MAINTENANCE CLINIC. F.H. Gawin*, T.R. Kosten*, , B. Schumann*, D. Wright*, P. Berger*. (SPON: L. Marks). Dept. of Psychiatry, Yale Univ., New Haven, CT 06519

Mazindol is a widely prescribed, well tolerated anorectic drug that shares several preclinical properties with cocaine. It is a potent dopamine uptake inhibitor, induces similar stereotopy and most notably is self-administered in a variety of species but has not been reported to be abused in man. Recently it was reported that there may be a receptor on the dopamine transporter mediating the self-administration of cocaine-like drugs. Because mazindol is thought to work preclinically through the same receptor as cocaine but clinically lacks abuse potential we decided to evaluate its effect on cocaine craving in an open pilot of 10 DSM-III cocaine addicts in our methadone maintenance program. Data will be presented on daily craving scales, biweekly urine toxicology, and weekly cocaine inventories. Our preliminary impressions are that mazindol is exceedingly well-tolerated with little abuse potential of its own. It appears to have some anti-craving efficacy. In those patients who have abused cocaine while on mazindol no adverse interaction was noted although mazindol did not antagonize cocaine euphoria.

268.17

TREATMENT OF ACUTE COCAINE TOXICITY. M.S. Gold, J.R.L. Ehrenkranz,* J.M. Jonas,* C.A. Dackis.* Fair Oaks Hospital, Summit, NJ 07901; Franklin Diagnostics, Inc., Morristown, NJ 07960.

Emergency room visits linked to cocaine have increased by 500% since 1982-1983 and a corresponding rise in fatal reactions to cocaine has occurred. We developed an animal model, using male beagles, to investigate the pathophysiology and therapy of acute cocaine toxicity. Twenty-eight animals received 1 mg/kg/min (-)-cocaine HCl (provided by the National Institute on Drug Abuse) in normal saline by intravenous infusion until a generalized motor seizure occurred while continuously electrocardiographically and neurologically-monitored for 15 minutes prior to, during and for 1 hour after each infusion. All 28 animals exhibited deranged temperature regulation, characterized by a progressive rise in core body temperature into the hyperpyrexic range occurring after cocaine infusion was discontinued and associated with peripheral vasoconstriction and absence of panting. We studied the effects of lowering core body temperature on cocaine toxicity by immersing the trunk and extremities of each animal in 12°C. water for 15 minutes following cocaine administration. Ventricular tachycardia, ventricular fibrillation, and death seen in controls was not seen in any of the immersed animals. Based upon this animal model we conclude that life-threatening cardiac effects and acute, fatal reactions to cocaine result from hyperpyrexia. Cocaine toxicity may be safely and effectively treated by physical cooling.

268.18

EFFECT OF COCAINE ON IN VITRO NORADRENERGIC- AND PHORBOL ESTER-STIMULATED HYPOTHALAMIC GnRH RELEASE. TS King, RS Schenken*, and MA Javors*. Depts of CSB, OB-GYN, and Psychiatry, Univ Texas Hlth Sci Ctr, San Antonio, TX 78284.

Although a common drug of abuse, cocaine's effects on hypothalamic neurotransmitter activities, GnRH secretion, and reproductive function have not yet been studied. The purpose of this study was to examine the effects of cocaine on in vitro noradrenergic- and phorbol ester-stimulated GnRH release in Sprague-Dawley rats. Bilaterally ovariectomized rats were injected subcutaneously daily for 2 weeks with saline vehicle or with 10 mg/kg cocaine. Each of 2 days prior to sacrificing the rats, each rat was "primed" by s.c. injection of 50 ug/kg estradiol. Hypothalamic tissue slices were prepared and placed in 0.1 ml microchambers and perfused with modified Krebs-Ringer buffer (pH 7.4) using a programmable perfusion system. Our results are as follows: (1) Ten min pulses of 10^{-7} M progesterone increased norepinephrine (NE) and serotonin (5HT), but not dopamine (DA), release in the control group. In the cocaine-treated animals, basal release of NE and 5HT was elevated over controls and pulsed progesterone caused increased NE, but not 5HT or DA, release compared to controls. (2) Ten min pulses of 100 nM NE increased GnRH release in control and cocaine-treated animals, but the response was less in the cocaine group. (3) A 10 min pulse of phorbol-12-myristate-13-acetate (PMA) induced dramatic increases in GnRH release in the control group, but had no effect in the cocaine-treated animals. These results suggest that cocaine may down-regulate pulsatile hypothalamic NE release and, thereby, pulsatile GnRH release. Additionally, cocaine inhibits GnRH released through a PMA-stimulated protein kinase C mechanism, possibly via cocaine's adverse effects on plasma membrane Na^+ transport. (Supported by NIH grant number HD-10102 [Neuroendocrine core]).

268.19

COCAINE INDUCED REDUCTIONS IN D1 RECEPTOR DENSITY AND BEHAVIOR DO NOT OCCUR IN THE PRESENSE OF MAGNESIUM. K.M. Kantak and M.J. Potegal. Dept. Psychol.,Boston Univ.,Boston, MA 02215 and Dept.Dev.Psychobiol., New York State Psychiat. Inst., New York, NY 10032.

Magnesium (Mg^{2+}) has behavioral actions similar to acute cocaine. One previous study showed the reversal of the disruptive effect of chronic cocaine on aggression following acute $MgCl_2$. This suggests an interaction between Mg^{2+} and cocaine at the dopamine receptor. Presently, aggression in mice was measured after 15 days of saline, cocaine, $MgCl_2$, or cocaine + $MgCl_2$. Parallel drugs treatments in rats were administered and D1 receptor density was determined autoradiographically in the substantia nigra (SN) and striatum (STR) using ^{3}H-SCH 23390 in 0.16 to 4.5 nM conc. Cocaine significantly reduced aggression to 53% of baseline. This disruption was not observed with Mg^{2+}. D1 receptor density in the SN and STR following cocaine showed a significant 10% mean reduction from control.Cocaine also induced a 14% reduction in individually determined K_d's in the STR. Such reductions were not found with Mg^{2+}. These data suggest that the disruption in behavior might be related to alterations in D1 receptors which are not present following Mg^{2+}.

MONOAMINES AND BEHAVIOR III

269.1

DIFFERENT MECHANISMS UNDERLIE THE ACQUISITION AND EXPRESSION OF HEROIN-INDUCED PLACE PREFERENCE. M. LE MOAL, L. STINUS* and T.H. HAND. INSERM U.259 - Université de Bordeaux II, Rue Camille Saint-Saëns, 33077 Bordeaux Cedex, France.

Heroin is known to produce a preference for stimuli reliably paired with its administration or predictive of its onset of action. However, little is known about how this conditioned place preference (CPP) develops, and there has been no investigation of what underlies the post- conditioning expression of this CPP.

Using a novel 3-compartment place conditioning apparatus, we confirmed that heroin (50-1000 μg/kg) produces a strong and long-lasting (8 weeks) CPP. Pretreatment on all 6 days of conditioning with naloxone (50 μg/kg), clonidine (20 μg/kg) or pimozide (100 and 200 μg/kg) prevented the development of CPP induced by 250 μg/kg heroin. We then attempted to determine whether these same treatments could disrupt an already established CPP. Animals were subjected to the conditioning procedure with 250 μg/kg heroin, and were given naloxone (50 μg/kg and 1 mg/kg), clonidine (10-100 μg/kg) or pimozide (50-200 μg/kg) prior to the test session only. Only the highest doses of pimozide and clonidine disrupted the established CPP ; naloxone failed to do so, even at 1 mg/kg. Together, these results suggest that while opiate and catecholamine systems are involved in the acquisition of heroin CPP, the post-conditioning expression of CPP in drug-free animals is not opiate-mediated, only partially catecholamine-mediated, and attributable to largely unidentified mechanisms. These data do not support the position that the conditioned effects of opiates are related in the release of endogenous opioid compounds.

269.2

DOPAMINE AND PREPARATORY FEEDING BEHAVIOUR: EFFECTS OF METOCLOPRAMIDE AND THIORIDAZINE. J.R. Blackburn[1], A.G. Phillips[1], and H.C. Fibiger[2], Depts. of Psychology[1] and Neurological Sciences[2], University of British Columbia, Vancouver, BC, Canada, V6T 1Y7.

A conditional stimulus (CS+) that has been consistently presented prior to the delivery of meals will elicit conditioned preparatory responses from rats. In particular, rats will enter the niche where food is to be delivered. Previous experiments have determined that these responses are severely attenuated by the dopamine (DA) receptor antagonist pimozide. We now report the effects of two additional DA receptor blockers. Metoclopramide (MET) is a weak antipsychotic agent associated with extrapyramidal side effects (EPSEs). In contrast, thioridazine (THIO), an "atypical" neuroleptic, is a potent antipsychotic agent that does not produce EPSEs at clinically effective doses. MET decreased entries into the niche (≥5.0 mg/kg) and the total time spent in the niche (≥5.0 mg/kg) prior to food delivery. In contrast, once food was delivered the animals entered the niche and consumed the meal in a normal manner. In a separate study, food intake was decreased only by ≥7.5 mg/kg MET. Thus consummatory behaviour, unlike preparatory responses, was not disrupted by MET. THIO (10-30 mg/kg) produced a non-significant attenuation of preparatory responding. The more pronounced impact of MET may be related to its putative preferential affinity for nigrostriatal DA receptors.

269.3

CONDITIONED LOCOMOTION FOLLOWING MICRO-INJECTIONS OF AMPHETAMINE INTO THE NUCLEUS ACCUMBENS.
G.D. Carr & A.G. Phillips. Department of Psychology, University of British Columbia, Vancouver, B.C., Canada. V6T 1Y7

Increased locomotor activity is a prominent unconditional effect of systemic amphetamine injections. Repeated pairings of amphetamine with salient stimuli result in conditioned locomotion upon subsequent exposure to the stimuli alone. Since unconditional locomotion can be produced by micro-injecting the drug directly into the nucleus accumbens, the present study examined whether conditioned locomotion would also result from these injections.

Male hooded rats had either amphetamine (10 ug in 0.5 ul) or a saline vehicle injected into the nucleus accumbens and were then placed in an activity chamber. Relative to saline, the intra-accumbens amphetamine injections produced significantly greater locomotor activity over six pairing days. When the rats were subsequently placed into the chambers without prior microinjections, those that had previously received the amphetamine injections displayed significantly greater activity than did the saline-injected group. This demonstrates that stimuli that are paired with intra-accumbens amphetamine can elicit conditioned locomotion.

269.4

AMPHETAMINE INJECTED INTO THE MEDIAL PREFRONTAL CORTEX ATTENUATES THE LOCOMOTOR ACTIVATING EFFECTS OF AMPHETAMINE IN THE N. ACCUMBENS.
J.-P. Tassin*, P. Vezina, G. Blanc* and J. Glowinski* (SPON: T.M. Jay). Chaire de Neuropharmacologie, INSERM U. 114, Collège de France, 75231 Paris Cedex 05, France.

Support for an inhibitory role of frontal cortical dopamine on locomotor behavior has come from lesion studies showing that depletion of dopamine in this site correlates well with the appearance of hyperactivity (e.g., Tassin et al., Brain Res., 141:267, 1978). In the present study, the effect of amphetamine injection into the medial prefrontal cortex (PFC) on the hyperactivity produced by a similar injection into the n. accumbens (NAC) was assessed. Rats were implanted with chronic bilateral injection cannulae aimed at both the PFC and NAC and their locomotor activity measured on four separate occasions: after injections of amphetamine into the NAC (1.5 µg/0.5µl saline/side) and saline into the PFC, amphetamine into the PFC (2.5µg/0.5µl saline/side) and saline into the NAC, amphetamine into both sites and saline into both sites (control). Order of injection was counterbalanced across subjects. Amphetamine injected into the PFC alone had little or no effect while in the NAC alone it produced a mean 314% increase in locomotion compared to control injections. However, when amphetamine was injected into both sites, PFC amphetamine reduced the hyperactivity produced by NAC amphetamine alone injections by a mean 42.2%. Although an effect of PFC norepinephrine cannot yet be ruled out, these results, together with those of lesion studies, support the view that PFC dopamine plays an inhibitory role in locomotor behavior.

269.5

UNILATERAL 6-OHDA LESION OF THE NIGROSTRIATAL SYSTEM OF THE RAT: DIFFERENT FUNCTIONAL CONSEQUENCES FOLLOWING NEONATAL AND ADULT LESION.
N. Abrous*, M. Le Moal and J.P. Herman* (SPON: L. Tapia-Arancibia). INSERM U.259 - Université de Bordeaux II., 33077 Bordeaux, France.

The nigrostriatal dopaminergic pathway was destroyed by the stereotaxic injection of 6-OHDA into the lateral hypothalamus of neonatal (PD2; 8 µg; NL group) or adult (2x6 µg; AL group) male rats. Rotational behavior, postural assymetry, lateralization of arm choice in a Y-maze, sensory neglect and paw use in a food-reaching task were tested 3 months after the lesion.

Biochemical measurement made four months after the lesion indicated a 97 % drop of dopamine content in the ipsilateral striatum as compared to the contralateral control side, 94 %, 54 % and 80 % in the nucleus accumbens, medial frontal cortex and amygdaloid complex resp. These value were comparable to those measured following adult age lesion. NL rats presented a contralateral spontaneous rotation following mild stress, which was potentiated by haloperidol. Animals of both groups presented a ipsilateral rotation upon treatment with d-amphetamine (5 mg/kg) and a contralateral rotation following injection of apomorphine (0.1 mg/kg). However the latter was less pronounced in the NL group. Animals of the AL group displayed a marked contralateral deficit: lack of contralateral choice in the Y-maze, contralateral sensory deficit and inability to use contralateral paw to retrieve food from a distant tray. These deficits markedly attenuated (paw use, sensory neglect) or missing (Y-maze). These results suggest a functional replacement by some other neuronal system following the neonatal lesion of the dopaminergic neurons.

269.6

NUCLEUS ACCUMBENS CONTRIBUTES TO THE DIRECTION OF DOPAMINE-DEPENDENT CIRCLING.
L.M. Colle and R.A. Wise. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec H3G 1M8.

Asymmetric activation of dopamine systems results in circling behavior. Direction and speed of circling are traditionally associated with caudate and nucleus accumbens (NAS) activation, respectively. Two experiments indicate a role for NAS in direction. First, animals with electrolytic lesions restricted to the NAS circled toward the lesion side when given d-amphetamine (d-A; 1.25 mg/kg i.p.) one day to 12 weeks post-lesion. The rate of circling ranged from 1 to 3 circles a minute in a 60 minute test. Second, NAS microinjections of d-A (2.5, 5.0, and 10.0 ug in 0.5 ul) caused circling away from the injection; l-amphetamine was ineffective. Ventral caudate injections caused behavioral activation but ipsilateral and contralateral circling were equally increased. Taken together these data suggest that asymmetric activation of the NAS is sufficient to induce a directional bias in animals, a role traditionally attributed to the caudate.

269.7

EFFECTS OF AMPHETAMINE AND APOMORPHINE BEFORE AND AFTER RECOVERY FROM BEHAVIORAL ASYMMETRIES INDUCED BY UNILATERAL VIBRISSAE REMOVAL
H.Milani*, H.Steiner*, R.K.W.Schwarting and J.P.Huston Inst. Physiological Psychology, Univ. Düsseldorf, D-4000 Düsseldorf, F.R.G.

Unilateral vibrissae removal (UVR) induces changes in the nigrostriatal projections, which are time-related to the recovery of asymmetries in spontaneous open-field behavior. Results of previous experiments suggest a close relationship between vibrissae function and dopaminergic systems in the basal ganglia. The present experiments were undertaken to analyze the influence of the dopaminergic agonists apomorphine (APO) and amphetamine (AMPH) on behavioral asymmetries after UVR.

Behavioral testing was performed either 4 hours after one UVR or after 9 days of daily UVR. Rats tested after 4 hours of UVR showed more peritaxis (facial scanning of the walls of the open-field) with the intact vibrissae side under APO and AMPH. Also, turning occurred towards the intact side under APO. After 9 days of UVR, however, the behavioral asymmetries were reversed as more peritaxis with the shaved side was observed under APO and AMPH.

These results are discussed in context of other results suggestive of an analogy between effects of unilateral 6-OHDA lesions and unilateral vibrissae removal.

Supported by DFG-grant Hu 306/3-3.

269.8

A BEHAVIORAL INVESTIGATION OF GBR 12909: EFFECTS ON MOTOR ACTIVITY, FIXED INTERVAL RESPONDING, AND CONDITIONED REINFORCEMENT.
A.E. Kelley and C.G. Lang*, Dept. of Psychology, Harvard University, Cambridge, MA 02138

GBR 12909 (GBR) is a potent and selective dopamine uptake inhibitor. In the present studies, the behavioral effects of GBR were investigated in rats. We first studied the locomotor stimulating properties of GBR. GBR (1,10,20 mg/kg) elicited dose-dependent enhancement of motor activity, lasting approximately 4 hours. Chronic treatment with a high dose (20 mg) resulted in a long-lasting subsequent sensitization to subthreshold (1,3 mg/kg) doses of GBR. In a test of fixed-interval responding for food reinforcement GBR (1,10,20 mg/kg)produced a dose-depedent increase in lever-pressing and disrupted timing behavior. This effect was not clearly rate-dependent, although low rates were affected more than high rates. In a final experiment, hungry rats were conditioned to associate presentation of a food pellet with a compound stimulus (light and click). After 2 weeks of training, two levers were present in the box for the first time. Responding on one (CR lever) resulted in presentation of the compound stimulus, but no food. Responding on the other lever (NCR lever) produced no consequences. GBR (1, 10, 20 mg/kg) selectively and markedly enhanced responding on the CR lever. These experiments demonstrate that GBR is a potent psychostimulant drug. GBR may be a very useful compound for studying the behavioral effects of cocaine-like substances.

269.9

CHANGES IN AMPHETAMINE STIMULUS GENERALIZATION FUNCTIONS PRODUCED BY APOMORPHINE, HALOPERIDOL, COCAINE AND ELECTRICAL STIMULATION OF THE VTA. J.P. Druhan, H.C. Fibiger and A.G. Phillips. Dept. Psychol.; Div. Neurol. Sci., Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Rats were trained to discriminate 1.0 mg/kg amphetamine (ip) from saline, and then tested for generalization to a range of amphetamine doses (0.0, 0.25, 0.5 & 1.0 mg/kg) administered either alone, or in combination with apomorphine (0.05, 0.1, 0.15 and 0.2 mg/kg, sc), haloperidol (0.1 & 0.125 mg/kg, ip), cocaine (2.5 & 5.0 mg/kg, ip) or electrical stimulation (15 & 20 uA) of the ventral tegmental area (VTA). Low doses (0.05 & 0.1 mg/kg) of apomorphine attenuated the amphetamine cues resulting in rightward shifts in the generalization functions, whereas higher doses of apomorphine (0.15 & 0.2 mg/kg) substituted for the amphetamine cues. Haloperidol antagonized the amphetamine cues, thus shifting the generalization functions to the right. Finally, cocaine and VTA stimulation potentiated the amphetamine cues, causing the generalization functions to be shifted to the left. These shifts in the amphetamine generalization functions are consistent with the actions of the drugs and VTA stimulation on dopamine neurotransmission, therefore confirming a role for dopamine neurons in mediating the cue properties of amphetamine.

269.10

WITHDRAWN

269.11

ENHANCEMENT OF DA ACTIVITY IN ACCUMBENS BUT NOT FRONTAL CORTEX FACILITATES SELF-STIMULATION IN MFB. M. E. Olds. Biol. Div., 216-76, Caltech, Pasadena, CA 91125.

The effects of selective enhancement of dopamine (DA) transmission in n. accumbens (ACC) or frontal cortex (FC) on self-stimulation (SS) of the medial forebrain bundle (MFB) and gross motor activity were investigated. Bilateral injections of 2.0 µl/side of a solution containing DA + pargyline + amphetamine into ACC of self-stimulators induced first a facilitation of responding lasting 90-120 min, then a depression lasting several hours, followed by transient facilitation/depression phases and a return to baseline rates. The same injections given to non-self-stimulators led to increases in lever-pressing which did not, however, meet SS requirements. Injections of amphetamine or in combination with pargyline in ACC induced similar effects but of smaller magnitude and duration. Injections of pargyline or DA, or their combination, in ACC were not effective in inducing a facilitation of SS or an increase in gross motor activity. Bilateral injections of DA + pargyline + amphetamine in FC of self-stimulators either had no effects or attenuated responding for the MFB reward. In non-self-stimulators, such injections reduced the already low response rate. These findings lend support to the notion that the DA projection to ACC, but not FC, has the capacity to regulate reinforcement produced by MFB stimulation.

269.12

BEHAVIORAL EFFECTS OF DOPAMINERGIC AGONISTS AND ANTAGONISTS IN THE PREFRONTAL CORTEX. G. E. Jaskiw, G. Christison*, W. J. Freed and D. R. Weinberger. Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032.

Though evidence for increased subcortical DA transmission following chronic dopaminergic (DA) denervation of medial prefrontal cortex (MPFC) has been presented (Pycock, CJ et al, Nature 286:74, 1980) the modulation of subcortical DA systems by cortical DA projections remains poorly understood. To elucidate this behaviorally, bilateral indwelling cannulae were implanted in the MPFC of male rats. Rotational bias (complete turns in one direction/total turns) was determined by administering d-amphetamine (1.5 mg/kg i.p.) (AMP) prior to one hour of rotometer testing. In different sessions over 21 days rats received 0.5µl injections of vehicle (VEH), sulpiride 10µg (SUL) or apomorphine 10µg (APO) into MPFC, following which either spontaneous or AMP accentuated rotational activity was used to reassess rotational bias. SUL and APO were administered unilaterally and accompanied by contralateral injection of VEH.

Rats who received SUL on the side opposite to their preferred side of rotation 10 min before AMP increased their rotational bias towards the injection side (non-preferred side), from .13 to .59 ($p < .004$). In contrast, following unilateral APO rats showed a greater spontaneous bias contralateral to the injection. Bilateral injections of VEH did not affect rotational bias. Though rotation is a complex phenomenon most studies suggest that DA activity in the striatum is a major determinant of rotational bias. Our findings confirm those of others (Morency, MA et al, J Neurosci 7:812, 1987) and suggest that under some conditions changes in DA activity within the striatum may parallel those within the MPFC.

269.13

EFFECTS OF COCAINE ON MULTIVARIATE MEASURES OF SPONTANEOUS LOCOMOTOR ACTIVITY IN MICE. D.M. Fritsch*, J.H. Porter and M.G. Hadfield. Depts. of Biology, Psychology, and Pathology, Virginia Commonwealth Univ., Richmond, VA 23284.

Adult, male mice were administered cocaine (0, 2.5, or 5.0 mg/kg, i.p.) for 7 consecutive days 15 min prior to a 10 min test session. Spontaneous motor activity was measured in a Digiscan Activity Monitor (Omnitech Electronics) that measured horizontal activity (number of horizontal beam interruptions), horizontal distance (cm), number of horizontal movements, stereotypy activity, number of stereotypies, revolutions, and vertical activity.

Analysis of the data revealed a significant dose-dependent increase in horizontal activity and horizontal distance travelled; however, there was no change in the number of horizontal movements (movement episodes separated by at least one sec). Cocaine also produced a significant dose-dependent increase in stereotypy activity, but the number of stereotypy episodes was not changed. The number of revolutions (clockwise and counter-clockwise combined) was significantly increased. There were no significant changes in vertical activity. Both horizontal activity and stereotypy activity increased over the 7 days of drug administration, but the other activity measures remained stable. These findings demonstrate the usefulness of automated systems for measuring motor activity.

269.14

AMPHETAMINE DISRUPTS PREPULSE INHIBITION OF ACOUSTIC STARTLE RESPONSE IN RATS: REVERSAL BY NUCLEUS ACCUMBENS DENERVATION. R.S. Mansbach (1) N.R. Swerdlow (1) M.A. Geyer (1), G.F. Koob (2) and L. Pulvirenti (2) (1) Dept. Psychiatry, UCSD Sch. of Med., San Diego, CA 92093; (2) Preclin. Neurosci., RISC, La Jolla, CA 92037.

The amplitude of the acoustic startle response [ASR] is depressed when the strong acoustic startle pulse is preceded by a weak acoustic prepulse. This "prepulse inhibition" [PPI] appears to be modulated in rats by brain dopamine [DA] activity, since DA agonists disrupt PPI, an effect reversed by DA antagonists. PPI is also disrupted in schizophrenic patients, where this loss may reflect endogenous DA overactivity. We examined the anatomical substrates of the DA modulation of PPI.

Rats (n=16) received infusion of either vehicle or 6-hydroxydopamine (6OHDA) bilaterally into the nucleus accumbens [NAC]. 1 wk later, animals were treated with either saline (SAL) or amphetamine [AMPH; 2.0 mg/kg sc free base] and ASR amplitude was measured during acoustic pulses (118 dB) alone, and during pulses preceeded 100 msec earlier by a weak (80 dB) prepulse. 1 wk later, the procedure was repeated, with treatments (SAL and AMPH) reversed within each animal. All animals were then sacrificed and analysis revealed 85% depletion of NAC DA in 6OHDA-infused rats.

AMPH injections significantly potentiated ASR amplitude in both vehicle- and 6OHDA-infused rats. AMPH injections resulted in a near-total disruption of PPI in vehicle-infused rats; this effect of AMPH was completely reversed in NAC 6OHDA-infused rats. These findings suggest a pharmacological dissociation of the effects of AMPH on ASR amplitude and PPI. These results are also consistent with our previous reports implicating NAC DA activity in the modulation of PPI, and support the notion that abnormalities of forebrain DA activity may underlie the loss of PPI in schizophrenic patients.

269.15

APOMORPHINE FACILITATES PENILE ERECTION IN RHESUS MONKEYS. S. M. Pomerantz. Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Although much is known regarding the role of dopamine in regulating male sexual behavior of rodents, very little progress has been made in evaluating the potential influence of this neurotransmitter on male sexual behavior of primate species. Based on a number of studies indicating that dopamine agonists facilitate male sexual behavior of rodents, studies have been initiated to examine the effects of the mixed D1/D2 dopamine agonist, apomorphine (APO), on male sexual behavior of rhesus monkeys. Erectile potency and male sexual interest was assessed by observing the monkeys' achievement of penile erections and their performance of courtship behavior to an estrogen-treated female rhesus monkey that they could see, hear, and smell, but not physically contact. Using this paradigm, APO treatment (100 µg/kg, IM) resulted in a 25-fold increase in the frequency of full penile erections and a 5-fold increase in the frequency of incomplete penile erections, but did not alter the frequency that the glans penis was visible outside of the penile sheath. In contrast to its effect on penile erection, APO treatment did not facilitate the display of male courtship behaviors. Thus, the beneficial effects of APO on male sexual behavior of rhesus monkeys appear to be limited to the stimulation of penile erections. Furthermore, in the absence of a female, no such stimulatory effects of APO on penile erection were observed. These results indicate that psychosocial factors exert a regulatory influence over the effectiveness of APO in facilitating penile erection. Future studies will examine the effects of APO on rhesus male sexual behavior by evaluating the monkeys' behavior at other dosages of APO and in different tests of sexual behavior.

269.16

THE EFFECTS OF CENTRAL ADMINISTRATION OF THE DOPAMINE AGONIST APOMORPHINE ON GENITAL RESPONSES IN THE RAT. E.A. Pehek, J.T. Thompson* and E.M. Hull. Dept. of Psychology, SUNY at Buffalo, Amherst, NY 14260.

The aim of the present experiments was to investigate the roles of CNS DA receptors in the regulation of penile reflexes and seminal emission in the restrained, supine rat.

Injections of apomorphine (APO) into the medial preoptic area (MPOA) facilitated penile reflexes (both erections and penile flips) but did not affect seminal emission. Similar injections into the paraventricular nucleus facilitated seminal emission and erection but not flips. APO injections into the subarachnoid space of the lumbosacral cord inhibited penile reflexes ex copula, impaired copulatory performance, and decreased the number of intromissions preceding ejaculation.

These results suggest that the populations of CNS DA receptors regulating seminal emission and penile reflexes may have largely separate anatomical distributions. DA receptors in sites such as the MPOA and lumbosacral cord may integrate the reflexive and voluntary motor aspects of male sexual behavior.

269.17

HYPERSENSITIVITY OF THE 6-HYDROXYDOPAMINE TREATED HEMISPHERE TO AMPHETAMINE PERSISTS FOR ONE WEEK POSTOPERATIVE. R.J.Carey and M.R.Lynch, VA and SUNY Med.Ctrs, Syracuse, New York 13210

Amphetamine elicits contralateral rotation in unilateral 6-OHDA rats during the initial 24-36 hrs postoperative when dopamine levels in the striatum increase but ipsilateral rotation is assumed to occur thereafter when striatal dopamine levels decline. Recently, contralateral rotation was observed to occur at 5 days postoperative when striatal dopamine levels are substantially reduced. Presently, we found that vigorous contralateral rotation could be elicited up to 7 days postoperative and at dose levels (0.5 mg/kg) not usually found to elicit reliable rotation in rats with chronic lesions. On day 8 and thereafter, only ipsilateral rotation was observed. This drastic change suggests an important alteration in catecholamine function between days 7 and 8 postoperative. This observation of amphetamine-induced circling, away from the dopamine deficient hemisphere, raises basic questions regarding the validity of using the direction of amphetamine-induced rotation to determine functional hemispheric dominance.

269.18

DIAZEPAM DEACTIVATION OF HALOPERIDOL-INDUCED CATALEPSY IN THE RAT. Dennis P O'Brien, Sergio M Pellis, Vivien C Pellis, and Philip Teitelbaum. Dept. Veterinary Medicine, University of Missouri, Columbia, MO 65211 and Dept. Psychology, University of Florida, Gainesville, FL 32611.

Behavioral studies of GABAergic drugs in catalepsy supported the theory of an inhibitory feedback to A9 dopamine cells. These studies relied on measures of immobility and are inconsistent with physiologic and other behavioral studies. An analysis of the effects of diazepam on all the various manifestations of haloperidol-induced catalepsy was performed. Rats were injected with either 0.5 or 5.0 mg/kg haloperidol followed by 0(vehicle), 0.5, 1.0, or 5.0 mg/kg diazepam then tested 15-120 m later. At the threshold dose of haloperidol, diazepam enhanced measures of immobility (ipsilateral crossed legs, awkward posture, four cork and open field latency). However, measures of catalepsy involving active responses (clinging, grasping, bracing, righting, placing, vocalizations or cataleptic jumping) were never enhanced and in some cases diminished. At the higher dose of haloperidol, diazepam diminished these active responses. This data shows that diazepam deactivates the enhanced postural support characteristic of haloperidol-induced catalepsy. Thus the behavioral evidence does not support an enhancement of feedback inhibition of A9 cells by diazepam.

269.19

DIFFERENTIAL EFFECTS OF HALOPERIDOL AND CLOZAPINE ON AMPHETAMINE-INDUCED CHANGES IN BEHAVIOR AND NEOSTRIATAL SINGLE-UNIT ACTIVITY IN FREELY-MOVING RATS. J. L. Haracz, J. T. Tschanz,* M. T. Ciancone,* J. Greenberg,* and G. V. Rebec. Dept. of Psychology, Indiana Univ., Bloomington, IN 47405.

All clinically-effective neuroleptics reverse amphetamine (AMP)-induced behavioral effects, but little is known of the underlying neurophysiological mechanisms. These were studied in freely-moving rats subjected to simultaneous videotaping of behavior and recording of neostriatal single-unit activity. To date, 19 lateral neostriatal neurons exhibited baseline firing rates of 3.6±1.6 spikes/sec during quiet rest, 10 of which increased during motor activity, such as locomotion (n=7) or head movements (n=3). These and other cells frequently altered their activity in relation to the probing of specific body regions. D-AMP (1mg/kg) was administered 30 min before the injection of either haloperidol (HAL; 0.1-1.0mg/kg) or clozapine (CLZ; 5-10mg/kg). AMP-induced stereotyped behaviors often developed in correspondence with increases or decreases in neuronal activity. Motor-related cells showed AMP-induced excitations (>300% of baseline) significantly more frequently than non-motor cells ($p<0.05$). HAL generally inhibited behavior while suppressing the activity of all 10 cells tested, usually to a rate well below the pre-AMP baseline. Paradoxically, 3 cells were inhibited by both AMP and HAL. In contrast, CLZ induced both decreases and increases in activity. These findings differ from results obtained from the immobilized rat preparation, in which acutely administered neuroleptics either elevated or did not affect neostriatal neuronal activity (Skirboll & Bunney, Life Sci. 25:1419, 1979; Rebec et al., Neuropharm. 19:281, 1980; Hu & Wang, Soc. Neurosci. Ab. 12:1389, 1986). The differential effects of HAL and CLZ may reflect mechanisms underlying the differential propensity of these drugs to produce motor side effects. Supported by PHS Grant DA 02451.

270.1

HOMOLOGOUS DOWNREGULATION OF ANGIOTENSIN II RECEPTORS ON DIFFERENTIATED MURINE NEUROBLASTOMA N1E-115 CELLS. S.J. Fluharty* and L.P. Reagan*. (SPON: L. Amini-Sereshki) Dept. of Animal Biology and Institute of Neurological Sciences, Univ. of Pennsylvania, Phila. PA 19104.

Murine neuroblastoma N1E-115 cells possess angiotensin II (Ang II) receptors and their density increases during in vitro differentiation. We have used this cell line to examine homologous regulation of Ang II receptors. Incubation of these cells with Ang II produces a rapid (< 15 min) decrease in the density, but does not alter the affinity, of these receptors. This apparent down-regulation is dose related with an ED_{50} of 1 nM, and maximal decreases were observed with 100 nM Ang II (B_{max}: control = 343.3 ± 12.3; Ang II = 27.3 ± 2.8 fmols/mg prot). Receptor loss was unaffected by postincubations of membranes with GTP for 30-120 min at 37°C to insure ligand dissociation. This loss of receptors was reversible with partial recovery in 4-6 hr. Ang III also produced this downregulation but the high affinity antagonist, $Sarc^1$, Thr^8-Ang II was ineffective except at doses exceeding 100 nM. These results suggest that differentiated N1E-115 cells are an ideal cell line to examine factors regulating the expression of neuronal Ang II receptors. Supported by NS 23986 and the Univ. of PA Research Foundation.

270.2

IN VITRO DIFFERENTIATION SELECTIVELY ALTERS ANGIOTENSIN II AND VIP RECEPTOR-EFFECTOR INTERACTIONS IN N1E-115 CELLS. L.R. DePalo* and S.J. Fluharty*. Dept. of Animal Biol. and CVP Div., Univ. of PA., Phila. PA 19104. (SPON: J. Hendricks).

Murine neuroblastoma N1E-115 cells were induced to differentiate by low serum (0.5%) and 1.5% DMSO. In vitro differentiation maximally increased Ang II receptors at 72 hr (B_{max}: cont. = 13.4 ± 1.4; diff. = 310 ± 38 fmols/mg prot). This upregulation increased the sensitivity of Ang II-induced cGMP production 1000x, whereas Ang II inhibition of adenylate cyclase (AC) did not change. On the other hand, VIP receptors were unaltered during differentiation (B_{max}: cont. = 46.7 ± 3.8; diff. = 55.9 ± 7.3 fmols/mg prot). Further, VIP (1 uM) stimulated AC from basal values of 17.9 ± 1.2 to 79.3 ± 2.1 pmols/min/mg prot and this also was unaffected by differentiation (81.5 ± 3.4); although forskolin (100 uM) stimulation of AC was increased (cont. = 298 ± 32.3; diff. = 460 ± 22.5 pmols/min/mg prot). Therefore, differentiation of N1E-115 cells selectivity enhances receptor-effector interactions both by upregulating specific receptors, as well as altering their coupling to biochemical effectors. Supported by NS 23986 and Univ. of PA Research Foundation.

270.3

CHARACTERIZATION OF ANGIOTENSIN II BINDING SITES ON MURINE NEUROBLASTOMA N1E-115 CELLS. L.P. Reagan* and S.J. Fluharty*. (SPON: J.M. Sprague) Dept. of Animal Biology and Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

The murine neuroblastoma N1E-115 cell line contains binding sites for the angiotensin II (Ang II) receptor antagonist [^{125}I]-$Sarc^1$,Ile^8-Ang II (SARILE). Binding of [^{125}I]-SARILE to N1E-115 membranes was rapid, reversible and specific for Ang II related peptides. The rank order potency of [^{125}I]-SARILE binding was: $Sarc^1$-Ang II = $Sarc^1$, Ile^8-Ang II > Ang II > Ang III = $Sarc^1$,Thr^8-Ang II >> Ang I. Bradykinin or Substance P did not compete for [^{125}I]-SARILE labelled sites. Scatchard analysis of membranes prepared from confluent monolayers revealed a homogenous population of high affinity (K_D = 383 ± 60 pM) binding sites with a B_{max} of 25.4 ± 1.6 fmols/mg protein. Moreover, the density, but not the affinity, of the binding sites increased as the cells progressed from logarithmic to stationary growth in culture. Finally, agonist- but not antagonist-binding to N1E-115 cells was regulated by guanine nucleotides. Collectively, these results suggest that the murine neuroblastoma N1E-115 cell line may provide a useful model in which to investigate the signal transduction mechanisms utilized by neuronal Ang II receptors. Supported by NS 23986 and Univ. of Pennsylvania Research Foundation.

270.4

LOCALIZATION OF ANGIOTENSIN II RECEPTORS ALONG THE VISCERAL AFFERENT PROJECTION SYSTEM. R.R. Miselis, X. Bao, and S.J. Fluharty. Dept. of Animal Biology, Inst. Neurol. Sciences, Univ. of Penna, Phila, PA 19104.

Using in vitro quantitative autoradiographic procedures and [^{125}I]-(Sar^1, Ile^8)-angiotensin II (Sarile) as the ligand, angiotensin II (AngII) receptors were mapped in the normal rat brain. In preliminary studies using brain homogenates we determined that SARILE labels a homogenous population of high-affinity binding sites that are specific for Ang-II related peptides. Binding of ^{125}I-SARILE reached equillibrium in 2 hrs at 25°C and was stable for at least 2 additional hrs. Autoradiographic analysis revealed AngII receptors in the circumventricular organs and nucleus of the solitary tract (20 fm/mg protein) and high densities in the nucleus medianus, paraventricular nucleus and subthalamus area (6-20 fm/mg). In addition there were very significant levels of AngII receptors in the gustatory NTS (3-8 fm/mg) and the parabrachial complex. AngII receptors were also high in parts of the lateral parabrachial nucleus (10-20 fm/mg) and moderate in the medial components (3-8 fm/mg). Thus AngII receptors are located along the projection system of visceral afferents in the hindbrain and may be identified with functional systems mediating sodium and fluid homeostasis. Support: GM27739, NS23986, MacArthur Foundation, Univ. of PA Res. Fdn.

270.5

REGULATION OF BRAIN ANGIOTENSIN II AND ATRIAL NATRIURETIC PEPTIDE RECEPTORS IN SPONTANEOUSLY HYPERTENSIVE RATS AFTER TREATMENT WITH A CONVERTING ENZYME INHIBITOR. A.J. Nazarali*, J.S. Gutkind, F.M. Correa and J.M. Saavedra Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

We have investigated the effect of chronic administration (25mg/Kg p.o. for 14 days) of converting enzyme inhibitor enalapril on the binding density of angiotensin II (ANG II) and atrial natriuretic peptide (ANP) in selected brain nuclei of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats employing receptor autoradiography. In specific brain areas, ANG II receptors were higher in the SHR than in WKY rats. Chronic treatment of the SHR selectively decreased brain ANG II receptors by almost 50% in only the subfornical organ. However no difference in ANG II receptor density was observed in the treated WKY. In many brain nuclei, significantly lower ANP receptors were found in the SHR compared to the WKY. Treatment resulted in a significant increase in ANP receptor density (almost 50%) in only the area postrema of treated SHR animals. Conversely treated WKY animals showed a 23-25% decrease in ANP receptors in both the subfornical organ and area postrema when compared to untreated WKY. These results indicate that in genetic hypertension, converting enzyme inhibitors may mediate part of their antihypertensive effects by regulating ANG II and ANP receptors in the circumventricular organs.

270.6

EXPRESSION OF ANGIOTENSIN II RECEPTORS IN *XENOPUS* OOCYTES FROM TRANSCRIPTS OF A cDNA CLONE. E. Butler* and F. Sutton* (SPON: B. Schrier). LDN/NICHD and LBG/NHLBI, NIH, Bethesda, MD 20892

Xenopus oocytes that have been injected with RNA transcripts of pMcAR-1, a mouse neuroblastoma-derived cDNA clone, become responsive to angiotensin II (AII). Responses are manifested as current fluxes that are elicited by perfusion of 10^{-7} M AII over voltage-clamped oocytes that have been injected with 1) mouse neuroblastoma NS20Y RNA, 2) SP6 transcripts of pMcAR-1, or 3) NS20Y RNA that has been purified by hybrid selection with filter-bound pMcAR-1. Uninjected or water-injected oocytes do not exhibit significant responses to AII under our conditions. Hence we consider it highly probable that pMcAR-1 encodes an angiotensin II receptor. More definitive assertions of the identity of pMcAR-1 may be possible when investigations with appropriate AII antagonists have been completed. RNA blot-hybridization analyses show that the major McAR-1 hybridizing species in NS20Y and mouse brain RNAs are ca. 4.7 Kb in length; however, less prevalent species of other sizes are detected also.

270.7

MODULATION OF OXYTOCIN BINDING BY ESTRADIOL AND PROGESTERONE. M. Schumacher*, H. Coirini* A.E. Johnson and B.S. McEwen. Lab.Neuroendocrinology, The Rockefeller University, New York, N.Y. 10021

One mechanism by which estradiol (E) and progesterone (P) may activate female reproductive behavior is to increase the sensitivity of the ventromedial nucleus of the hypothalamus (VMN) to excitatory neurotransmitters. Thus oxytocin (OT) facilitates lordosis behavior and E-treatment increases OT-binding in the VMN. By using quantitative autoradiography, we showed that 3H-OT binding is most efficiently increased by estrogen in the ventrolateral part of the VMN, Treatment of ovariectomized females with 10 ug of estradiol-benzoate (EB) for 2 days resulted in a twofold increase in OT-binding in this part of the VMN. A recent study suggests that OT-induced facilitation of lordosis behavior is P-dependent. Experiments are now in progress to determine the effect of P alone or in combination with EB on OT-binding in the VMN. We found that in addition to their genomic effects, E and P also exert direct effects on OT-binding in the uterus. These effects are dependent on the hormonal status of the animal. When added to the incubation medium, 10-7 M of E and P decrease OT-binding in the uterus of EB+P treated animals but increase OT binding in females treated with EB alone. We are now investigating if E and P also exert such effects in the brain.

NIH Grant NS 07080 (BMC), EMBO fellowship (MS).

270.8

OXYTOCIN RECEPTORS IN RAT BRAIN: CHARACTERIZATION WITH THE SELECTIVE ANTAGONIST ^{125}I-d(CH$_2$)$_5$Tyr(Me)^{2}Thr^{4}Tyr(NH$_2$)^{9}OVT (OTA). J. Elands*, C. Barberis* and E.R. de Kloet* (SPON: H. Rigter). Rudolf Magnus Institute for Pharmacology, University of Utrecht, The Netherlands.

Central nervous system receptors for oxytocin (OT) and vasopressin (AVP) have been shown with tritiated OT and AVP. Because of the restricted localization, the limited binding capacity and the cross-specificity (for AVP and OT) of these receptors it has been difficult to assess their characteristics with the tritiated ligands. Iodination of OT or AVP was accompanied by a dramatic loss of affinity for their respective receptors. OTA, the Tyr^9 substituted OT analogue, however exhibited an enhanced OT receptor affinity (Kd=0.038 nM) after iodination. While having a 300-500 fold lower affinity for V_2 and V_1 receptors it appeared to be one of the most selective OT receptor ligands. Autoradiography revealed a great number of rat brain regions containing specific OTA binding sites. Due to the high selectivity of OTA it can now be concluded that these binding sites represent OT receptors. Identification of OT receptors in the brain was confirmed with membrane receptor assays. A heterogeneity in affinity of AVP/OT analogues for OT receptors in the hypothalamic ventromedial nucleus and the ventral hippocampus was demonstrated. Heterogeneity and regional distribution of the OT receptors as well as the availability of highly selective OT receptor ligands have provided the criteria to specify the role of the peptide in centrally regulated functions.

270.9

EFFECTS OF SOMATOSTATIN-28 ON SUPRAOPTIC MAGNOCELLULAR NEURONS IN THE RAT. W.N. Raby, C.W. Bourque, R.A. Benoit and L.P. Renaud. McGill Univ. Centre for Research in Neuroscience, Montreal, Canada H3G 1A4.

Somatostatin-14 (SS-14) and somatostatin-28 (SS-28) are the two main bioactive forms of somatostatin within the rat hypothalamus. Fibers displaying SS-28-like immunoreactivity have recently been visualized in and around the supraoptic nucleus (Sawchenko et al., J. Chem. Neuroanat. 1988). During intracellular recordings in hypothalamic explants, certain supraoptic cells displayed a prolonged hyperpolarization within 0.5-2 minutes exposure to 0.1-10 µM SS-28. In 6/10 phasic neurons, a gradual reduction in burst length and intraburst firing frequency lead to a complete cessation of spontaneous activity. In 3/6 continuously firing cells on-going activity also stopped. Full recovery from hyperpolarization required 2-5 minutes. Similar results were obtained in TTX (0.5 µM)-containing media. Therefore, it appears that SS-28 depresses the excitability of rat supraoptic neurosecretory neurons. Supported by FCAR and MRC.

270.10

SOLUBILIZATION AND PARTIAL PURIFICATION OF BRAIN SOMATOSTATIN RECEPTORS. H-T. He* and T. Reisine, Dept. of Pharmacology, Univ. Pennsylvania Sch. Medicine, Phila. PA 19104

Somatostatin (SRIF) is a neurotransmitter in the central nervous system. Its physiological actions are mediated by cell surface receptors. In order to investigate the physical properties of brain SRIF receptors, we have attempted to purify the receptor. Photocrosslinking studies employing a SRIF analog, [125I] CGP 23996, the crosslinking agent HSAB and UV light suggest that the size of the brain SRIF receptor is 60 kilodaltons (kd). Treatment of brain membranes with detergents solubilized the [125I] CGP 23996 labelreceptors. The solubilized and membrane bound SRIF receptors have the same size. To attempt to purify the SRIF receptor, brain membranes solubilized with CHAPS were applied to a sepharose column coupled to Trp8-SRIF. Following extensive washing, two major protein components could be eluted from the affinity column by lowering the pH of the column buffer with sodium acetate. The size of the proteins eluted were 60 kd and 45 kd. Treatment of the solubilized brain proteins with Trp8-SRIF prior to their application to the affinity column specifically reduced the amount of these two proteins that subsequently bound to the column. The size of the 60 kd protein eluted from the Trp8-SRIF affinity column is similar to the size of the brain SRIF receptor covalently labeled with [125I] CGP 23996. Proteolysis of brain SRIF receptors yields a major protein component of 45 kd suggesting that the smaller protein eluted from the affinity column is a degradation product of the SRIF receptor. Presently, attempts are being made to sequence these putative SRIF receptors. Supported by NIH grants DK37404 and GM34781.

270.11

COVALENT LABELLING OF THE SOMATOSTATIN RECEPTOR FROM RAT ANTERIOR PITUITARY. J.F. Bruno* and M. Berelowitz, Div. of Endocrinology, SUNY Stony Brook, NY 11794.

Somatostatin (SRIF) inhibits pituitary growth hormone secretion by binding to a high affinity G protein-linked membrane receptor. In the present study, the molecular characteristics of this receptor were investigated by covalently cross-linking ^{125}I-Tyr^{11}-SRIF to its putative receptor in rat anterior pituitary membranes using four cross-linking agents: ANB-NOS, HSAB, SANPAH and DSS.

When membranes were crosslinked with ANB-NOS, a broad band was labeled with an apparent molecular weight(M_r) of 68,0000; however, with HSAB, the broad band was resolved into a doublet with M_r's of 66,000 and 69,000. SANPAH covalently labeled the M_r 69,000 protein and a minor species with a M_r of 45-47,000. Crosslinking with DSS labelled only the M_r 66,000 band. Labelling of both bands was specific since affinity labelling with each of the 4 agents was abolished when 1uM cyclic SRIF was included in the binding reaction. Increasing concentrations of unlabelled SRIF resulted in dose-dependent inhibition of specific membrane binding and of affinity labelling of both the 66,000 and 69,000 proteins. Competition curves constructed from binding and cross-linking studies were superimposable. Experiments are now in progress to determine the molecular basis for the difference in M_rs.

270.12

DEVELOPMENTAL PATTERN OF FUNCTIONAL RECEPTORS FOR SOMATOSTATIN (SRIF) IN NEURONAL AND GLIAL CELL CULTURE DERIVED FROM FOETAL MOUSE HYPOTHALAMUS. J. Epelbaum, R. Rasolonjanahary*, M. Fodor*, C. Kordon, C. Loudes*, A. Tixier-Vidal*, A. Faivre*. U. 159 INSERM et Groupe de Neuroendocrinol. Mol. et Cell., Paris, France.

Embryonic cells were cultured in a defined medium. In neurons, SRIF levels increased steadily up to 21 days while they were undetectable, at all times, in glial cells. At 13 days in culture, SRIF cells, visualized with an antibody raised against SRIF28(1-12), amounted to 3.6 % of total cell number. SRIF binding sites, assessed by 125I-TyrO-DTrp8-SRIF binding in saturation experiments, were four time more concentrated on neurons than on glia (606 vs 156 fmol/mg protein) ; both preparations displayed similar affinities (neurons : Kd = 2.2 nM ; glia : Kd = 2.3 nM). Using SMS 201 995 as competitor, two sites were detected on neurons (K1 = 5 pM and K2 = 4.5 nM) while only the lower affinity site was present in glia (K = 1.7 nM). SRIF inhibited basal and forskolin or VIP stimulated adenylate cyclase activity up to 52 % in neurons and 23 % in glia. 24 h pretreatment with pertussis toxin (100 ng/ml) blocked completely these effects of SRIF.

The results demonstrate that SRIF receptors develop on hypothalamic neurons and glia, being more concentrated on the former. As soon as 6 days in vitro receptors are functional since they are coupled with inhibition of adenylate cyclase activity.

270.13

SELECTIVE REDUCTIONS IN CEREBROCORTICAL SOMATOSTATIN RECEPTOR BINDING DENSITY IN ALZHEIMER'S DISEASE. D.L. Knight*, G. Bissette, C.D. Kilts, G.P. Reynolds*, C.B. Nemeroff (Spon: J. McCubbin). Depts. of Psychiat. & Pharmacol., Duke Univ. Med. Center, Durham, NC 27710.

High affinity somatostatin (SRIF) receptor binding sites were measured in post mortem human brain tissue using [^{125}I]-Tyr$_{11}$-SRIF. Although there have been consistent reports of reductions in the concentration of SRIF-like immunoreactivity in the cerebral cortex in Alzheimer's disease (AD), there is some controversy surrounding changes in the number (Bmax) of SRIF receptors. Beal et al. (Science 229: 289-291, 1985) demonstrated significant reductions in the Bmax of SRIF receptors in frontal pole (BA 10), frontal cortex (BA 9), premotor cortex (BA 6), middle temporal gyrus (BA 21) and the hippocampus in AD. Whitford et al. (Reg. Pept. 15: 198, 1986) reported an increase in SRIF receptors in hippocampal tissue in AD, and Crow et al. (Brit. J. Pharmacol. 84(suppl): 8P, 1985) reported no changes in SRIF receptor Bmax in the frontal or temporal cortices in AD.

The present study confirmed and extended the findings of Beal et al. There was a 40% reduction in the Bmax of SRIF receptors in the frontal pole (BA 10) and the temporal pole (BA 38). Small decreases in SRIF receptor number in the hippocampus and caudate did not attain statistical significance. No changes were found in the amygdala or hypothalamus.

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270.14

DEGLYCOSYLATION OF RAT BRAIN AND PITUITARY CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS. D.E. Grigoriadis and E.B. De Souza. Neurosci. Branch, NIDA Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224.

CRF receptors in brain and pituitary have been previously shown to migrate as two distinct bands of different molecular weights (MW) (brain, 58 kDa; pituitary, 75 kDa). Since both proteins exhibit similar pharmacological characteristics, we determined whether the difference in the MW was due to differential glycosylation of the proteins in the two tissues. Using the homobifunctional crosslinking reagent dissuccinimidyl suberate, covalently labeled CRF receptors from brain and pituitary were solubilized in 6 mM CHAPS, adsorbed onto columns of wheat germ agglutinin (WGA) and specifically eluted using N-acetyl glucosamine. This indicated that both labeled proteins were glycoproteins containing complex sugar moieties. In order to partially define the glycoprotein nature of the CRF receptors in both brain and pituitary, covalently labeled receptors were treated with the enzyme neuraminidase (1 U/ml) which specifically cleaves terminal sialic acid residues. In the frontal cortex, neuraminidase treatment caused an increase in the mobility of the labeled protein thus decreasing the apparent MW of the CRF receptor from 58 to 52 kDa. Similar decreases were observed in the anterior pituitary. In summary, although CRF receptors in brain and pituitary have different MW, both appear to be glycoproteins containing terminal sialic acid residues.

270.15

CHARACTERIZATION OF CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS IN THE INTERMEDIATE LOBE OF THE PITUITARY. E.B. De Souza and D.E. Grigoriadis. (SPON: N.S. Buckholtz) Neurosci. Branch, NIDA/Addiction Res. Ctr., Baltimore, MD 21224.

CRF is the major physiological regulator of proopiomelanocortin (POMC)-derived peptide secretion in the anterior pituitary. In addition, CRF has also been shown to regulate the synthesis and secretion of POMC-derived peptides in the intermediate lobe. Autoradiographic studies have identified specific binding sites for CRF in the intermediate lobe of pituitary, however, their characteristics remain undefined. The binding of ^{125}I-oCRF to intermediate lobe homogenates was guanine nucleotide-sensitive, saturable and of high affinity (K_D = 400-600 pM; B_{max} = 100-120 fmoles/mg protein). The pharmacological rank order of potencies for various analogs or fragments of CRF was characteristic of the well-established CRF receptor in the anterior pituitary. Affinity cross- linking studies revealed that the molecular weight of the CRF binding protein in the intermediate lobe was identical to that of the anterior lobe (74,800 Da). The autoradiographic localization of CRF receptors in rat, porcine and bovine pituitaries will also be presented. In summary, CRF receptors in the intermediate lobe appear to have virtually identical binding characteristics to those in the anterior lobe. These data further substantiate the physiological role of CRF in regulating intermediate lobe hormone secretion.

270.16

EFFECTS OF CHRONIC ANTIDEPRESSANT AND BENZODIAZEPINE TREATMENT ON CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS IN RAT BRAIN. D. Pearsall*, D.E. Grigoriadis and E.B. De Souza. Neuroscience Branch., NIDA Addiction Research Center., Baltimore, MD 21224.

Recent clinical data suggest that CRF may play a major role in several neuropsychiatric disorders including major depression and panic/anxiety disorders. CRF administration in rats has been reported to produce several behavioral changes characteristic of anxiogenic compounds. In addition behavioral and biochemical data suggest that CRF and benzodiazepines may interact in brain. The present study was designed to examine the effects of chronic treatment (s.c. administration 21 or 28 days) with antidepressants (imipramine or desipramine) or benzodiazepines (diazepam, alprazolam or adinazolam) on modulation of CRF receptors in discrete areas of rat brain and in anterior pituitary. As previously reported, we found that chronic antidepressant treatment down regulated 5HT$_2$ serotonin and beta adrenergic receptors in cerebral cortex. While there was a trend toward increased CRF binding in cerebral cortex, brain stem and striatum following antidepressant treatment, these changes were not statistically significant. In addition, no significant changes were seen in CRF binding in other brain regions including cerebellum, olfactory bulb, hypothalamus, hippocampus and in anterior pituitary. Following chronic benzodiazepine treatment, CRF receptors were not significantly altered in cerebral cortex, cerebellum or brain stem. These data demonstrating minimal effects of chronic antidepressant and benzodiazepine treatment on CRF receptors in discrete regions of rat brain suggest that alternate mechanisms may be involved in the actions of these drugs on CRF neurotransmission in brain.

270.17

CORTICOTROPIN-RELEASING FACTOR (CRF) STIMULATES ADENYLATE CYCLASE ACTIVITY IN MOUSE SPLENIC MACROPHAGES. Elizabeth L. Webster*, George Battaglia, and Errol B. De Souza (SPON: M.S. Kafka). Neuroscience Branch, Addiction Research Center, National Institute On Drug Abuse, Baltimore, MD 21224.

We have previously identified a high affinity, magnesium-dependent, and guanine nucleotide-sensitive binding site for CRF in mouse splenic macrophages comparable to the well-characterized CRF receptors in pituitary and brain. The regulation of ^{125}I-ovine CRF binding by divalent cations and guanine nucleotides indicates that splenic CRF receptors are coupled to a guanine nucleotide regulatory protein. In the present study, we examined adenylate cyclase activity in splenic homogenates in order to determine the transduction mechanism(s) through which CRF produces its effects in mouse spleen. CRF receptor-mediated stimulation of adenylate cyclase activity was dependent on time, and tissue protein concentration. Guanine nucleotide facilitation of basal and CRF-stimulated adenylate cyclase activity was dose-dependent and nucleotide specific. The pharmacological potencies of various CRF analogs and fragments in stimulating adenylate cyclase correlated with their relative affinities for CRF receptors in spleen, brain, and pituitary. These results suggest that at least some of the actions of CRF on immune function may be due to CRF receptor mediated stimulation of cAMP production in mouse splenic macrophages.

270.18

FUNCTIONAL ALTERATIONS IN LOCAL CEREBRAL GLUCOSE UTILIZATION FOLLOWING CENTRAL ADMINISTRATION OF CORTICOTROPIN-RELEASING FACTOR (CRF) IN RATS. J. Sharkey*, N. M. Appel and E. B. De Souza (SPON: P.A. Schueler). Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD 21224.

CRF is the primary physiological regulator of basal and stress-induced proopiomelanocortin-derived peptide secretion from the pituitary gland. In addition, recent data suggest that CRF may act as a neurotransmitter in brain to coordinate the endocrine, behavioral and autonomic responses to stress. In order to further elucidate the neuroanatomical substrates underlying the actions of CRF in brain, we have examined the effects of intracerebroventricular administration of CRF (5.25 nmol in 10 µl of saline) on function-related glucose utilization (GU) in 50 anatomically discrete regions of rat brain using the ^{14}C-2-deoxyglucose quantitative autoradiographic technique. CRF differentially affected GU in discrete regions of rat brain. Consonant with the hypophysiotropic role for CRF, pronounced increases in GU were seen in the median eminence and lateral nucleus of the hypothalamus. In contrast, reductions in GU were observed in cingulate cortex and nucleus accumbens, brain regions which have been implicated in mediating stress responses. Other stress-related brain areas that showed increased GU included the locus coeruleus and median raphe nucleus. Punctate increases in GU were noted in the cerebellar cortex and vermis substantiating the functional role of CRF in the cerebellum. Additional brain areas showing elevated GU in response to CRF included the fornix, red nucleus and ependymal lining of the ventricles. The changes in GU in response to CRF administration provide additional evidence for a neurotransmitter role for CRF in brain and further support the importance of this neuropeptide in coordinating responses to stress.

270.19

EFFECTS OF CHRONIC STRESS ON BRAIN AND PITUITARY CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS. G.J Kant, S.M. Anderson and E.B. De Souza. Dept of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307 and Laboratory of Neuroscience, Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD 21224.

CRF is thought to be involved in the integration of neuroendocrine, autonomic and behavioral responses to stress. In the present study, CRF receptors were measured in membrane homogenates prepared from anterior pituitary, frontal cortex, motor cortex, somatosensory cortex, mesolimbic area, caudate, hypothalamus, midbrain and cerebellum of rats stressed for 3 or 14 days (around-the-clock intermittent footshock), which could be avoided or escaped on 90% of the trials presented).

Plasma corticosterone levels were significantly elevated in both the 3 day and 14 day stress groups as compared to controls. [^{125}I]oCRF binding was decreased in anterior pituitary and frontal cortex following 3 days of chronic stress. After 14 days of chronic stress, hypothalamic CRF binding was decreased as compared to control animals but no other differences were seen. We suggest that the decrease in CRF receptors in the anterior pituitary following 3 days of stress may be due to increased plasma corticosterone levels and/or increased CRF release. The down-regulation of frontal cortex and hypothalamic receptors is more likely to be primarily in response to sustained stress-induced CRF release at those sites.

270.20

ANTIBODIES TO A PEPTIDE COMPLEMENTARY TO EGG-LAYING HORMONE ARE SPECIFIC FOR A PROTEIN FROM THE OVOTESTIS. J.V.A. Choate, T.E. Kruger* and J.E. Blankenship. Marine Biomed. Inst., Univ. Tex. Med. Br., Galveston, TX 77550.

Egg laying in Aplysia californica has served as a model for studying the control of a behavior by a peptidergic neuroendocrine system. Several peptides, including egg-laying hormone (ELH) participate in the regulation of this behavior. ELH is thought to effect its targets by acting on a specific receptor. In an attempt to isolate and characterize the ELH receptor, we have generated antibodies by immunizing rabbits with a peptide encoded by the mRNA sequence complementary to the mRNA of residues 1-19 of ELH (see Proc. Natl. Acad. Sci. USA, 82:1372-1375, 1985). This complementary peptide antibody (anti-HLE) shows high specificity for HLE using an immunodot blot assay. The antibody was purified on a DEAE column, coupled to Reactagel 6X and used as an immunosorbant for Triton X-100-solubilized ovotestis tissue. Preliminary results suggest that this antibody specifically recognizes a high molecular weight, multisubunit ovotestis protein, as determined by polyacrylamide gel electrophoresis. The specificity of the antibody for this protein is supported by analysis of the column eluate by ELISA. Supported by NINCDS NS 23169(JEB), 07185(TEK), 11255, and NSF BBS8711368.

PEPTIDES: ANATOMICAL LOCALIZATION II

271.1

TRANSMITTER IMMUNOCYTOCHEMISTRY USING WHOLEMOUNTS OF THE DEVELOPING SPINAL CORD OF Xenopus. J.W. Gazzerro* and R.H. Nordlander (SPON: T. Whittingham). Dept. Oral Biol., Case Western Reserve Univ., Cleveland, OH 44106.

We examined the distribution of several neurotransmitters known to be present in sensory pathways of the spinal cord. Antibodies to substance P (SP) were applied to the dissected embryonic and larval CNS of Xenopus (stages 24-48). Antibodies to somatostatin (SS), vasoactive intestinal peptide (VIP) and calcitonin gene related peptide (CGRP) were applied to the CNS of mid-larval stages (47-48). Our eventual goal is to use these antibodies to examine the distribution and time of onset of immunoreactivity in order to follow the development of subsets of sensory neurons and their central processes.

Clear marking of some sensory and cranial nerve ganglion cells occurred with each of the four antibodies as did staining of fibers in the dorsolateral fiber tract occupied by ascending Rohon-Beard and sensory ganglion fibers. Only one antibody (CGRP) was restricted to this pathway in its CNS distribution. The rest marked many other longitudinal axons and showed stereotyped cellular distributions within the spinal cord and brain. Anti-SP stained axonal growth cones of a descending pathway that appeared early in development.

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271.2

NEURONAL LOCALIZATION OF CHOLECYSTOKININ mRNA IN RODENT BRAIN WITH IN SITU HYBRIDIZATION HISTOCHEMISTRY. S.M. Ingram*[1], R.G. Krause II*[2], F. Baldino, Jr.[3], L.C. Skeen*[1] and M.E. Lewis[3] (SPON: B. Wolfson[2]) [1]Dept. of Psychology and the Institute for Neuroscience, University of Delaware, Newark, DE. [2]Medical Products Dept., E.I. du Pont de Nemours & Co., Wilmington, DE., [3]Cephalon, Inc., West Chester, PA

The distribution of cholecystokinin (CCK) mRNA in the rat and mouse brain was determined using in situ hybridization histochemistry with a synthetic oligonucleotide probe 3' end labelled with [^{35}S]. Our results demonstrate a widespread distribution of CCK mRNA throughout the rodent brain. Individual cell bodies containing CCK mRNA were identified in the neocortex, claustrum, amygdala, the dentate gyrus and regions CA1-CA3 of the hippocampus, and several subnuclei of the thalamus and hypothalamus. Heaviest concentrations of CCK label were in the endopiriform/piriform cortex and the ventral tegmental area of the mesencephalon. These results expand the boundries of CCK maps previously made using immunocytochemistry and radioimmunoassay by demonstrating the existence of CCK-synthesizing cells in regions such as the tenia tecta and periaqueductal grey where the presence of CCK cell bodies was uncertain. The distribution of CCK mRNA is consistent with that of neuronal CCK-like immunoreactivity and is reflective of the diverse physiological actions of this peptide.

271.3

IN SITU HYBRIDIZATION HISTOCHEMISTRY USING ENZYME LABELED SYNTHETIC OLIGONUCLEOTIDE PROBES. R.G. Krause II*[1], F. Baldino, J.L. Ruth*[2] and L.G. Davis[1] [SPON: K. Wilcox]. [1]Medical Products Dept., E.I. Dupont de Nemours & Co.,Inc., Wilmington, DE 19898, and [2]Molecular Biosystems, Inc., 10030 Barnes Canyon Rd., San Diego, CA 92121.

Conventional in situ hybridization protocols use radiolabeled DNA and RNA probes to identify specific mRNAs within individual cells. This histochemical technique involves hybridizing fixed tissue sections with a radiolabeled probe whose sequence is complementary to the mRNA of interest. After washing, the sections are exposed to X-ray film to determine the effectiveness of the hybridization and to localize the identified mRNA on a regional scale. The sections are then dipped in photographic emulsion to ultimately obtain cellular resolution.

Synthetic oligonucleotide probes have proved very useful in these procedures. In order to improve cellular resolution, we have developed oligonucleotides coupled to an enzyme which replaces the radioisotope as the reporter system. The signal is obtained by providing the enzyme with an appropriate substrate. This allows the colorimetric visualization of the mRNA-related signal. We have successfully used alkaline phosphatase or horseradish peroxidase labeled probes to detect various endogenous mRNAs at the cellular level within twenty-four hours of hybridization rather than the extended autoradiographic exposure sometimes needed (e.g. tyrosine hydroxylase).

A number of advantages exist for these new probes which include the ability to identify mRNAs in a day versus the weeks or months of autoradiographic exposure needed for some mRNAs, an improved cellular resolution, and ease of use. Most importantly, the detection of multiple mRNA species in a single section using a combination of probes differentially labeled will more readily allow studies on co-localization and differential gene expression.

271.4

IMMUNOCYTOCHEMISTRY IN RAT FOREBRAIN USING A CRF ANTI-IDIOTYPIC ANTIBODY. D.T. Piekut and K.M. Knigge. Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642.

A polyclonal anti-idiotypic antibody was generated by immunization of a rabbit with IgG of a primary (idiotype) CRF antiserum. In the present study, the anti-idiotypic antibody is used to demonstrate and localize immunoreactive images in rat forebrain. Immunocytochemistry was performed on vibratome-cut sections of fixed rat brain. CRF anti-idiotypic antisera revealed punctate granules, 1.2-1.6μm in diameter, associated with cell bodies and fibers or present in dense fields in which there are no visible perikarya or processes. These immunoreactive images are seen in several regions of rat forebrain including cerebral cortex, caudate-putamen, nucleus accumbens and olfactory areas. These areas possess abundant CRF-immunostained fibers and terminal fields. Under the present conditions of tissue fixation and immunocytochemical procedures, no immunoreactive images are associated with perikarya of CRF-immunoreactive neurons. Preliminary characterization of the anti-idiotypic antisera has been conducted. Pre-incubation or co-incubation of the antisera with rat neural membranes or synthetic CRF reduced or eliminated staining of the tissue sections in a dose-dependent manner. Immunostaining was eliminated when 0 3mg neural membrane protein was incubated with 1ml diluted antisera (1:1000); neural membranes were completely inactive after heat treatment. High concentrations of synthetic CRF were needed in competition studies to eliminate staining. The distribution of immunostained images visualized with the anti-idiotypic antibody and the concurrent distribution of receptors for CRF in brain as revealed by biochemical assay and morphologically by autoradiographic methods suggests that the anti-idiotypic antibody may recognize a receptor or receptor-associated structure for CRF in rat forebrain. Supported by NIH grants NS18626 and NS00869.

271.5

IMMUNOCYTOCHEMISTRY OF RAT BRAINSTEM AND WESTERN BLOT ANALYSIS USING A PUTATIVE CRF ANTI-IDIOTYPIC ANTISERUM. G. J. Michael, S. A. Joseph, and K. M. Knigge. Neuroendocrine Unit, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

A polyclonal antiserum generated against an ammonium sulfate precipitated IgG fraction of CRF antisera was used in immunochemical studies in the rat brainstem. This antiserum specifically recognizes an antigen on the plasma membrane as determined by the ability of rat neural membranes to eliminate staining following preabsorption. This preabsorption effect is eliminated by heat treatment. By western blot, a membrane protein of relative molecular weight 110-120,000 is recognized by the antiserum. Following preincubation of the antiserum with the 110-120 kD nitrocellulose-bound protein, a reduction in histologic labling is noted. These results suggest the protein labeled on western blots is antigenically related to a protein labeled in fixed brain sections. Immunocytochemistry reveals an extensive distribution of labeled neuronal perikarya and apparent dendritic processes in defined anatomic regions. A high density of labeling is found within the superior and inferior colliculi, periaqueductal grey, dorsal raphe nucleus, ventral tegmental area, interpeduncular nucleus, laterodorsal tegmentum, pedunculopontine tegmental nucleus, nucleus of the solitary tract and the nucleus of the spinal tract of the trigeminal nerve. A comparison between this staining pattern and reports of CRF receptor localization supports the hypothesis that neurons postsynaptic to CRF are labeled with our antibody.

271.6

OPIOMELANOCORTIN PROJECTIONS TO RAT BRAINSTEM NUCLEI. L.J. Sim and S.A.Joseph, Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642.

Physiological and pharmacological evidence indicates that the opioid peptides function in pain modulation at both spinal and supraspinal levels. In the brain, the periaqueductal gray (PAG) and nucleus raphe magnus (nRM) produce analgesia upon electrical stimulation or morphine microinjection. We have demonstrated that opiomelanocortin neurons in the arcuate nucleus project to nocioceptive brainstem nuclei (PAG and nRM) by using combined horseradish peroxidase-wheat germ agglutinin (HRP-WGA) retrograde transport and peptide immunocytochemistry (ICC). In order to identify the brainstem neurons that may interact with efferent projections from hypothalamic opiomelanocortin neurons, *Phaseolus vulgaris*-leucoagglutinin (PHA-L) was iontophoretically injected into discrete regions of the arcuate complex in the rat. Animals were sacrificed 5-10 days after injection and the PHA-L was visualized using routine immunocytochemistry. Terminals were observed in various forebrain and brainstem nuclei, including the PAG and raphe complex. Double ICC staining of PHA-L transport and peptide/neurotransmitter localization was then utilized to determine the neurochemical content of the terminal neuronal field. These studies further characterize arcuate opiomelanocortin participation in the descending pain pathway.

271.7

THE PARAVENTRICULAR NUCLEUS IN THE HUMAN BRAIN: IMMUNOCYTOCHEMICAL ORGANIZATION OF CRF, OXYTOCIN, AVP AND PUTATIVE VASOPRESSIN RECEPTORS. S.A. Joseph and W.H. Pilcher. Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642

Antisera generated against corticotropin releasing factor (CRF), oxytocin (OXY), and arginine vasopressin (AVP) were used to immunocytochemically map the localization of neuronal elements containing these neuropeptides in the human paraventricular nucleus (PVN). The peptidergic pattern of organization was related to the magnocellular and parvocellular divisions of the PVN as determined by routine cresyl violet Nissl staining. Dual staining methods revealed that while colocalization exists among these neuropeptides no coexistence within perikarya could be demonstrated.

A polyclonal vasopressin anti-idiotype antibody was generated by immunization with an ammonium sulfate precipitated IgG fraction obtained from primary anti-vasopressin antiserum. Anti-idiotype antisera have been shown to recognize and interact with specific receptor proteins. Initial characterization of the AVP anti-idiotype antiserum showed that it specifically reacted (western blot) with the 62 and 55 kilodalton proteins of rat brain, which had been demonstrated to be associated with the AVP recognition site. In addition the antibody reacted with similar proteins in bovine posterior pituitary (Abood et al). In the human PVN, immunocytochemistry with the AVP anti-id revealed a distribution pattern similar to that seen with vasopressin antisera. Double stained (PAP/GAG) immmunocytochemical analysis using this anti-id antiserum plus antiserum generated against vasopressin revealed a coexistence of vasopressin and a putative vasopressin autoreceptor in vasopressinergic perikarya. Supported by grants NS #21323 and AHA #87 1011.

271.8

OXYTOCINERGIC SYSTEMS IN THE PREOPTIC AREA ARE SENSITIVE TO ESTROGEN, Caldwell, J.D., Brooks, P.J., Jirikowski, G.F. & Pedersen, C.A. Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, NC 27599

The effect of estrogen in sensitizing animals to the facilitative effects of oxytocin (OXT) on maternal and female sexual behaviors may be the result of its extensive influence on central OXT physiology. Implanting 10 mm silastic tubing filled with estradiol into ovariectomized (OVXed) females significantly ($p<.05$) increased immunoreactive levels of OXT in extracts from the preoptic area (blank implanted = 418 $\pm$ 90pg vs. estradiol = 798 $\pm$ 148pg; $t_{16}=2.19$). Estrogen implants of this size increased immunostain intensity of oxytocinergic cells in the dorsal preoptic lateral subcommissural nucleus (LSN), while 4 mm implants had no effect after 10 days. Analysis of immunostaining patterns 1 or 2 days after 20 µg estradiol benzoate (EB) injection found similar increased immunostain intensity in the LSN. Cells immunostained for OXT in the preoptic area also expressed OXT mRNA. An I^{125}-labelled 38-base oligodeoxyribonucleotide probe was hybridized to 30 µm thick sections for 16 hours at 37° C. Slides were washed to a final stringency of 0.1X SSC at 37° C, dried, dipped in emulsion and exposed for 36-60 hours. The number of hybridizing cells in the LSN, the periventricular nucleus and the lateral hypothalamus increased 1 and 2 days after an im injection of 20 µg EB versus oil in OVXed animals. Estrogen affects immunoreactive levels of OXT, oxytocinergic distribution and OXT-oligomer hybridization in the preoptic area.

271.9

LOCALIZATION OF CCK AND METHIONINE-ENKEPHALIN (m-ENK) IMMUNOREACTIVITY WITHIN CELL BODIES IN THE PREOPTIC AREA AND ANTERIOR HYPOTHALAMUS OF THE BRAZILIAN GRAY SHORT-TAILED OPOSSUM. C.A. Fox, D.E. Adam, G.E. Hoffman, R.E. Watson, Jr. and C.D.Jacobson. Dept. Veterinary Anatomy, Iowa State University, Ames, Iowa 50011; Dept. Physiology, University of Pittsburgh, Pittsburgh, PA. 15261; and Dept. Anatomy, University of Kentucky, Lexington, Ky. 40536.

Sex differences in the distribution of CCK and m-ENK have been observed in the rat. In this study, we have studied the anatomical localization of CCK and m-ENK immunoreactive cell bodies in the medial preoptic area (MPOA) and anterior hypothalamus (AH) of adult colchicine treated male and female Brazilian gray short-tailed opossums, (Monodelphis domestica) using the avidin-biotin, nickel enhanced immunocytochemical technique. CCK immunoreactive cell bodies are scattered along the third ventricle, and within the paraventricular (PVN) and suprachiamatic (SCN) nuclei. In addition, males have more CCK positive cell bodies than females rostral to and within the MPOA. Also there is a group of m-ENKergic cell bodies in the dorsal MPOA, PVN and SCN. These results indicate that the localization patterns of CCK and m-ENK like immunoreactivity are in part similar to those observed previously in the rat. In addition, to our knowledge, this is the first evidence of a sex difference within the brain of a marsupial and support the use of this animal in studies investigating sexual differentiation of the brain.

271.10

CCK AND SUBSTANCE P FIBER DENSITY WITHIN THE MEDIAL PREOPTIC NUCLEUS: SEX DIFFERENCES AND CASTRATION EFFECTS. C.W. Malsbury & D.M. Nance. Dept. Psychology and Div. of Basic Med. Sci., Memorial U. of Newfoundland, St. John's, NF, Canada, A1B 3X9, and Dept. Anatomy, Fac. Med., Dalhousie U., Halifax, Nova Scotia, Canada, B3H 4H7.

Computer-based video microscopy was used to measure optical density from alternate sections stained using rabbit antibodies to CCK-8 and substance P (SP) with the PAP technique. The average optical density within the medial preoptic nucleus was taken from representative sections (one for each peptide from each rat) located rostral to Gorski's sexually dimorphic nucleus. No rats were colchicine-treated, thus stained perikarya were not visible within the nucleus. Using 4 male-female pairs we found that both CCK and SP fiber staining was denser in males than in females, but this sex difference was significant only for CCK. The effects of castration were examined at 2, 4 and 8 wks post-surgery in three groups of males (N=4/group), either sham operated (SHAM), castrated (C), or castrated and given a 45 mm silastic capsule of testosterone at the time of surgery (C+T). At 8 wks CCK staining was significantly less in group C as compared to group SHAM (30% less) and group C+T (24% less). CCK staining was also less dense in the castrated groups than in the other groups at 2 and 4 wks, but these differences were not significant. SP fiber staining was reduced slightly by castration at 8 wks: C vs SHAM, 9% lower; C vs C+T, 14% lower. Testosterone capsules maintained the density of staining for both peptides at levels comparable to those in sham operated controls.

Supported by NSERC (to CWM) and MRC (to DMN) of Canada.

271.11

INNERVATION OF RAT HYPOTHALAMIC PARAVENTRICULAR (PVN) PRO TRH SYNTHESIZING NEURONS BY IMMUNOREACTIVE NEUROPEPTIDE Y. R.Toni*,I.M. Jackson,R.M.Lechan.Div. of Endo., New England Med Ctr.Boston,MA 02111 and R.I.Hosp.,Providence,R.I.02902.

To determine whether neuropeptide Y (NPY)-containing afferents to the PVN terminate on thyrotropin releasing hormone (TRH)synthesizing neurons, we performed double-immunolabeling studies at light and ultrastructural levels using antisera recognizing NPY and proTRH. SD rats were perfused through the aorta with 0.2% glutaraldehyde/4% paraformaldehyde for 20 min and the brains postfixed 2 hs at 4C. Some animals were studied after 3 weeks of hypothyroidism(0.02% methimazole) to increase perikaryal immunostaining of pro-TRH. Immunocytochemistry was performed on 40 um sections through the PVN by the ABC technique using antiserum to NPY (1:2000,provided by Dr.J.Polak) and the DAB chromogen was amplified by silver-gold intensification. In a sequential step, antiproTRH(#351 or #373,1:500) was incubated with the same tissue section and identified by the brown DAB chromogen.Sections were prepared for light microscopic evaluation or embedded in Epon resin for ultrastructural analysis.NPY-immunoreactive (IR) axons densely innervated the PVN and enveloped proTRH-IR neurons in all subdivisions of the PVN making both axo-somatic and axo-dendritic contacts. Synapses were also observed between NPY-IR axons and unlabeled neurons.These studies demonstrate that the NPY afferents to the PVN innervate proTRH synthesizing neurons and provide a morphologic basis to suggest NPY-mediated regulatory function over tuberoinfundibular TRH.PHS Fellowship 1F05TW03924

271.12

RADIOIMMUNOHISTOCHEMICAL LOCALIZATION OF ANGIOTENSIN II-LIKE IMMUNOREACTIVITY IN RAT BRAIN. D.P. Healy. Department of Pharmacology, Mount Sinai School of Medicine, New York, NY 10029.

Ang II-like immunoreactivity has been localized by immunohistochemistry to discrete populations of neurons throughout the brain. The immunoperoxidase and immunofluorescent staining procedures, however, are not quantitative, such that only qualitative assessments can be made on the levels of immunoreactivity in various brain regions under normal or altered physiological states. In this report semi-quantitative radioimmunohistochemistry has been utilized in order to measure relative levels of Ang II in whole brain sections. Slide-mounted sections of rat brain were incubated with rabbit antisera directed against Ang II followed by donkey anti-rabbit [125I]-Ig-Fab secondary antibody. The sections were exposed to autoradiographic film and grain densities converted to quantities of radioactivity bound per unit area by microdensitometry. The highest concentrations of Ang II were found within the median eminence and the central nucleus of the amygdala. High densities of Ang II were found within the bed nucleus stria terminalis, supraoptic nucleus, paraventricular nucleus, spinal trigeminal nucleus and nucleus of the solitary tract. These results suggest that radioimmunohistochemistry can be utilized to measure changes in brain Ang II content within discrete nuclear groups.

271.13

RENIN AND ANGIOTENSIN-RELATED CARBOXYPEPTIDASE (ARC) ACTIVITIES CO-LOCALIZED IN SYNAPTOSOMAL FRACTION FROM RAT BRAIN. R.S. Levy, D.G. Changaris* & N.W. Lesousky*. Depts. Biochemistry, Surgery, & Lab. Biol. Psychiatry, Univ. Louisville Sch. of Med., Louisville, Ky 40292.

Des-leu angiotensin I (AI-dl) is a biologically-active peptide with markedly greater activity within the brain as opposed to the vascular system. Sucrose gradient fractions of a crude synaptosomal preparation of whole rat brain showed the highest concentrations of both renin and ARC activities within a 1.4 M sucrose pellet. This consistent finding, despite many experimental manipulations, suggests that these enzymes may be localized to the same subcellular structure. Synaptosomes were isolated from whole rat brain according to the method of V. P. Whittaker et al. (Biochem. J., 90:293, 1964). The S_3 pellet was applied to a discontinuous (0.6, 0.8, 1.0, 1.2, 1.4 M) sucrose gradient. The layers and pellet were separated, frozen, sonicated, and assayed for renin and ARC activity. Each sample was incubated either with 0.3 mM tetradecapeptide or with angiotensin I, pH 5.5. The products were assayed by HPLC. The renin and ARC activities were concentrated 83- and 43-fold, respectively, within the 1.4 M lysed pellet (absolute values: 175 and 48 nmoles/mg/min, respectively). These data suggest that AI-dl can be generated from angiotensin I within neurons without the presence of converting enzyme activity.

271.14

A NEW GASTRIN 34(1-9)-LIKE PEPTIDE IN THE HYPOTHALAMO-HYPOPHYSIAL SYSTEM OF THE FROG. F. Vandesande and S. Marivoet* Laboratory for Neuroendocrinology, Zoological Institute, Naamsestraat 59, B-3000 Leuven, Belgium.

An immunocytochemical study using mice antisera specific for the NH2 terminal of h-gastrin 34(1-9) and an anti-CCK-8 (Vanderhaeghen), recognising the penta-gastrin terminal, was performed in the hypothalamo-hypophysial system (HHS) of the frog. With the anti-CCK, immunoreactive (ir) perikarya projecting to the external region of the median eminence (EME), were observed in the nucleus infundibularis ventralis. ir-fibres were absent in the posterior lobe (PL). A strong ir was present in the cells of the pars intermedia (PI) and in some unidentified cells of the pars distalis (PD). With our anti-gastrin antisera ir-perikarya were found in the nucleus preopticus (NPO). ir-fibres were present in the EME and a very strong ir-staining was observed in the PL and the PI-cells. Some unidentified cells in the PD were ir. As the ir-cells in the PI and the PD are recognised by the anti-CCK and our anti-gastrin 34(1-9) antisera, our results suggest that in the frog a gastrin 34 like peptide or two processed peptides related to gastrin 34 are present in the PI and the PD. The material in the NPO and the PL is only ir with anti-gastrin 34(1-9) and not with anti-CCK. Therefore we conclude that in the frog HHS an unknown peptide related to gastrin 34(1-9) is present.

271.15

SOMATOSTATIN AXONS DIRECTLY SYNAPSE ON GROWTH HORMONE RELEASING HORMONE SYNTHESIZING NEURONS IN THE HYPOTHALAMIC ARCUATE NUCLEUS OF THE RAT. Zs. Liposits*, I Merchenthalar*, B. Flerko* and W.K. Paull*. (SPON: J. Dexter). Depts. of Anatomy, Univ. Med. Sch. Pecs, Pecs, Hungary and Univ. of Missouri-Columbia, Columbia, MO, USA, 65212.

Physiological studies have suggested that hypophysiotropic somatostatin (SS) and GHRH systems are functionally interrelated (Lumpkin et. al., Science 211:1072, 1981: Wehrenberg et. al., Biochem. Biophys. Res. Comm. 109:562, 1982). This immunocytochemical (ICC) double labelling study (SS antiserum 669, gift from S. Reichlin; GHRH antiserum R567) demonstrated that within the arcuate n., fusiform and multipolar GRH-synthesizing neurons demonstrating indented nuclei and immunopositive rER and 80-100nm dia. neurosecretory granules received SS-IR positive afferent fibers on their dendrites and cell bodies. Ultrastructural ICC showed SS-IR axons frequently surrounded the GRH neurons and formed direct synapses. These data support the view that the central somatostatin system is directly interconnected with the hypothalamic GRH-IR system and has the capability of influencing the activity of GRH-IR cells. Supported by grants from the U.S. NIH (NS19266), NSF (INT8703030) and (INT8602688) and the Hungarian Academy of Sciences (OTKA 104).

271.16

ULTRASTRUCTURAL LOCALIZATION OF NEUROTENSIN IN THE VENTRAL TEGMENTAL AREA AND CENTRAL NUCLEUS OF THE AMYGDALA. *V. E. Bayer, V. M. Pickel, and A. C. Towle.* Dept. of Neurology, Divs. of Neurobiology and Molecular Cornell Univ. Med. Coll., New York, NY 10021.

We determined the ultrastructural localization of a rat polyclonal antiserum to neurotensin within the ventral tegmental area (VTA) and central nucleus of the amygdala (CNA) in the rat using the peroxidase-anti-peroxidase method. Immunoblot analysis showed that this antiserum recognizes neurotensin and known neurotensin-related peptides (LANT-6, neuromedin N, Gln-neurotensin, neurotensin 8-13). In the VTA, neurotensin-like immunoreactivity (NTLI) was detected almost exclusively in the dense-core vesicles (DCV's) occurring throughout the soma and processes of labeled neurons. In the CNA, different populations of neurons were characterized either by (1) NTLI localized primarily to the DCV's, or (2) NTLI present both in the DCV's and heterogeneously throughout the cytoplasm. Neurons with NTLI from these two regions also differed in relative density of somatic contacts (more in the CNA), and relative density of glial investments (greater in the VTA). Labeled terminals from both regions displayed a number of axo-axonic appositions. The differences between the NTLI neurons in these regions may reflect contrasting distributions of dopamine (or other neurotransmitters) in the perikarya and terminals of the VTA and CNA, respectively. (Supported by NIMH grants MH00078 and MH40342 to V.M.P.).

271.17

AUTORADIOGRAPHIC DISTRIBUTION OF SOMATOSTATIN RECEPTORS IN MOUSE BRAIN DURING NORMAL DEVELOPMENT AND AFTER THE NEONATAL LESION OF BASAL FOREBRAIN. G.Forloni, C.F. Höhmann and J.T. Coyle. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD, 21205.

Somatostatin (SOM) appears early in CNS development, and several studies suggest that it plays a role in the early organization of the brain. We previously showed that the immunocytochemical distribution of SOM in the cortex during postnatal development could be modified by neonatal lesion of the cholinergic basal forebrain (nBM). In the present study, we extended our investigations to the SOM receptors by the analysis of the autoradiographic distribution of [^{125}I]SOM binding sites during normal development and after nBM lesion. BALB/c mice received unilateral electrolytic lesion to nBM within 24 hours of birth. Animals were killed postnatal days (PD) 1, 3, 7, 14, 21, 30. Brain coronal sections were incubated with 0.5-1 nM of [^{125}I] SOM; the non-specific binding was determined in the presence of 100 μM SOM or SOM analog SMS-201-995. At PD 1, there was a high density of SOM receptors in the hippocampus, pyriform cortex and layers 5 and 6 of cerebral cortex. At PD 7, the amygdala, habenula and cingulate cortex also showed high levels of [^{125}I] SOM binding. During development and in adulthood, the concentrations of SOM receptors in thalamus appeared low. At PD 7, a decrease of [^{125}I]SOM binding sites was evident in the frontal cortex ipsilateral to the nBM lesion. The present study demonstrates the early appearance of SOM receptors during postnatal development and support a functional relationship between cortical SOM and nBM cholinergic innervation.

271.18

HIPPOCAMPAL SOMATOSTATIN HYBRIDIZATION HISTOCHEMISTRY. **R.S. Greenwood, S King*, R. Meeker, and J.N. Hayward. Dept of Neurol. and Neurobiology Prg., UNC Sch. of Med., Chapel Hill, NC 27599**

In situ **hybridization histochemistry and immunocytochemistry detect mRNA and proteins coded by mRNA, respectively and may offer alternative ways of mapping peptides. To compare these techniques in an anatomically well-defined region of the brain, the distribution of preprosomatostatin (PPSRIF) mRNA in the septal hippocampus was compared to that of somatostatin-like (SRIF-L) immunoreactivity.**

Coronal sections of the septal hippocampus from adult Sprague Dawley rats were processed by *in situ* hybridization using a ^{125}I-labeled 30-mer oligonucleotide directed against the 3' coding region of PPSRIF mRNA. Grain counts from the resulting autoradiograms were made in each of the subdivisions of the hippocampus and over individual cells. Sections from some animals were also processed for combined *in situ* hybridization and immunocytochemistry for localization of PPSRIF and SRIF-L immunoreactivity in the same section. *In situ* hybridization of adjacent sections with a PPSRIF mRNA nonsense probe were used as controls. The distribution of PPSRIF mRNA in the septal hippocampus corresponded well with SRIF-L immunoreactivity. In sections undergoing combined *in situ* hybridization and immunocytochemistry cells labeled by the oligonucleotide probe had SRIF-L immunoreactivity.

We conclude that the distribution of PPSRIF mRNA in the septal hippocampus is the same as SRIF-L immunoreactivity. These results provide validation that *in situ* hybridization of PPSRIF may be an alternative means for localizing and perhaps quantifying SRIF-L neurons in the hippocampus.

Supported in part by Javits Award NS-13411.

271.19

DIFFERENTIAL REGULATION OF STRIATAL NEUROPEPTIDE Y AND SOMATOSTATIN CONTAINING NEURONS IN THE RAT. R.J. Boegman, Y. Smith, A. Parent, J-C. Martel, and R. Quirion. Departments of Pharmacology and Toxicology, Queen's University; Laboratoire de Neurobiologie, University Laval, Douglas Hospital Research Centre; and Department of Pharmacology, McGill University.

Neuropeptide Y (NPY) has been localized to a subpopulation of medium-sized aspiny interneurons in the striatum also containing somatostatin (SS) and NADPH-diaphorase activity. Previously, Kerkerian et al. (Neurosci. Lett. 66: 106, 1986) reported an increase in striatal NPY immunoreactive neurons following a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigra. Our study sought to establish whether following a unilateral 6-OHDA nigral lesion the striatal NPY and SS immunoreactivity increased in parallel. In addition, binding of NPY and SS to striatal tissue was evaluated. Rats showing a behavioural response to dextroamphetamine were used. Six weeks after the lesion, animals were perfused and serial sections stained for NPY or SS immunoreactivity. While striatal NPY staining increased by 35% ipsilateral to the lesion, SS immunoreactivity remained unaltered. In striatal tissue ipsilateral to the lesion, there was a 27% decrease in NPY binding and a 76% increase in SS binding. Our data indicate that striatal NPY immunoreactive neurons are under tonic dopaminergic control.

(Supported by the Medical Research Council of Canada)

271.20

DISTRIBUTION OF SUBSTANCE P-CONTAINING FIBERS IN THE HUMAN PREFRONTAL CORTEX. K. Satoh, S. Iritani*, M. Fujii*, and S. Takahashi*. Dept. of Psychiatry, Shiga Univ. of Medical Sciences, Otsu 520-21, Japan.

As shown in previous neuroanatomical studies, the prefrontal cortex of mammalian brain possesses unique substance P (SP)-containing neuronal systems, and the existence of SP-containing fibers has been briefly reported in the human neocortex. In the present study, a detailed morphological description of SP-immunoreactive cell bodies and fibers in the human prefrontal cortex is given using normal post-mortem materials. The brains were sliced and fixed with Zamboni mixture immediately after being dissected out, and standard ABC-immunoperoxidase histochemistry was conducted using a monoclonal antibody against SP. Many SP-immunoreactive fibers were observed throughout the layers of prefrontal cortex. The laminar distribution of SP fibers was similar to those of the monkeys (Macaca fuscata), and fine immunoreactive puncta were seen throughout the superficial layers, I-III, particularly dense in the molecular layer.

271.21

CHEMOARCHITECTONIC PATTERNS OF PEPTIDES IN HUMAN BASAL FOREBRAIN: EVIDENCE FOR A SYSTEM COMPRISING THE BED NUCLEUS, SUBSTANTIA INNOMINATA, AND CENTRAL AMYGDALA. L.J. Martin*, V.E. Koliatsos, R.G. Struble, R.E. Powers*, and D.L. Price SPON: (M.V. Wagster). Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205-2182

Chemoarchitecture peptidergic systems in the human basal forebrain were studied to investigate relationships between the bed nucleus of the stria terminalis (BST), the substantia innominata (SI), and the central amygdala (CEA). Acrolein-fixed autopsy material (postmortem delay 5-11 hours) from six controls (27-66 years of age) was processed immunocytochemically for the presence of leucine-enkephalin (LENK), somatostatin (SOM), and neurotensin (NT). In the BST and CEA proper, three similar types of coextensive immunoreactivities were shown: LENK-, SOM-, and NT-positive, medium-sized, ovoid, and pyriform somata bearing 2-4 primary dendrites; LENK- and SOM-positive woolly fibers; and LENK-, SOM-, and NT-positive puncta. In the SI, a dense plexus of LENK/SOM woolly fibers and LENK, SOM, and NT puncta extended from the ventral BST to dorsal CEA; peptide-positive perikarya with the same morphology as those in BST/CEA were rarely observed. These results indicate that human BST and CEA exhibit similar chemoarchitectonic features and are interconnected through SI and that this continuum may represent a reciprocal en passant system with punctate terminations on unidentified target neurons.

271.22

DETECTION OF VASOPRESSIN mRNA IN CELLS OF THE MEDIAL AMYGDALOID NUCLEUS BY IN SITU HYBRIDIZATION. J. H. Urban, M. A. Miller* and D. M. Dorsa. Depts. Pharmacol. and Med., Univ of WA and GRECC, VAMC, Seattle, WA 98108.

Vasopressin (VP)-immunoreactive cell groups have been reported in the amygdala. The purpose of this study was to verify the presence of VP synthesizing neurons in the medial amygdala using in situ hybridization histochemistry.

Brain sections (15um) from 3 adult male Wistar rats were hybridized using an oligonucleotide probe that is complementary to the glycopeptide portion of the mRNA encoding vasopressin. The sections were dipped in NTB$_2$ emulsion, exposed for 10 days and developed. Semiserial sections of the medial amygdala were analyzed under dark-field microscopy and cells were quantified using an automated grain counting system.

Cells expressing VP mRNA were localized in the medial amygdala from the anterodorsal through the posterodorsal aspect of the nucleus. Twelve to 32 cells were found per section with the heaviest density of cells occurring at 2.12 to 3.40mm caudal to bregma (Paxinos and Watson, 1986). Labeling intensity of cells averaged 53.1 ± 3.3 grains/cell and was relatively constant throughout the nucleus. This data demonstrates the presence of AVP synthesizing cells in the medial amygdaloid nucleus and provides a method for quantifying their activity.

271.23

DISSOCIATION OF 5-HT FROM THYROTROPIN RELEASING HORMONE (TRH) IN RAT FOREBRAIN REGIONS. A. Sattin, M.J. Kubek, W.C. Low, C.J. Staley*, and J.R. Simon. Depts. of Psychiatry, Anatomy and Physiology, VA Med. Center and Indiana U. School of Med., Indianapolis, IN 46223.

TRH is known to be co-localized with 5-HT in a portion of the raphé neurons that project to the spinal cord of rat. Co-localization was explored in forebrain by use of chemical lesioning with 5,7 dihydroxytryptamine (DHT) and by surgical lesioning of fiber tracts projecting to the hippocampus (HC). TRH was assayed by specific RIA and 5-HT and 5-HIAA by HPLC. Three weeks after 100 µg DHT (free base injected in 10 µl over 1 min) i.c.v. (desipramine 25 mg/kg i.p. at -45 min) 5-HT and 5-HIAA were depleted in anterior and pyriform cortex and dorsal and ventral HC, but TRH was unchanged in all regions. Results of surgical lesions of fimbria-fornix (FF) alone and FF plus dorsal or ventral perforant path (PP) revealed, after 3 weeks, a major 5-HT input via FF and a smaller one via PP (undetectable 5-HT and reductions in 5-HIAA). TRH was also significantly reduced. Previous immunohistochemistry has localized TRH to the intrinsic CA and granule cells of HC and to other forebrain neurons, but extrinsic TRH is also possible in HC. Since 5-HT axons terminate on CA cells, granule cells and other forebrain neurons these results suggest that 5-HT and TRH are not co-localized in the forebrain, but might, instead, interact indirectly in cell-cell regulation. Supported by VA, MDA and AG5575.

271.24

INSULIN-LIKE IMMUNOREACTIVITY IN HUMAN BRAIN. **C.A.Bennett-Clarke*, G.C.Budd*, B.Pansky* and M. Velasco (SPON: A.McGrady). Depts. Anatomy, Physiology & Pathology, Medical College of Ohio, Toledo, OH 43699.**

Several reports indicate that insulin and its receptors are present in the mammalian CNS. In this study, adult human brain was examined for insulin localization using routine immunocytochemical techniques. Frontal cortex and hypothalamus fixed in formalin were sectioned with a vibratome or a sliding microtome, pretreated in 3% H_2O_2, rinsed in PBS and stained immunocytochemically. We used a monoclonal antibody (purified ascites fluid) to human insulin (Biogenex Labs.). Sections were sequentially incubated in primary antiserum (1:100 - 1:500), biotinylated anti-mouse IgG (1:100),streptavidin /HRP (1:100) and DAB. Rat and human pancreas sections were used as positive controls. Negative controls included (1) absence of the primary antisera or (2) immunoabsorbed ascites fluid. In both areas positive staining was limited to neuronal perikarya in two patterns. The first was a diffuse brown cytoplasmic stain, while the second pattern appeared as a group of punctate granules within the cytoplasm or maybe on the cell surface. Within the cortex, immunoreactive cells were usually pyramidal. Hypothalamic insulin-IR neurons occurred mostly in the paraventricular and lateral regions.
This project supported by the Ohio Dept.of Aging

271.25

LOCALIZATION OF SPINALLY PROJECTING M-ENK-LIKE IMMUNOREACTIVE LOCUS COERULEUS NEURONS IN THE CAT. V.K. Reddy*, S.J. Fung, H. Zhuo* AND C.D. Barnes, Dept. of VCAPP, Washington State University, Pullman, WA 99164

Studies in the past have revealed the presence of several peptidergic and cholinergic somata in relation to catecholaminergic cell bodies in the locus coeruleus (LC) complex of the cat. Most catecholaminergic cells are also said to contain enkephalin. The purpose of this study was to localize spinally projecting neurons containing either M-ENK-, SP-, TH- or ChAT-like immunoreactivity. In anesthetized cats, a 30% solution of HRP was injected either into cervical or lumbar enlargements followed by intraventricular injection of colchicine 48 hrs later. The animals were perfused after a 24 hr survival period and appropriate levels of the brainstem were sectioned (25 - 30 µm) on a freezing microtome. The sections were reacted for HRP using DAB following heavy metal intensification. The sections were then incubated in antisera raised to either M-ENK, SP, TH or ChAT and processed by peroxidase immunochemistry.

Spinally projecting M-ENK- and TH-like immunoreactive neurons were frequently evident in various nuclear groups of the LC complex. On the other hand none of the SP and ChAT positive neurons appeared to project to the spinal cord. Quantitative analyses are currently underway to ascertain the extent of the descending M-ENK- and TH-like immunoreactive neurons. (Supported by NIH grant NS24388).

271.26

Computer-aided mapping of specific neuronal populations in the human brain. Edward T. Koh*, Edward G. Stopa, Joan C. King, Wade T. Rogers, and James S. Schwaber. Dept. of Anesthesiology, UMass Medical Center, Worcester, MA 01605, Depts. of Neurosurgery, Pathology, and Anatomy & Cell Biology, Tufts Univ. Medical School, Boston, MA, and duPont Experimental Station, Wilmington, DE (SPON: A. Tischler)

It has recently become possible to stain human autopsy brain tissue with informative marker substances. Analyzing such tissue effectively has been very difficult using conventional manual charting methods because of scaling problems resulting from the sheer size of the brain. We have used a computer-aided microscopic mapping system to study the distributions of several peptide-containing neuronal populations in this tissue. To our knowledge, these are the first high-resolution panoramic maps of specific neuronal populations in the human brain.

Relatively large (2x2x1 cm) blocks encompassing the preoptic area, hypothalamus, and portions of the amygdala were sectioned and stained with antibodies to either mammalian LHRH, lamprey LHRH, neurotensin, or neuropeptide Y. With computer assistance, maps were generated which represent the positions of labeled cell bodies and axons, to a resolution approaching one micron in the X and Y dimensions. Computer-aided three-dimensional reconstructions help to clarify the organization of the projection systems. The use of IBM-compatible microcomputer-based systems to perform such mapping and reconstruction will be discussed.

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY III

272.1

SELECTIVE IRBP ACCUMULATION ON THE SURFACE OF CULTURED MOUSE PHOTORECEPTORS: EVIDENCE FOR THE EXISTENCE OF IRBP BINDING SITES? **L. Politi*, L. Lee*, B. Wiggert*, G. Chader* and R. Adler, (SPON: E. Adler-Graschinsky) Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD. and NEI, Bethesda, MD.**

The retinoid binding protein IRBP is synthesized by photoreceptor cells, and may function in vitamin A transport between them and the pigment epithelium. We are investigating mechanisms regulating IRBP synthesis, secretion and binding, using cultures of isolated mouse retinal neurons and photoreceptors in retinoid-free, chemically-defined medium. Western and slot blot analysis of the cultures demonstrated the presence of IRBP both in cell extracts and conditioned medium. IRBP immunoreactivity was exclusively associated with photoreceptors, and was not detectable in non-photoreceptor neurons. IRBP appeared to accumulate in photoreceptors in a developmentally regulated manner, both in detergent-permeated and in unpermeated cultures. In the latter, IRBP-like immunoreactivity was restricted to the surface of the photoreceptor inner segment. This surface labeling became undetectable within 6 hrs after switching the cultures to fresh, IRBP-free medium, suggesting a correlation between the presence of IRBP in the medium and its accumulation on the photoreceptor cell surface. Thus, our studies indicate that isolated mouse photoreceptors accumulate and secrete IRBP even when grown in the absence of retinoids, and suggest that they may also have cell surface binding sites for this molecule.

272.2

NICOTINIC ACETYLCHOLINE RECEPTORS AND CHOLINE ACETYLTRANSFERASE IN THE DEVELOPING CHICK RETINA. K.T. Keyser*, T.E. Hughes, P.J. Whiting*#, J.M. Lindstrom# and H.J. Karten, (SPON: N.Lugo-Garcia). Dept. of Neurosciences, UCSD, 92093 and Receptor Biology Laboratory#, Salk Institute for Biological Studies, La Jolla, CA 92138.

A population of amacrine cells in the vertebrate retina is thought to use acetylcholine as a neurotransmitter. Putative cholinergic neurons in the chick retina have been studied using antisera directed against choline acetyltransferase (ChAT). Similarly, putative *cholinoceptive* cells were recently identified with monoclonal antibodies directed against the nicotinic acetylcholine receptor (nAChR). We have used these markers in combination to visualize presumptive pre- and postsynaptic elements in the developing chick retina.

Embryonic day 6 (E6): In the central retina, near the vitreal surface, both ChAT-positive and nAChR immunoreactive cells were present. **E8:** Two rows of cells, separated by the nascent inner plexiform layer (IPL), exhibited ChAT immunoreactivity. nAChR positive somata were present in both rows interspersed among the ChAT positive cells. **E10:** ChAT immunoreactivity was visible in two laminae of the IPL adjacent to the rows of immunoreactive somata. Many of the nAChR-positive somata in the inner nuclear layer (INL) were tear-drop shaped and some gave rise to short processes. **E14:** A few large nAChR immunoreactive cells, possibly displaced ganglion cells, were visible in the INL. nAChR-positive processes were visible in two laminae of the IPL. **E18:** The distribution of both ChAT and nAChR-positive cells was comparable to that seen in the hatchling with immunoreactive cells present in both the INL and GCL and a bilaminar distribution of processes in the IPL. These results suggest that ChAT and nAChRs are present in discrete populations of cells in the embryonic eye from at least E6 onward. The populations are present well before the onset of synaptogenesis. Supported by EY06890 and NS24560 to H.J.K.

272.3

DEVELOPMENT OF THE INNER PLEXIFORM LAYER OF THE NORTH AMERICAN OPOSSUM. [1]T.M. Fischer,[2]M.A. Kirby, and [1]P.D. Wilson. [1]Dept. of Psych., Univ. of Cal. Riverside, Riverside CA 92521, [2]Depts. of Ped. and Anat., School of Med., Loma Linda Univ., Loma Linda, CA 92350.

We have examined synaptogenesis and formation of the IPL in opossum pups at light and electron microscopic levels.

The presumptive IPL is first apparent at postnatal day 12 (P12, gestation = 13 days) as a narrow zone separating the cytoblast layer from the developing ganglion cell layer. The width of the IPL remains relatively constant until P30, when it begins to exhibit rapid growth. At the EM level, opposing membrane densities lacking vesicles can be seen at P17. Contacts resembling conventional synapses can be detected at P26, near the time of peak axon counts in the developing optic nerve, and prior to the period of the highest rate of axon elimination. Contacts resembling bipolar cell ribbon synapses appear to be present at P51, 9 days prior to eye opening.

In general, the pattern of synaptogenesis observed in the opossum is similar to that seen in placental mammals, with a possible exception being the occurrence of conventional synapses prior to the period of peak axon elimination.

272.4

A GRADIENT OF CELL ADDITION TO THE OUTER NUCLEAR LAYER MAY INFLUENCE DIFFERENTIAL RETINAL EXPANSION. A.D. Springer, M. Berk*, J. Vogel*, and B. Wilson*. Dept. Of Anatomy, New York Medical College, Valhalla, NY 10595.

An intraocular injection of tritiated-thymidine labels cells born over a 1 week period. The number of labeled cells in the goldfish retinal ganglion cell (GCL) and outer nuclear (ONL) layers increases for 1 week post-injection. Excluding the germinal zone, about 20,000 cells are added across the entire GCL and 16,000 cells to the ONL over the 1 week period. By 16 weeks post-injection, only 400 labelled cells remain in the GCL, but 8,000 cells (presumably rods) remain in the ONL. Densitometric analysis of silver grains overlying cells in the GCL indicates that the disappearance of these cells is not related to multiple divisions diluting out the label. Instead, most of these cells may die within 2 weeks of being born. Reconstruction of the position of the labelled ONL cells indicates that there is a peripheral-central gradient of cell addition to the ONL. Excluding germinal zone cells, 43% of the cells added to the ONL are in the outer 8% of the retina. Comparable areas located more centrally contain a progressively smaller proportion of the new cells: 12% and 5%. The central 76% of the retina contains only 40% of the new ONL cells. More cells being added peripherally to the ONL may cause the periphery of the other retinal layers to expand more than their centers.

272.5

ANALYSIS OF THE OPTIC NERVE FIBER DIAMETER SPECTRUM IN THE WL-WABBLER-LETHAL MOUSE. E.W.CARROLL*, R.L.CURTIS, AND J.L.MELVIN* DEPT.BASIC SCI.MARQUETTE U. SCH. OF DENT. AND DEPTS. OF ANAT. & CELL BIOL., PHYSICAL MED. & REHAB., MED. COLL. OF WIS., MILWAUKEE, WI 53233.

The purpose of this investigation was to contrast optic nerve fiber diameter spectra in C57BL/6J wl-wabbler lethal mice (wl/wl) with controls (+/+). We have demonstrated that the pathology observed in wl/wl optic nerves fits the criteria of primary axonal (Wallerian) degeneration (Carroll & Curtis, Anat. Rec. 202:28A, 1982). The ALICE image processing system at the Argonne National Laboratory (Potts et. al., Invest. Ophthal., 11:980, 1972) provided total counts and cross sectional area of all myelinated optic nerve axons in 10 control and 7 wl/wl mice at 28 days post-partum.

No significant difference was found in either the total cross-sectional area of optic nerves in +/+ vs wl/wl ($0.05>p>0.025$) or in the total number of myelinated optic nerve fibers in +/+ ($\bar{X}$=37,357) vs wl/wl ($\bar{X}$=34,890) ($0.3>p>0.02$). Analysis of axonal diameter spectra indicates that while no significant differences exist for fibers less than 1μm in dia. (90% of total), wl/wl optic nerves exhibited fewer fibers greater than 1 μm in dia. ($p<0.01$ after Bon Feronni correction).

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272.6

MOUSE RETINAL GANGLION CELL MORPHOLOGY. S.D.Schlussman*, S.C.Sharma and A.Bernstein*(SPON. S.Fraley). Depts. Anatomy and Ophthalmology, N.Y.M.C., Valhalla N.Y.10595 and Medical Genetics, Univ. of Toronto.

The intricate pattern of retinal neuronal connectivity is refined through a process of developmentally regulated cell death. Previous studies have demonstrated that this naturally occuring cell death can be reduced experimentally, indicating that this process is at least partially dependent on competition between ganglion cell (g.c.) axons for terminal sites. Analysis of g.c.morphology in these experimentally manipulated "crowded" retinae reveals a general reduction in soma size and dendritic field area. It has been suggested that the size of g.c. dendritic fields is influenced by interactions with nieghboring cells. Thus there appears to be both intra and extra retinal factors determining cell size and density.

Recently a transgenic microphthalmic mutant mouse has been described, and it may provide a useful model for analysis of g.c. and dendritic field size. In order to ascertain whether the transgenic mouse g.c.have reduced dendritic arborizations it was neccesary to first characterize the normal mouse g.c. morphology. Following HRP injection into the superior colliculus, retinal g.c. were analyzed based on soma size and dendritic morphology. This report details the characterization of the normal and transgenic mouse retina. Supported by NEI 01426

272.7

DENDRITIC GROWTH OF IMMATURE GANGLION CELLS FOLLOWING THE DEATH OF MATURE ONES IN THE GOLDFISH'S RETINA. P. F. Hitchcock, Department of Ophthalmology, The University of Michigan, Ann Arbor, MI 48105

The retina of the goldfish grows throughout its life, in part, by the continual addition of new neurons at the margin. New ganglion cells, on average, grow a minor proportion of their dendritic arbor toward central retina (Hitchcock and Easter, J. Neurosci., 6:1037, 1986). Hitchcock and Easter (op. cit.) proposed that this lack of dendritic growth into more central regions resulted from the dendrites of older ganglion cells excluding the dendrites of the younger ones.

I tested this proposal by killing existing ganglion cells with retrogradely transported propidium iodide, allowing the retina to continue growing up to 8 months, and observing the dendritic morphology of the cells located near the margin. The dendritic arbors were stained by filling them intracellularly with lucifer yellow.

Two results were found: 1) In the experimental retinae, those ganglion cells residing near the margin grew their dendrites preferentially toward central retina. 2) The branching pattern of these cells' dendrites was often abnormal. These results suggest that interactions among the growing dendrites of adjacent ganglion cells (see Wassle et al., Nature, 292:344, 1981) control both their orientation and branching pattern.

Supported by grants BNS 86-07886 and EY07060.

272.8

RETINAL GANGLION CELL (RGC) DEATH AFTER AXOTOMY IS INFLUENCED BY THE DISTANCE BETWEEN THE LESION AND THE NEURONAL SOMATA M.P. Villegas-Perez*, M. Vidal-Sanz*, G.M. Bray and A.J. Aguayo (SPON: S.A. Keirstead). Neurosciences Unit, The Montreal General Hospital and McGill University, Montréal, Québec, Canada, H3G 1A4.

Axotomy close to the perikaryon, which is a requisite for the regrowth of CNS axons into peripheral nerve grafts, also causes the death of large numbers of neurons (Villegas-Pérez et al., J.Neurosci. 8:265, 1988). To quantitate the effect of distance of axotomy from the cell body on neuronal survival, we retrogradely labelled RGCs with diI applied to the superior colliculus and dLGN of adult Sprague-Dawley rats, transected the optic nerve (ON) at four points along its course in different groups of animals, and determined the densities of surviving (diI labelled) RGCs in 12 standard areas of each retina.

In control retinas, the mean density of diI-labelled RGCs was 2288 +66; one month after ON transection, these values were 18% of controls for ON lesions 0.5 mm from the eye; 31% for 3 mm, 55% for 8 mm, and 71% for 10 mm. These results indicate that small changes in the location of ON lesions can lead to substantial differences in RGC survival. Variations in a source of trophic influences available in ON stumps of different lengths may be one of several possible explanations for the observed relationship between RGC survival and the distance of the axotomy from the cell body.

272.9

A SINGLE CELL ANALYSIS OF THE EARLY STEPS OF RETINAL GANGLION CELL DIFFERENTIATION IN *XENOPUS*: FROM SOMA TO AXON TIP.

Christine E. Holt. Dept. of Biology, B-022, UCSD, La Jolla, CA 92093.

Intracellular dye injections of Lucifer yellow (10%) were made into retinal ganglion cells (RGCs) of embryonic *Xenopus* retina in part to determine whether the growth cones of these cells make dye-coupling junctions with other cells along the primoridial optic pathway. Wholemount preparations were treated with α-LY Ab after injection, reacted for HRP with DAB and sectioned at 40 μm. No evidence of dye-passing contacts could be found at any stage of the pioneering growth cone's route suggesting that junctional mediated intercellular signalling does not play a major role in pathfinding.

Quantitative analysis of the dimensions and complexity of growth cones at different points in the pathway revealed that those at the optic nerve head (onh), where growth cones turn to leave the retinal surface, are significantly larger than elsewhere and possess more filopodia than growth cones in the rest of the ipsilateral stretch of the pathway (retinal surface, optic nerve, brain entry point). Growth cones in the contralateral stretch of the pathway (chiasm and optic tract) show a similar degree of complexity as those in the onh but are smaller.

To determine whether dendrite elaboration is coordinated with target arrival, dendrite genesis was examined in single RGCs whose axon tips were at varying positions along the pathway. 20% of RGCs whose axon tips were located within the retina have begun to elaborate dendrites. This figure increases linearly with respect to axon length such that ~90% possess dendrites by the time their growth cones reach the dorsal optic tract. This study also revealed the surprising finding that ~5% of RGCs send more than one axon into the optic pathway.

Support: NIH (NS23780) and a McKnight Award.

272.10

EM ANALYSIS OF SINGLE RETINAL GANGLION CELL TERMINALS IN DEVELOPING *RANA PIPIENS*

Lai-Hsing Yen*and M. Constantine-Paton. Dept. of Biology, Yale University, New Haven, CT 06511

Retinal ganglion cell (RGC) terminals in developing frog tecta are dynamic structures that continually make new synapses at distal sites and retract established branches in proximal parts of the arbor. We are asking whether these dynamic processes are reflected in either the morphology or distribution of synapses within single arbors.

Forty micron vibratome sections of aldehyde fixed tecta containing a few HRP filled RGC terminals are reacted with DAB, post-fixed with osmium ferricyanide, dehydrated and embedded in Spurr's medium. The entire morphology of well isolated individual terminals is reconstructed at the light microscope level. Semi-thick and intervening ultra-thin sections through these terminals are used to localize HRP labeled profiles at the EM level. Vesicle density, synaptic distribution, and organelles of these profiles are recorded.

We find that most labeled RGC synapses are made on small clear profiles, many of which in turn synapse onto other clear profiles. Less frequently, the clear profile post-synaptic to a RGC terminal receives an additional synapse from an unlabeled process. Post-synaptic profiles rarely contain glycogen but frequently show irregularly shaped vacuoles. The preceeding properties are common to proximal and distal regions of the terminals. The synaptic density does not vary greatly between these two regions. However, the density of synaptic vesicles is clearly higher in distal regions, suggesting that vesicle accumulation precedes morphologically discernable synapses. (Supported by NIH grant EY06039)

272.11

SYNAPTIC OVERLAP FOLLOWED BY SEGREGATION OF DIFFERENTIALLY LABELED OPTIC TERMINALS IN THE GOLDFISH OPTIC TECTUM.

R.L. Meyer & G.H. Kageyama. Dev Bio Center, UC Irvine, CA 92717.

When optic fibers from both eyes are made to grow simultaneously into one tectum in goldfish, regenerating fibers from the two eyes initially overlap, then segregate into discrete columns. In order to determine if the early overlapping fibers formed transient synapses we developed a method by which we could differentially label terminals from the two eyes so that they could be distinguished from each other at the E.M. level. The optic nerve of one eye was crushed and the fish returned to its tank for 2 days, then a tube filled with HRP was applied to the cut end of the left optic nerve and allowed to survive for another 2 days, during which time HRP was allowed to fill the optic axons of the opposite eye. In this way one set of optic terminals was labeled by electron dense degeneration while the other set was labeled with HRP. During the early phase of regeneration (1 mo post deflection + optic nerve crush) HRP labeled fibers were readily observed at the light microscope level. Axons from either eye formed a diffuse overlapping projection in anterior to middle and eventually posterior tectum. At the E.M. level the differentially labeled optic fibers are intermingled and form overlapping synapses. After column formation the optic terminals undergoing electron dense degeneration were clearly segregated from those labeled with HRP. This is a clear example of synaptic plasticity in adult CNS. Support:NIH NS-16319.

272.12

REGENERATING OPTIC AXONS FORM TRANSIENT TOPOGRAPHICALLY INAPPROPRIATE SYNAPSES IN GOLDFISH TECTUM:A WGA-HRP EM STUDY.

GH Kageyama, RL Meyer. Dev Bio Center, UC Irvine, CA 92717.

Crushed optic axons in the mature goldfish will gradually reinnervate the contralateral tectum and restore correct retinotopography. Regenerating axons often follow abnormal routes to reach their topographically correct position. Along the way, axons may branch and form early terminal-like arborizations. Since normal numbers of optic synapses are observed as early as 1 mo postcrush when optic axons are not yet refined, it is likely that many early synapses are both transient & topographically inappropriate. Small 2nl injections of WGA-HRP were made in ventronasal retina to label a corresponding set of axon terminals that normally terminate within a topographically discrete & predictable 200 um diam spot in the contralateral medial posterior tectum. These spots were revealed with a modified TMB method. At the EM level the TMB reaction product was readily identified almost exclusively in optic terminals located within the spots. Similar injections in ventronasal retinae of early 3 wk regenerates labeled only transient ectopic synapse-forming terminals in anterior tectum. Most of these early regenerating fibers were small & formed immature en passant type synapses. No label was found in the topographically appropriate (medioposterior) area of tectum. By 5wk postcrush, large tectal areas were labeled including both appropriate and inappropriate regions. After 2 mo, label was again confined to small spots. Support:NIH NS-16319.

272.13

THE EFFECTS OF GLUTAMATE RECEPTOR AGONISTS AND ANTAGONISTS ON THE EVOKED TECTAL POTENTIAL IN *RANA PIPIENS*.

E.A. Debski and M. Constantine-Paton. Dept. of Biology, Yale University, New Haven, CT 06511.

The NMDA receptor, a subclass of glutamate receptors, appears to play a role in the establishment of retinal-tectal topography in tadpoles (Cline et al., PNAS, 84: 4342, 1987). We have used our cannulated tadpole preparation (Debski et al., Neurosci. Abstr. 13: 1691, 1987) to examine the effects of NMDA and the antagonist APV on the tectal potential evoked by bipolar stimulation of the optic nerve. Unexpectedly, we found that post-synaptic activity is blocked reversibly by NMDA in a dose-dependent manner: perfusion of the preparation with 6 μM NMDA blocks approximately 50% of the post-synaptic response; perfusion with 12 μM NMDA, essentially all of the response. In contrast, APV at physiological doses (60μM) increases slightly the positive component of the post-synaptic response. This effect too is fully reversible and as with NMDA, the changes are stable and complete within 15 minutes of drug application.

We are currently investigating if NMDA decreases synaptic transmission by affecting directly retinal ganglion cell terminals and/or by increasing tectal inhibition. This work was supported by NIH grants EY05829 and EY06039.

272.14

NMDA RECEPTOR ANTAGONIST, APV, DISORGANIZES THE RETINOTECTAL MAP.

H.T. Cline & M. Constantine-Paton. Dept. Biology, Yale Univ. New Haven, CT 06511.

The NMDA receptor plays a role in eye-specific segregation of retinal ganglion cell (RGC) terminals in the optic tecta of three eyed tadpoles (Cline et al, 1987, PNAS 84: 4243). We have shown that chronic application of the NMDA receptor antagonist, aminophosphonovaleric acid (APV), to the optic tectum results in the desegregation of the RGC terminals without blocking pre- or post-synaptic activity and without altering arbor morphology as seen with TTX treatment.

We have mapped the retinotectal projection in APV-treated tadpoles by focally injecting HRP into the rostro-medial tectum to label the RGCs projecting to that tectal site. Care was taken to inject uniform amounts of HRP. HRP-labeled RGCs were located primarily in the ventral-temporal retina, but in APV-treated tadpoles, labeled RGCs were dispersed over a greater area of the retina than in staged control tadpoles. In addition, individual labeled RGCs were occasionally seen in the nasal retina. These data support the hypothesis that the NMDA receptor is involved in the activity-dependent fine-tuning of the retinotectal projection. This work is supported by NIH grants EY05818 and EY06039.

272.15

NMDA BLOCKERS PREVENT BOTH RETINOTOPIC SHARPENING AND LTP IN REGENERATING OPTIC PATHWAY OF GOLDFISH. J.T. SCHMIDT, Dept. Biol. Sci., SUNY Albany, NY 12222.

The regenerating retinotectal projection of goldfish has an increased capacity for LTP that may be related to the activity driven sharpening of the retinotopic map. In normals, field potentials elicited by optic nerve shock are very stable. In newly regenerated projections (20-40 days), responses are initially small (<1mv), but giving 20 stimuli at 0.1Hz results in a 100-200% increase in amplitude that is stable for hours. Topical application of NMDA blockers AP5 or AP7 at 25μM prevents potentiation without decrementing ongoing responses (AP6 does not). To test whether NMDA blockers also prevent retinotopic sharpening, I infused AP5 or AP7 into the ventricle for 2-3 weeks during regeneration using osmotic minipumps (3-4μl/day of 500μM soln; approx. 50X dil. in CSF). Electrophysiological mapping at 53-107 days postcrush showed that AP5 and AP7 (but not AP6) blocked the sharpening. At each tectal point, responsive areas in the visual field were enlarged to 28°(vs 10° normally). This was comparable to data from fish regenerating with activity blocked (TTX) or strobe synchronized and indicates uncorrected errors in targeting of regenerated arbors (Schmidt, Cell & Molec. Neurobiol. 5:65, 1985). In the current model, retinotopically appropriate synapses (and branches) are stabilized because the normally correlated firing of neighboring ganglion cells should result in the summation of postsynaptic responses and in effective activation of NMDA receptor channels triggering Ca^{++} entry.(NIH grant EY03736).

272.16

THE ROLE OF NMDA RECEPTORS IN THE DEVELOPMENT OF BINOCULAR MAPS IN XENOPUS TECTUM. W.S. Scherer* & S.B. Udin. Dept. of Physiol., SUNY, Buffalo, NY 14214.

The tectum of Xenopus frogs receives input from both eyes. The contralateral eye's projection reaches the tectum directly, via the optic nerve, and the ipsilateral eye's projection reaches the tectum indirectly, via the nucleus isthmi. The isthmotectal (IT) map shows great plasticity during development and it will reorganize in response to rotation of one eye.

The effects of chronic treatment with the agonist NMDA and antagonists APV and CPP on development of binocular tectal maps in Xenopus reared with 90° rotation of the left eye were examined electrophysiologically. Drugs were administered topically via slow release from Elvax polymers implanted below the tectal pia. Animals treated with NMDA exhibited congruent, rotated maps on the right tectum (5 out of 6). In 4 out of 4 APV-treated animals, the retinotectal map was rotated 90° as expected, but the isthmotectal map from the unrotated eye was unrotated; and thus the two maps were approximately 90° out of register. CPP-treated animals (5 out of 6) also had appropriately rotated contralateral maps but the IT map was incongruent and only the central 40% of the ipsilateral visual field was represented. These results indicate that NMDA receptors play a role in the topographic organization of binocular tectal maps. Supported by USPHS Grant EY-03470 to S.B.U.

272.17

MICROSTIMULATION IN THE AREA OF NUCLEUS ISTHMI ENHANCES TECTAL RESPONSES TO OPTIC TRACT STIMULATION IN GOLDFISH. W.M. King & J.T. Schmidt, Biol. Dept., SUNY Albany, NY 12222

Although the retinotectal transmitter is most likely an excitatory amino acid, a cholinergic modulation is likely since 1) nicotinic receptors are present on retinal terminals 2) cholinergic agents affect transmission and 3) ChAT pos. fibers are present in the same lamina of tectum and originate from both tectal pyriform neurons and the Nucleus Isthmi (NI) which is reciprocally connected with tectum. We have used an in vitro preparation of goldfish tectum and brainstem to test whether activity from NI may modulate retinotectal transmission. We used metal microelectrodes to stimulate in the area of NI and verified the sites histologically. Retinotectal field potentials were similar to those recorded in vivo and stable for hours. The short latency monosynaptic response was followed by a polysynaptic response about 15 msec later. Single shocks to the optic tract also evoked repetitive field potentials in NI, as observed by Vanegas et al. When optic tract stimulation was preceded by a shock to NI, the mono- and polysynaptic tectal responses were larger (about 15 and 50% respectively) and the latency of the polysynaptic response decreased. The effect was apparent at intervals of 10 to 100msec following NI stimulation and maximal when optic tract stimulation was submaximal. In summary, we have found that single shocks to the optic tract elicit repetitive field potentials in NI and that stimulation of NI area enhances subsequent tectal responses to optic tract stimulation. (NIH grant EY03736).

272.18

FIBRE ORDER IN THE RODENT'S OPTIC TRACT: DISTRIBUTION OF OPTIC AXONS, GENESIS OF RETINAL GANGLION CELLS, AND THE POSITION OF GROWTH CONES IN DEVELOPMENT **B.E. Reese* and R.J. Colello* (SPON: F.A.W. Wilson) University of Oxford, Dept of Human Anatomy, Oxford, U.K.**

The generative periods for neuronal sub-populations in the retinal ganglion cell layer were examined by combining tritiated thymidine autoradiography with horseradish peroxidase histochemistry, and were then compared with the relative distributions of their axons in the optic tract of adult rats. The distributions of axon-like and growth cone-like profiles in the developing optic tract was also examined during the neurogenetic period in embryonic rats and mice.

In adult rats, the largest optic axons are found deep in the tract, and their cells of origin (Type I) are generated early (E.14-16). The finer optic axons are found at all locations across the optic tract, and their parent cells (Types II/III) are generated simultaneous to and well after Type I cell genesis (E.14-20). Uncrossed optic axons are found relatively deep in the optic tract, but with very few positioned at the deep and superficial borders; genesis of their cells peaks relatively early (E.16), with few cells generated before (E.14) or after (E.18). During this neurogenetic period, the developing optic tract continues to accumulate axonal profiles, yet the profiles of presumed growth cones are found only along the tract's superficial (sub-pial) border. These results suggest that fibre position within the adult optic tract is an index of axonal arrival during development, and indirectly, a chronology of neurogenesis.

272.19

IS THERE SUBSTANTIAL TOPOGRAPHIC ORDER DURING DEVELOPMENT OF THE RAT RETINOCOLLICULAR PROJECTION? J.P.A. Yhip* and M.A. Kirby (SPON: S. Yellon). Depts. of Pediatrics and Anatomy, Sch. of Med., Loma Linda University, Loma Linda, CA 92350.

It is well demonstrated that during development retinal projections are both more extensive and numerous than at maturity. At present, however, little is known of the topographic order of these projections. To explore this, we have examined the distribution and density of labeled retinal ganglion cells following placement of discrete deposits of a tracer into the superior colliculus (SC) of neonatal and adult rats.

Small deposits of rhodamine filled latex microspheres (0.02-0.1 μl using glass pipettes, tip diameter 25-30 μm) were placed into the SC of pigmented rats ranging in age from the day of birth to adulthood (hypothermia or barbiturate induced anesthesia). Following a 36-48 hr survival period, the animals were deeply anesthetized with barbiturate and perfused through the heart with saline, followed by a 10% formal-saline solution. The location and density of labeled ganglion cells were plotted on drawings of retinal wholemounts.

In all ages examined where deposits were localized to the SC, an area of high density labeled ganglion cells was observed in a restricted portion of each retina. The area of labeled cells was always topographically appropriate in relation to the location and extent of the SC deposit. At all ages examined this pattern was most pronounced for deposits into the peripheral-caudal pole of the SC (which avoids the problem of fibers of passage). Also present in each retina were a small number of labeled ganglion cells that were widely dispersed and distant to the high density area and are presumed to represent topographic errors. These results suggest a high degree of topographic order is present during neonatal development of the rat retinotectal projection.

272.20

A RETROGRADE LABELLING STUDY OF THE UNCROSSED RETINOFUGAL PATHWAYS IN DEVELOPING NORMAL RATS AND RATS WITH UNILATERAL NEONATAL THALAMECTOMY OR EYE REMOVAL. S.O. Chan* and L.S. Jen. Department of Anatomy, The Chinese University of Hong Kong, Hong Kong.

The present study has compared the numbers of ipsilaterally projecting retinal ganglion cells(IPRGCs) observed in developing Sprague-Dawley albino rats and rats which received left thalamectomy(T) or monocular enucleation(E) at birth, with WGA-HRP introduced to their right thalami.

The results obtained from normal rats showed that the average total number of IPRGCs decreased from 3700 cells/retina on day 0(day of birth) to reach the adult level of 1200 cells/retina on postnatal day 5. The number of IPRGCs in T cases however increased to about 4500 cells/retina on postnatal day 1 before it decreased rapidly to achieve the adult figure of about 2400 cells/retina on postnatal day 5. Similar trend of changes in the total number was also observed in E cases, with the maximum reaching about 4000 cells/retina on day 1.

These results indicate not only that the elimination of IPRGCs in one eye during development can be prevented by a contralateral thalamic lesion or eye removal made at birth, but also suggest that there is misrouting or rerouting of optic axons at the optic chiasma following the lesion, which probably leads to retention of some of the IPRGCs which normally do not persist into adulthood.

273.1

ISOLATION AND CHARACTERIZATION OF HUMAN MUSCLE cDNA CLONES IMMUNOREACTIVE TOWARDS ANTIBODIES PRODUCED IN HUMAN MYASTHENIA GRAVIS. M. Mihovilovic*, A.E. Waters*, A.D. Roses. Duke Univer. Med. Ctr., Durham, NC., 27710.

In human myasthenia gravis (MG) circulating auto antibodies to several uncharacterized antigens are produced. We have found MG antibodies that bind to at least 5 (non-cholinergic) muscle proteins resolved by SDS electrophoresis and also to different muscle structures in muscle tissue sections.

We have used MG antibodies to screen an expression muscle cDNA library and isolated 4 immunoreactive cDNA clones.

Nucleotide sequence analysis (of about 300 base pairs) indicate that 3 of them (8/3, 9/3 and 7/9) carry identical cDNA sequences. The cDNAs have a length from 1.38 to 1.9 Kb. Northern blot analysis employing mRNA purified from different human tissues have shown that these cDNAs exclusively hybridize to mRNA species expressed in human muscle.

The fourth clone (7/3) carries a 1.8 Kb insert which hybridizes to at least 3 muscle mRNA species and to a unique mRNA species present in MG thymoma.

Studies are currently in progress to obtain the complete sequence of these clones and to characterize the fusion proteins that they encode. These cDNAs will be further used to study the relationship that exists between circulating antibody to non-cholinergic muscle antigens and disease expression of myasthenia gravis.

273.2

GENETIC LINKAGE STUDIES IN FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS (ALS). D. A. Figlewicz*[1,2], G.R. Rouleau*[1], J.L. Haines*[1], R.H. Brown, Jr.*[1] and the Collaborative Familial A.L.S. Study Group. (SPON: J.A. Nathanson). [1] Neurology Service, Ma. General Hosp., Boston, MA 02114; [2]CNS Research, Cambridge, MA 02139.

The etiology of motorneuron death in ALS is unknown. In the 5-15% of cases which are familial, genetic analysis may lead to the identification of the defective gene(s) and provide insight into the molecular basis of the disorder. We are evaluating 36 multi-generational ALS families in which the inheritance pattern appears to be autosomal dominant. Blood specimens have been obtained and lymphoblastoid lines established for 160 family members including 24 individuals with ALS. Potential informativeness of families was evaluated using the LIPED program, varying parameters such as penetrance, polymorphism information content and map position. In these families, a random search of the entire genome is underway using standard genetic linkage techniques employing highly polymorphic DNA markers. Results of the genetic linkage search are analyzed by LIPED. Updated results will be presented.

273.3

MATHEMATICAL SIMULATION OF THE EFFECTS OF AXIAL DIFFUSION AND NON-ZERO-ORDER KINETICS ON OXYGEN DELIVERY IN PERIPHERAL NERVE. T. D. Lagerlund and P. A. Low. Department of Neurology, Mayo Clinic, Rochester, MN 55905 USA

We have extended our previous mathematical model of the oxygen supply of peripheral nerve tissue to include additional effects which make the model more realistic. Our simulation of the release, diffusion, and consumption of oxygen in the capillaries and surrounding tissue of peripheral nerve now includes axial diffusion in blood and in surrounding tissue in addition to bulk flow of blood and radial diffusion of oxygen out of the capillary. Also, our simulation assumes that the oxygen consumption of nerve tissue obeys Michaelis-Menten kinetics rather than zero-order kinetics as had been assumed in the Krogh model. With these assumptions, we could calculate via numerical solution of the relevant differential equations, the oxygen tension at all points in the capillary and surrounding tissue, as a function of distance from the center of the nearest capillary and distance along the capillary from the arterial to the venous end. Using average measured values for microcirculatory parameters in rat nerve, we calculated a distribution of oxygen tension values which agrees reasonably with experimentally measured distributions. The effects of axial diffusion and of Michaelis-Menten kinetics on the oxygen distributions were noticeable under normal conditions, but these effects were even more important in situations in which oxygen delivery was adversely affected.

273.4

MUSCULAR DYSTROPHY: ISOPROTERENOL-DEXAMETHASONE SYNERGISM IN CHICKENS. R.K. Entrikin, R.T. Abresch*, R.H. Lantz*, D.B. Larson*, and D.P. Bradford*. Dept. of Physical Medicine & Rehab., Univ. of California, Davis, CA 95616.

Earlier we showed that glucocorticoids (GLU) increase muscle function (ES, exhaustion score) in genetically dystrophic chicks. More recently, a GLU improved muscle strength in Duchenne dystrophy patients. Unfortunately, adverse effects (such as weight loss in chicks) were noted at effective doses. In this study we administered (i.p.) dexamethasone (DEX), a GLU, in combination with isoproterenol (ISO), which reduces body weight less than DEX at effective doses. DEX alone (0.1 mg/kg/day) and ISO alone (1.0 mg/kg/day) produced 22-day ESs (mean $\pm$ SEM) of 4.2 $\pm$ 1.3 (n = 23) and 1.8 $\pm$ 0.7 (n=24), respectively. (Mean ES for untreated normal chicks is about 20; for dystrophics only about 1.5.) In combination, the same doses of DEX and ISO increased the 22-day ES to 15.4 $\pm$ 2.5 (n = 11) and produced weight reduction similar to that with DEX alone. In brief, ISO potentiates the beneficial effects of GLUs, but does not ameliorate the adverse effects in avian dystrophy. (Supported by MDA and NIDRR Grant # H133B80016.)

273.5

ULTRASTRUCTURE OF SKELETAL MUSCLE IN MULTIPLE SCLEROSIS. A. Márquez*, C. García*, H.J. Finol and I. Mosquera*. Medicine and Sciences Faculties, Universidad Central de Venezuela. Apartado 50587, Sabana Grande, Caracas, Venezuela.

Although motor disturbances are common in multiple sclerosis and muscle atrophy may occasionally be present in this disease, its skeletal muscle pathology has not been studied. In this work we report the findings observed in a muscle biopsy made for diagnosis purposes in a multiple sclerosis patient, a 20 years old woman who presented blurred speech, ataxic gait, bilateral ptosis of eyelids, tongue fasciculations, increased deep tendon reflexes, and mental disorders. EMG study was no conclusive. Alterations found were: atrophy which varied from slight to severe, disorganized contractil and sarcotubular systems, bizarre mitochondria, presence of glycogenosomes some of them limited by mitochondrial membranes, muscle basement membrane thickened and reduplicated in some areas. Satellite cells were abundant and proliferative. Capillary alterations included thickening and reduplication of basement membrane and occlusion of lumen. Macrophages were present in the extracellular space and next to capillaries. Multiple sclerosis is not considered a muscle disease but the histopathological changes we observed can not be explained only in a neurological basis. Because of that, the presence of a primary miopathic component could be part of the etiopathogenesis in this entity.

This work was supported by grants from CDCH of UCV (No. C-03-17-86), Fundación Polar and The British Council Venezuela.

273.6

EVIDENCE AGAINST FUNCTIONAL DENERVATION AT THE WOBBLER MUTANT MOUSE NEUROMUSCULAR JUNCTION J.H. LaVail, K.P. Irons* and D.C. Bowen* Dept. Anatomy and Neuroscience Program, U.C.S.F., San Francisco, CA 94143

Based on the extensive degeneration of motoneurons in the cervical spinal cord, the wobbler (*wr/wr*) mutant mouse has served as a model for human neurodegenerative diseases, such as Werdnig-Hoffman disease. In the course of the first ultrastructural investigation of neuromuscular junctions (nmj) in the lateral rectus muscles of *wr/wr*, we found the major distinguishing feature of the *wr/wr* muscle compared to control muscle was that the postjunctional folds of endplates were shallower and less numerous than those of age-matched controls (Soc. Neurosci. Abst. 1986, 262). To investigate the nmjs further we have now used a variety of techniques. Labeling of dissociated muscle fibers with ^{125}I-a-bungarotoxin showed that acetylcholine receptors (AChR) were clustered at the nmj, but there was a 1.4 to 1.9-fold increase in extrajunctional binding within 100 μm of the endplate; beyond 100 μm the level of binding was indistinguishable from that of control muscles. Indirect immunocytochemical studies using antibodies against AChR subunits (donated by Z. Hall and Y. Gu, UCSF) indicated the presence of mature subunits clustered at the nmjs. Although we have no quantitative estimates, our histochemical evidence indicates that acetylcholinesterase is present in the cleft of the *wr/wr* nmj. Since the nerve terminal shows no degenerative features, there is no increase in extrajunctional binding beyond 100 μm of the vicinity of the endplate, and the AChR are apparently composed of mature subunits, we conclude that the changes in the endplate membrane of the *wr/wr* lateral rectus muscle are not due to denervation. Furthermore, the maintenance of postjunctional folds and the insertion of mature AChR at the *wr/wr* nmj are apparently independent events. (Supported in part by N.I.H. NS 13533).

273.7

ALTERATION OF SODIUM CHANNEL INACTIVATION IN A RAT MODEL OF MYOTONIA PRODUCED BY REDUCING EXTRACELLUAR CHLORIDE. R.L. Ruff. Dept. of Neurology, Cleveland Veterans Administration Med. Ctr. and Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106

Several forms of myotonia in humans and animals including myotonia congenita, Becker's form of myotonia and myotonia in goats are associated with reduced muscle chloride conductance. Myotonia can be produced in vitro by reducing skeletal muscle membrane chloride conductance. The effects of reducing extracellular chloride concentration on sodium channel gating in rat extensor digitorum longus muscle was studied using the loose-patch voltage clamp. Cells were studied in solutions with 132.5 mM or 14 mM chloride concentration. Chloride was replaced by methylsulfate. In 14 mM chloride muscle cells were hyperexcitable with depolarizations of about 10 mV from the resting potential producing repetitive action potentials. Sodium channel fast inactivation was shifted by +15 mV and the rate of fast inactivation was slower in low chloride solutions. Slow inactivation appeared to be shifted by +18 mV, and the shift in slow inactivation increased the current available for depolarizations from the resting potential by 287% in 14 mM chloride. In contrast, the voltage dependence of sodium channel activation did not appear to be altered. The changes in sodium channel inactivation could contribute to the hyperexcitability seen in this model of myotonia. However, it is not clear if similar changes occur in any of the natural forms of myotonia.

Supported by Merit Reviewed funding from the United States Veterans Administration.

273.8

A COMPARATIVE STUDY OF DISEASED HUMAN MUSCLE XENOGRAFTS IN ATHYMIC RATS AND MICE. A.K. Gulati*, M.H. Rivner* and T.R. Swift* (SPON: D.S. Feldman). Departments of Anatomy and Neurology, Medical College of Georgia, Augusta, GA 30912.

We studied the fate of skeletal muscle biopsied from patients with Duchenne muscular dystrophy, amyotrophic lateral sclerosis and polymyositis after transplantation into athymic (i.e. immunodeficient) rats and mice. In each recipient rodent, the extensor digitorum longus (EDL) muscle was removed. The biopsied human muscle was then placed in the site previously occupied by the EDL muscle and sutured in place under slight tension. Muscles were removed at one, 3, 6 and 10 months after transplantation for morphological analysis. In nude mice, the muscle transplants survived, underwent successful regeneration and innervation by host motor neurons. In contrast, muscles in nude rats after initial regeneration underwent regression and extensive replacement by connective tissue. It is concluded that human muscle xenografts do not survive in nude rats but do in nude mice. This difference in the acceptance of transplanted human muscle may be related to differences in the immunocompetency between the two species. Transplantation of human muscle into nude mice offers an in vivo model to study the etiology of various neuromuscular disorders.

273.9

CLINICAL AND ELECTROMYOGRAPHIC RESPONSES TO INDUCED PARALYSIS IN INHERITED CANINE PERIODIC PARALYSIS. G.A. Hegreberg, M. Paloheimo*, M. Moore*, S. Jenkins* and B. Rigor*. Washington State University, Pullman, WA 99164 and University of Louisville, Louisville, KY 40292.

An autosomal recessive disorder of dogs is accompanied by generalized muscular weakness and progressive muscle atrophy with extreme sensitivity to cold, exercise and excitement stress. The severe and intermittent muscle weakness associated with the canine condition has clinical similarities to the periodic paralyses of people, especially hypokalemic periodic paralysis. Clinical and electromyographic studies were performed to compare this canine condition to the human hypokalemic periodic paralysis. These studies included 1) induction of skeletal muscle paralysis, 2) measurement of evoked compound muscle action potentials (ECMAP) before and after induction of paralysis and 3) measurements of serum electrolytes levels before and after the induction of paralysis. Muscle paralysis was induced in three affected adult male dogs by the intravenous administration of glucose and insulin with subsequent metabolic alkalinization with sodium bicarbonate. The ECMAP's were measured from the interosseous muscles of the forelimb after stimulation of the ulnar nerve. Clinically, a profound generalized muscle paralysis was produced in the affected dogs after administration of gluocose, insulin, and sodium bicarbonate. The post-administration ECMAP's were depressed to 40-60% of baseline levels. Serum potassium and sodium levels were within normal range prior to the induction of paralysis. Potassium levels were decreased to 1.5-3.0 mmol/liter during the height of the paralysis. Respectively, sodium levels were elevated to 162-179 mmoles/liter. The clinical and electromyographic changes found in this canine disorder are similar to human hypokalemic periodic paralysis and this canine disease provides an animal model for further characterization of the pathophysiology of this disease. Supported in part by NIH RR00515, Washington State University, the University of Louisville, Datex, and Cadwell Laboratories.

273.10

A QUANTITATIVE ANIMAL MODEL FOR STUDYING THE EFFECTS OF SHORT TERM ELECTRICAL STIMULATION ON THE REHABILITATION OF DENERVATED MUSCLE PARALYSIS. W.Bedingham* and D.G.Ericson*. (SPON: J.Heltzel). 3M Biosciences Laboratory, St. Paul, MN 55144.

The clinical utility of electrotherapy in the treatment of flaccid muscle paralysis remains controversial. The present study was designed to quantify and evaluate the rehabilitative efficacy of electrical stimulation on the fundamental electrophysiology and pathology of denervated muscle in controlled animal studies (Chinchilla sp.). The animals were divided into 3 primary groups: (1) unilateral denervation of the tibialis anterior (TA) muscle, (2) bilateral denervation of the TA muscle, and (3) control - no denervation. Each primary group was subdivided into a stimulated or nonstimulated subset, and each subset was further subdivided according to the method of stimulation (intramuscular, epysmisal), duration of stimulation, and stimulus pattern. After the prescribed stimulation period, each TA muscle was examined by quantitative electrophysiological and histochemical techniques. The treatment methods were compared by nonparametric analysis between independent subgroups and within subgroups, using the test and contralateral TA muscle in each animal as a matched pair.

Denervation caused significant reduction in muscle tissue weight and fiber diameter, decreased tetanic tension and fatigue resistance, and prolonged single twitch contraction times. Depending on the treatment methodology used, it was shown that electrical stimulation could provide significant improvement in these deficits. Comparison between matched denervated TA pairs showed that 20 minutes of electrical stimulation per day provided significant increase in muscle weight, fiber diameter, and tetanic tension. Only a limited increase in benefit was observed in animals treated with longer daily stimulation times. When compared to matched normal TA muscles, however, the electrically stimulated denervated TA muscles remained significantly inferior in mechanical performance and morphology.

273.11

MYOBLAST INJECTION TREATMENT FOR MUSCLE WEAKNESS. P.K. Law, T.G. Goodwin*, H.J. Li* and M. Chen*. Depts. of Neurology and Physiology/Biophysics, University of Tennessee Memphis, Memphis, TN 38163

A treatment has been developed to prevent hindlimb and intercostal muscle weakness in murine dystrophy. Injection of histoincompatible normal myoblasts/fibroblasts (co-culture) into dystrophic muscles improved the structure and function of the muscles to almost normal. Immunosuppression of the C57BL/6J-$dy^{2J}dy^{2J}$ hosts was by way of daily subcutaneous injection of cyclosporin-A. Injected dystrophic muscles exhibited greater cross-sectional area, total fiber number, wet weight, and twitch and tetanus tensions six months postoperatively. Fiber typing was more defined and they contained more normal-appearing and less abnormal-appearing fibers than non-injected controls. Eleven out of nineteen mice that received myoblast injections on both sides showed such behavioral improvement that their locomotive patterns were indistinguishable from normal. Using dimeric isozymes as genotype markers for host and donor cells, the demonstration of parental and hybrid isozymes inside the injected muscles substantiated the survival and development of donor myoblasts into normal myofibers, and the fusion of normal myoblasts with dystrophic satellite cells to form genetically mosaic myofibers. Since the treatment design is based on muscle developmental processes universal to all mammals, it has potential for clinical application. (Supported by USPHS NS-20251 and NS-26185).

274.1

LAYER VII OF RODENT CEREBRAL CORTEX. G.S. Goodwin and R.L. Reep. Department of Neuroscience, J-244 JHMHC, University of Florida, Gainesville, FL 32610.

The cerebral isocortex is usually considered to be a six-layered structure. However anatomical findings suggest that a seventh layer be recognized in rodent isocortex. Injections of retrograde fluorescent tracers into medial agranular cortex produce labeled cells in somatic sensory and visual cortex. These occur as sheets or clusters in layers II/III, V and the deepest portion of the gray matter immediately adjacent to the underlying white matter. Injections in lateral agranular, somatic sensory, or visual cortex result in similar laminar labeling patterns in those isocortical regions containing labeled neurons. Adjacent stained sections reveal that the deep lying cells reside in a thin continuous layer closely adherent to the underlying white matter and separated from layer VI by a cell-sparse fiber plexus zone (which we have previously demonstrated to contain corticocortical axons). It is this deep cell layer to which we refer as layer VII. All isocortical regions of the rat brain exhibit a well defined layer VII with overlying cell sparse zone. Maximilian Rose identified layer VII in several other species of rodents as well.

Developmental studies (including our own) indicate that layers VII and I originate from an early primordial plexiform layer which is later split by the cortical plate that forms layers II-VI.

We suggest that layer VII be considered a distinct entity on cytoarchitectural, developmental and connectional grounds. It remains to be seen to what extent layer VII is definable in all rodents and among other mammalian orders.

Supported by the Maxwell Fund.

274.2

RAT MEDIAL AGRANULAR CORTEX CONNECTIVITIES. Sherry L. Stuesse and Donald B. Newman, NEOUCOM, Rootstown, OH, 44272 and USUHS, Bethesda, MD 20814-4999.

Rat medial agranular cortex (AGm) may be homologous to monkey frontal eye fields (FEF). To test this hypothesis, AGm connections were determined by use of a Phaseolus lectin, WGA-HRP, or the Wiitanen/Fink-Heimer method. AGm projected to cortex: orbital, AGm, motor, anterior cingulate, retrosplenial, and perirhinal. AGm received input from dorsopeduncular cortex and contra AGm. Subcortical projections were similar to those of monkey FEF and targeted gaze control and visual orientation areas: rostral interstitial nucleus of the medial longitudinal fasciculus, paramedian pontine reticular formation (reticularis pontis oralis, caudalis and reticularis gigantocellularis), nuclei cuneiformis and subcuneiformis, subthalamicus, pretectalis, raphe centralis superioris, and superior colliculus. Reciprocal connections were made with the following thalamic nuclei: intralaminar, mediodorsal, ventrolateral, ventromedial, parafascicular, and posterior. AGm projected to caudate-putamen, basilar pontine gray, central gray, and zona incerta. AGm received input from raphe dorsalis and globus pallidus. Differences in connections between rostral and caudal injections were observed: caudal AGm projected more heavily to those regions implicated in eye movement control. These experiments confirm that AGm in rat contains the homologue of primate FEF. AGm may also be homologous to the primate supplementary motor area as evidenced by connections with globus pallidus, caudate-putamen, and motor cortex.

274.3

DISTRIBUTION, ONTOGENY AND CONNECTIONS OF CHOLECYSTOKININ (CCK) mRNA NEURONS IN THE RAT CORTEX. J-M. Burgunder*, W.S. Young III Lab of Cell Biology, Natl. Inst. Mental Health, Bld36, Rm 3A17, Bethesda, MD 20892.

CCK-immunoreactive neurons are located in all cortical layers. These neurons participate in local circuitry but a role in more distal innervation is debated. In the present study, CCK neurons were studied by *in situ* hybridization (using a synthetic, 48base oligonucleotide probe, labeled with [S35]dATP using terminal deoxynucleotidyl transferase) and fluorogold retrograde labeling. Different neuronal types containing CCK mRNA were present in all cortical layers: moderately labeled neurons were few in I, plentiful in II to superficial IV, and few in deeper IV and superficial V, whereas many, more heavily labeled neurons, were present in deep V and VI. In addition, some very heavily labeled neurons were found scattered in all layers.

In the developing cortex, CCK mRNA was seen for the first time in some cells of the primordial plexiform layer at E15, followed two days later by cells in the cortical plate. This pattern matured gradually, in parallel to the cortical morphogenesis, reaching adult-like patterns at the 20th postnatal day.

After several injections of the fluorescent dye fluorogold into cortical and subcortical target areas of the neocortex of adult rats, many cortical neurons with CCK mRNA were retrogradely labeled. These results indicate that cortical CCK neurons establish extensive associational (e.g. parieto-frontal), commissural (e.g. frontal) and corticofugal (e.g. corticostriatal) pathways.

274.4

CORTICOSPINAL (CS) NEURONS MAY SEND MULTIPLE AXONAL BRANCHES THROUGH THE PYRAMIDAL TRACT. D.L. O'Donoghue, M.T. Cox and D.R. Humphrey. Lab. of Neurophysiology, Emory Univ., Atlanta, GA 30322

The number of CS cells has been estimated after placements of Fast Blue (FB) in the mid-cervical spinal cord of adult rats (over 3 weeks of age; n=3). Dye placements were maximized by transecting the dorsal funiculus and placing gelfoam soaked in 5% FB within the transection. After a week survival, rats were sacrificed and the cerebral cortex was sectioned coronally at 50um. The numbers of FB labeled cells in 500um bins were counted and sections were sampled every 400um. Cell distributions were constructed and the number of FB labeled CS cells was estimated.

The estimated total number of CS cells was 28,293 (± S.E.6933) in one hemisphere. Even if this represents an underestimate, there are still far more axons in the rat CS tract: 80,000 cervical CS fibers (Leenan et al. '85, Brain Res. 359:65). Electron microscopic studies are underway to verify the number of fibers in the cervical CS tract. Because of the disparity between the number of labeled CS cells and the reported numbers of CS fibers, we suggest that many CS neurons have 2 or more axonal branches in the CS tract. (Supported by NIH Grant NS20146).

274.5

COLLATERAL PROJECTIONS OF SENSORY CORTICAL NEURONS IN HOODED RATS THAT PROJECT TO THE TECTUM AND THE PONS. L.E. Hallman, B.R. Schofield, and C.-S. Lin. Dept. Anatomy, Duke Univ. Med. Cntr., Durham, NC 27710.

We have used the retrograde transport of Fluoro-Gold and rhodamine-coated beads to determine the incidence of sensory cortical neurons which send axon collaterals to both the tectum and the pons. Recently, we have used this combination of dyes to show that in primary visual cortex over 60% of the neurons that project to the superior colliculus also project to the pons (Hallman et al., J. Comp. Neurol., in press).

We examined 442 cells from four cases. The data from auditory and extrastriate cortices were similar. Over 60% of the labeled layer V neurons had collaterals to both the tectum and the pons. Of the labeled corticotectal cells over 75% were double-labeled and of the labeled corticopontine cells over 74% were double-labeled. Similar precentages were found in somatosensory cortex, even though the pontine injection may have labeled corticospinal and corticobulbar, as well as corticopontine cells. Thus, corticotectal and corticopontine projections arise to a large degree from the same cells in each of these cortical areas. This property may be a general characteristic of all cortical areas.

Supported by NSF BNS 86-06570 and NIH EY05777.

274.6

INTERACTION OF SEROTONIN AND GABA IN CEREBRAL CORTEX AND HIPPOCAMPUS. I. Törk* and J.-P. Hornung. (SPON: C. Straznicky). School of Anatomy, University of New South Wales, Kensington, NSW 2033, Sydney, Australia and Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

The cerebral cortex is innervated by two distinct and morphologically different serotonergic subsystems (Kosofsky and Molliver, *Synapse* 1:153, 1987; Mulligan and Törk, *J. Comp. Neurol.* 270:86, 1988). One of the fiber systems is characterized by large axon terminals, and is in synaptic contact with the somata and dendrites of a subpopulation of non-pyramidal neurons in the neocortex and hippocampal formation. A significant percentage of cortical non-pyramidal neurons is GABAergic (Gabott and Somogyi, *Exp. Brain Res.* 61:323, 1986), and we have used double labelling immunohistochemical techniques to demonstrate the direct interaction between serotonergic axons and glutamic acid decarboxylase- (GAD), or GABA-immunoreactive neurons, in the cat. We have surveyed large populations of cortical GABAergic neurons in sections of the auditory, somatic sensory and prefrontal cortex in which we have also demonstrated serotonergic axons using a monoclonal antibody against serotonin. All layer I neocortical neurons densely innervated by serotonergic axons were also immunoreactive for GAD or GABA. The innervating axons made multiple synaptic contacts with their target cells and many single boutons exhibited multiple synaptic densities. However, many layer I GABAergic neurons did not receive serotonergic input. Densely innervated GABAergic neurons were less common in layers II and III and could not be observed in any of the deeper layers. Further evidence for interaction of serotonergic axons with GABAergic neurons was found in the dentate gyrus in which a distinct layer of serotonergic axons innervated the dendrites and somata of basket cells at the interface between granule and polymorph layers. These results strongly suggest that the brainstem reticular formation has direct access to some of the key neurons in cortical circuitry and could modulate cortical inhibition via the ascending serotonergic pathways.

274.7

INHIBITION SHAPES THE SIZE OF MOTOR CORTEX REPRESENTATIONS K.M. Jacobs and J.P. Donoghue Center for Neural Science, Brown University, Providence, RI 02912

Intracortical electrical stimulation mapping reveals a somatotopically organized representation in cerebral motor cortex (MI). We have shown that this representation reorganizes within hours of a peripheral nerve transection that disconnects one part of the representation from its target muscles. In the present experiments we examined the hypothesis that intracortical inhibitory circuitry blocks the expression of connections that can effectively alter the MI representation pattern.

The MI representation pattern was mapped using standard intracortical electrical stimulation techniques in 8 ketamine anesthetized rats. Vibrissa (Vib) and forelimb (FI) sites were localized by stimulating with pulse trains (60 μA) at 11-40 sites spaced 250-500 μm apart. The FI area was defined by recording the electromyogram (EMG) from the elbow flexor and wrist extensor muscles contralateral to the mapped cortex. After initial mapping the stimulating electrode was fixed at a single site 0.25 to 1.0 mm medial to the Vib-FI area border. At this site Vib movements were observed, but no FI EMG was elicited at 60 μA. Next, a glass micropipette (tip diameter 1-2 μm) containing 10 mM bicuculline methylbromide (BMB) was placed at a depth of 1.8 mm in the FI area, 1.0 to 2.0 mm lateral to the stimulating electrode. After 15-75 minutes of continuous BMB ejection (50-150 nA) FI EMG (as well as Vib movement) was elicited by stimulating at the Vib site; 30-290 minutes after stopping the ejection only Vib movements were evoked at this site. Similar iontophoretic ejections of either AChCl or Na glutamate were applied separately from the BMB at the FI site for up to 2 hours. Neither caused a shift in MI representation. In no case was a spread of distant representations into the stimulated region observed.

These experiments indicate that the strength of excitatory connections between somatic representations and thus, the amount of cortex involved in the control of individual muscle groups is regulated by intracortical inhibition. It is not yet clear whether bicuculline unmasks circuits that are already present or whether secondary time-dependent processes are involved. However, it appears that increasing intracortical excitation alone is not sufficient to produce map shifts. Finally, these results demonstrate that a cortical substrate exists for producing shifts in MI organization similar to those we have observed following peripheral nerve lesions.

Supported by NIH NS 22517 and March of Dimes

274.8

REGIONAL DIFFERENCES IN DISTRIBUTION AND STAINING INTENSITY OF ASPARTATE-LIKE IMMUNOREACTIVITY IN RAT CEREBRAL CORTEX. C. Toomim, P. Petrusz* and J. Caffrey*. University of North Carolina, Chapel Hill, NC 27599 and Baylor College of Medicine, Houston, TX 77030.

Major divisions of the rat cerebral cortex were examined for differences of distribution and intensity of staining with an antiserum formed against aspartate-glutaraldehyde-hemocyanin (Hepler et al., J. Histochem. Cytochem., 36:13, 1988). Rats were anesthetized with chloral hydrate or pentobarbital and perfused with 1% paraformaldehyde (PF) followed by 4% PF-0.5% glutaraldehyde. Coronal vibratome and paraffin sections were incubated with anti-Asp and stained by standard immunoperoxidase methods. Staining was seen in perikarya and dendrites in all layers. Sections were analyzed visually and given intensity values from 0 (no stain) to 3 (dark specific stain). Staining patterns in various cortical areas include: Cingulate: gradual increase from light rostrally to dark caudally. Retrosplenial: dark throughout. Frontal: light at the medial border, moderate laterally. Parietal: light ventrolaterally, dark dorsomedially. Occipital: light medially and laterally, moderate to dark centrally. Temporal: light to moderate. Insular and perirhinal: light. Piriform and entorhinal: little or no stain. These results suggest that there are substantial differences in the distribution and intensity of Asp-like immunoreactivity between topographically and functionally distinct areas of the rat cerebral cortex.

274.9

ZINC-CONTAINING AXONAL TERMINALS IN RAT NEOCORTEX ARE PREDOMINANTLY CORTICAL IN ORIGIN. S. D. Hill and C. J. Frederickson, Lab. for Neurobiology, Univ. of Texas at Dallas, Richardson, TX 75080.

Laminae I-III and V of the neocortex are innervated by zinc-containing axonal systems, the boutons of which can be stained by zinc:TSQ fluorescence or neo-Timm's silver methods. These boutons have been shown to have Gray's Type I morphology (Perez-Clausell and Danscher, Brain Res., 1985, 335: 91-98), but the cells of origin have not been determined.

We have studied the zinc staining in the neocortex of the rat after either (a) surgical isolation of cortical "islands" or (b) destruction of local cortical neurons with ibotenic acid. The results indicate that the zinc-containing boutons are predominantly of intrinsic origin. Thus, in 2-4 mm diameter cortical islands (undercut through the white matter and surrounded by a circular knife cut from pia to white matter) the zinc staining in laminae I-III and V persisted with minor alterations throughout 7 day survival periods. In contrast, after focal destruction of cortical neurons by ibotenic acid (10 ug in 1 ul), zinc staining was completely abolished throughout most of the lesioned area. Efforts to identify specific cells of origin by retrograde transport of ZnSe from the boutons are in progress. Supported by MH 42798.

274.10

ANTIBODY TO A SOLUBLE PROTEIN PURIFIED FROM BRAIN SELECTIVELY LABELS LAYER V CORTICOFUGAL PROJECTION NEURONS IN RAT NEOCORTEX. B. B. Stanfield and D. M. Jacobowitz. Laboratory of Clinical Science, NIMH, Poolesville, MD 20837.

Protein 36 is a soluble protein isolated and purified from rat brain. In the rat neocortex, antibody to this protein labels the cell bodies and processes of pyramidal cells within layer V (Fukuda, T., and Jacobowitz, D.M., *Brain Res.*, 441:185-194, 1988). We have used the fluorescent retrograde tracer, Fast Blue (FB), in combination with FITC immunocytochemistry to determine the projection sites of the cortical neurons detected by this antibody. FB was injected (0.3-0.5 μL of 2% FB) into either the pyramidal decussation, the pyramidal tract in the pons, the inferior colliculus or the parietal cortex on one side. Six days later the rats were perfused, the brains sectioned and the sections processed for immunocytochemistry.

Following injections into the pyramidal decussation or into the pyramidal tract many of the FB labeled pyramidal tract neurons in the parietal cortex were also FITC labeled. Similarly, after inferior colliculus injections many FB labeled corticotectal neurons in the auditory cortex were FITC labeled. In these cases there were, in addition, many FITC labeled neurons which were not FB labeled, but in only a relatively small number of FB labeled neurons was FITC labeling not detected. In contrast, after injections into the contralateral cortex, FB labeled callosally projecting neurons in layer V were not FITC labeled. Thus, antibody to protein 36 may selectively detect within the neocortex a particular neuronal cell type, the corticofugal projection neurons of layer V.

274.11

QUANTITATIVE ANALYSIS OF CHOLINERGIC FIBERS IN THE RAT CEREBRAL CORTEX. H. Ojima*, T. Yamasaki*, H. Kojima*, and A. Akashi* (SPON: Y. Shinoda). Research Institute, Daiichi Seiyaku, Co., Ltd., Tokyo 134, JAPAN

No systematic investigation of the regional heterogeneity in the distribution pattern of cortical cholinergic fibers has yet been carried out. We determined their density over various regions in the rat cerebral cortex.

Cholinergic fibers were visualized using a monoclonal antibody to choline acetyltransferase. The number of positive fibers crossing the ruled lines of a counting grid in the microscope ocular was counted. Measurement was made at regular intervals from the pial surface of the sections passing through six anteroposterior levels.

In the frontal cortex, the density of positive fibers was constant throughout the layers. In the parietal, temporal, and occipital cortices, the density in the superficial and deep layers was 1.5 to 3 times higher than that in the middle layer. In the anterior cingulate cortex, the density in the middle layer was 1.5 times higher than that in the superficial and deep ones, but this difference became obscure at more posterior level. In the piriform cortex (PC), the middle density was 2.5 to 3 times higher than the superficial and deep one. The entorhinal cortex showed a laminar distribution pattern similar to that in the PC, but the middle density was only 1.5 times higher than the superficial and deep one. These results show the region-specific distribution pattern of cholinergic fibers.

274.12

TRANSNEURONAL RETROGRADE TRANSPORT OF TETANUS TOXIN C FRAGMENT TO MOTOR CORTEX FOLLOWING INJECTIONS IN RAT VIBRISSAL MUSCLES. E. McGuinness and J. Allman. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

The C fragment of tetanus toxin (TTC) retains its parent molecule's capacity for retrograde transneuronal transport but lacks its toxicity. We injected TTC in the vibrissal muscles in rats and, following a 24 hour survival time, mapped the retrograde transport of TTC in the brain using immuno-peroxidase labeling of monoclonal antibody to TTC developed by Evinger and Erichsen (Brain Res. **380:** 383, 1986). Dense labeling was present in the lateral part of the facial nucleus ipsilateral to the injection site, which had previously been demonstrated with HRP to project directly to the vibrissal muscles (McGuinness, Neurosci. Abstr. **7:** 986, 1981). The most densely labeled neurons resulting from retrograde transneuronal transport were located throughout the reticular formation. There were clearly labeled cells bilaterally in the nucleus of the solitary tract and in some cases in the contralateral red nucleus. There also was consistent labeling of neurons in the deeper layers of the portion of the contralateral motor cortex corresponding to the vibrissal representation (Hall and Lindholm, Brain Res. **66:** 23, 1974). Since in rats, the motor cotex does not project directly to the facial nucleus, the TTC presumably passed through at least one intermediate neuron between the facial nucleus and the motor cortex. In the transneuronal retrogradely labeled neurons, the TTC-immuno-peroxidase reaction product was located in the cytoplasm of the cell body and proximal dendrites. Labeled dendrites could sometimes be traced for several hundred microns. The cell nucleus stood out as an unlabeled structure.

We thank Dr. Craig Evinger for providing the antibody to TTC. This research was supported by a Markey Foundation Grant in Developmental Biology (CIT).

274.13

MORPHOLOGICAL IDENTIFICATION OF DENDRITIC BUNDLES IN PRIMARY VISUAL AND MOTOR CORTEX OF GALAGO CRASSICAUDATUS, HEDGEHOD AND RAT. H.Ginzler and K.Fleischhauer*, Anatomical Institute, Univ. of Bonn, D 5300 Bonn and Neurological Hospital Köln-Merheim, D 5000 Köln 91, F.R.G.

Dendritic bundles formed by apical dendrites emerging from layer 5 large pyramidal cells were identified on Klüver stained 10µm paraffin sections in a prosimian primate, insectivor and rat.The apical dendrites of clustered neighbouring pyramidal cells converge and lay in close apposition and traverse the upper layers in form of tightly apposed bundles. In visual cortex these bundles reach the upper part of layer 2 without branching appearing as parallel vertical structures. In contrast dendritic bundles in motor cortex display extensiv and regular branching and form secondary bundles within layer 3 with apical dendrites from neighbouring primary bundles. These two bundling types with parallel unbranched bundles in primary visual cortex and regularly branched apical dendrites and secondary bundles in motor cortex are almost identical in all three examined species.

The functional significance of these different branching patterns may correlate with the difference in functional organization between visual and motor cortex: the branching pattern in motor cortex may allow integration over a larger area of cortex, whereas the close apposition of apical dendrites without branching may reflect the preoccupation of the visual system with high spatial resolution.

TRANSMITTERS UPTAKE, STORAGE, SECRETION AND METABOLISM II

275.1

ACUTE EFFECTS OF LITHIUM ON CATECHOLAMINES, SEROTONIN AND THEIR MAJOR METABOLITES IN DISCRETE BRAIN REGIONS. **T.A. Reader, E. Gottberg* and L. Grondin***. Département de physiologie, Université de Montréal, Montréal, Québec, H3C 3J7, Canada.

The acute effects of lithium on the central monoamine systems were investigated in the anterior cingulate (CIN), the primary visual (VIS) and the piriform-entorhinal (PiEn) cortices as well as in the hippocampus (HIP), the neostriatum (CPU; caudate-putamen) and the olfactory bulbs (OB). One hour after the administration of LiCl (2 and 10 mEq/kg; i.p.) the endogenous noradrenaline (NA) levels increased in the CIN and PiEn cortices, in the HIP and the CPU, while dopamine (DA) only increased in the CIN and PiEn regions. These changes in endogenous DA were accompanied by reductions in the metabolites DOPAC and HVA. The levels of HVA and DOPAC, but not 3-MT, were also found to be decreased in the CPU in spite of an unchanged DA content. The serotonin (5-HT) contents was increased in all regions, except in the OB, but with 5-HIAA levels which were either normal or decreased (CIN and VIS cortices). These results are compatible with a reduced turnover and/or with an inhibition of monoamineoxidase. Alternatively, the particular properties (synthesis regulation and release) of the terminal NA, DA and 5-HT fibers in the different regions could account for this differential action of lithium on steady state monoamine levels and turnover rates.

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275.2

RELEASABLE PROTEINS FROM THE BASAL GANGLIA OF THE RAT. A.M. Sierra-Honigmann,*B.Antón,*P.Leff†, M.Sordo,*N.Jiménez,*J.Calderón* and A. Bayón. Inst. Invest.Biomédicas,UNAM,04510 México,D.F.; Dept.Biol.Celular,CINVESTAV,IPN; and Inst. Mexicano Psiquiatría, S.S. México D.F.

The release of unidentified protein material during perfusion of the rat striatum has been documented (Bayón, et al.in:"In vivo Perfusion and Release of Neuroactive Substances", Acad. Press, 1985). The release of this material is stimulated by tissue depolarization and is, in part, calcium-dependent suggesting that it could be stored in and secreted from synaptic vesicles. Preliminary analysis show that this releasable protein material is very heterogeneous and the individual components are collected in too small amounts to facilitate traditional purification strategies. In a first approach we immunized mice with the soluble contents of enriched preparations of striatal synaptic vesicles -and more recently with material obtained from perfusates. We have produced monoclonal antibodies; several of these antibodies recognize components present both in synaptic vesicles and perfusates from rat striatum and are used to identify the releasable proteins.

275.3

ALTERATION OF THE STRIATAL DOPAMINERGIC TRANSPORT SYSTEM BY ASCORBIC ACID. E.A. Debler, A. Hashim*, A. Lajtha, and H. Sershen*. Center for Neurochemistry, N.S. Kline Inst. Ward's Island, NY, NY 10035.

Previously we demonstrated that 0.1 mM ascorbic acid (AA) reduced the uptake of 1-methyl-4-phenylpyridine (MPP^+) into striatal synaptosomes in a noncompetitive fashion, but did not alter the transport of dopamine (DA), a phenomenon unique to AA and not due to lipid peroxidation (J. Neurosci. Res. 17:298, 1987; Soc. Neurosci. Abstr. 13:1110, 1987). With the thought that this effect of AA may be related to its oxidative capacity, the effects of succinate and NADPH on DA and MPP^+ uptake were studied. Both compounds were ineffective (IC_{50} > 10 mM) in inhibiting either DA or MPP^+ transport. In an effort to localize the site of AA action, we evaluted its effects on the binding of mazindol to the DA transporter. AA increased the K_d (3.5-fold) of binding of [^{3}H]mazindol to the dopamine transporter, but reduced the K_i (a factor of 4 or more) of MPP^+ in inhibiting [^{3}H]mazindol binding. The effect of AA in increasing MPP^+ affinity to the DA transporter is in discord with the AA-induced inhibition of MPP^+ transport, and demonstrates that AA affects components in the transport system other than the recognition site for DA uptake blockers. Thus, AA-induced inhibition of MPP^+ transport continues to appear unique and probably relates to an action of AA on events occurring after the binding of MPP^+ to the carrier. (Support: The Council for Tobacco Res.-USA).

275.4

VERY FAST PHOSPHORYLATION AND DEPHOSPHORYLATION OF SEVERAL Mr < 20000 PROTEINS FROM PLASMA MEMBRANE FROM BOVINE CHROMAFFIN CELLS. W.De Potter*, J.Cosaert* and J.De Block*. (SPON: A. Dahlström).Dept. of Med., Lab. of Neuropharmacology, University of Antwerp(UIA) Antwerpen, B2610 Belguim.

Chromaffin cells secrete catecholamines by exocytosis. Phosphorylation, triggered by an increase in free Ca^{2+}-concentration has been postulated to play a role in this process.(Trifaro et al., J. Mol. Pharmacol., 7:52, 1971) We therefore studied phosphorylation and dephosphorylation of chromaffin cell plasma membrane proteins.

Plasma membranes were prepared according to Zinder et al.,(Zinder et al.,Cell Tiss. Res., 188:153, 1978).They were incubated with (γ32P)ATP for 15", 1' and 2' at 0°C, 22°C and 17°C as well as in varying incubation media.Proteins were separated by SDS polyacrylamide gelelectrophoresis and phosphorylated proteins were detected by autoradiography.Several proteins (Mr < 20000) were labeled within 15", even at 0°C.The proteins were labeled intensively and the bands disappeared upon further incubation at 37°C (within 2').Incubation with EDTA instead of EGTA at lower free Ca^{2+}-concentration showed only minor labeling.A partial characterization of these proteins revealed that none of these fast phosphorylated proteins are identical with myosin light chain.

These data suggest the presence in the chromaffin cell membrane of a Mg^{2+}-dependent protein kinase as well as a divalent cation dependent protein phosphatase.

275.5

ENDOGENOUS ADENOSINE REGULATES NEURONAL EXCITABILITY IN LOW CALCIUM MEDIUM. J.C. Fowler. Life Sciences Division, Los Alamos National Lab., Los Alamos, NM 87545.

Levels of neuromodulators, in contrast to those of classically defined neurotransmitters, need not be regulated by calcium dependent synaptic release mechanisms. It is not clear whether basal and/or evoked levels of the putative neuromodulator adenosine are necessarily dependent on the presence of calcium and functional synapses. In the hippocampal slice superfused with medium containing sufficient calcium to support synaptic transmission, adenosine is present in adequate concentration to exert a tonic inhibitory influence on neuronal excitability. The adenosine antagonist theophylline and adenosine deaminase, an enzyme that converts adenosine to the inactive inosine, increase the amplitude of the extracellularly recorded synaptic response in the brain slice presumably by reducing the tonic inhibitory influence of endogenously released adenosine (Dunwiddie and Hoffer, Br. J. Pharmac. 69:59,1980). Experiments were designed to determine if endogenous adenosine could be demonstrated in the hippocampal slice superfused in a modified low Ca^{++}(.2 mM), high Mg^{++} (4 mM) medium that blocks synaptic transmission. Standard extracellular stimulating and recording techniques were used.

At a flow rate of 2 ml/min, stimulation of the alvear fibers elicited an extracellularly recorded antidromic population spike in CA1 pyramidal neurons which was usually followed by 2-4 afterpotentials. Reductions in flow rate were associated with a marked depression of the afterpotentials with little change in the initial antidromic population spike. The depression of the afterpotential at the lower flow rates was largely reversed by theophylline (100 µM) and by adenosine deaminase (10 µg/ml). The selective depression of the afterpotential was mimicked by the application of the adenosine reuptake blocker, dipyridamole (100 µM) and this depression was reversed with theophylline. These results suggest that lowered flow rates favor accumulation of endogenous adenosine. Since synaptic transmission was blocked, it is concluded that sufficient endogenous adenosine exists in the absence of synaptic function to alter neuronal excitability.

275.6

BIOSYNTHESIS AND RELEASE OF N-ACETYLASPARTYL-GLUTAMATE FROM AMPHIBIAN RETINAL NEURONS. L.C. Williamson and J.H. Neale. Dept. of Biology Georgetown University, Washington, D.C. 20057.

N-Acetylaspartylglutamate (NAAG) is a nervous system specific dipeptide which we have localized in the visual system neurons in several species. In a previous study, we identified NAAG-IR within vesicles in synaptic endings of amacrine and bipolar neurons in the inner plexiform layer of the amphibian retina as well as in ribbon synapses of photoreceptor cells.

In the present study, we have examined the biosynthesis and release of NAAG from these retinal neurons. Grass frogs were injected intraocularly with ^{3}H-glutamate. Retinas were removed and incubated in a series of physiological buffers with varying amounts of calcium and potassium. The radiolabeled molecules released by these retinas under control and depolarized conditions were purified by cation exchange chromatography followed by anion exchange HPLC. ^{3}H-NAAG was found to be released by the retinal cells upon depolarization with elevated potassium and this release process required extracellular calcium. These results support the hypothesis that the neuropeptide, NAAG, functions in synaptic communication.

275.7

LONG-TERM INHIBITION OF THE DOPAMINE UPTAKE COMPLEX BY METAPHIT. I. Zimányi*, A.E. Jacobson*†, K.C. Rice†, A. Lajtha, and M.E.A. Reith (SPON: I. Wajda). Center for Neurochemistry, N.S. Kline Institute for Psychiatric Research, Ward's Island New York, NY 10035, †NIH, NIDDKD, Bethesda, MD 20892

Metaphit (MET) an isothiocyanate derivative of PCP was shown to acylate PCP receptors and irreversibly inhibit the binding of labeled ligands to the dopamine uptake complex. The present study examines the effect of MET on [^{3}H]mazindol binding in mouse striatum in vitro and ex vivo. MET inhibited the [^{3}H]mazindol binding with an IC_{50} of 6.4 uM. The effect of 10 uM MET was still present after removal of MET by washing and centrifugation. A 15-min exposure to 10 uM MET was sufficient to inactivate all susceptible sites. The long-term nature of the effect of MET is suggested by the findings that at least in 3 hr MET did not dissociate significantly from its binding site. In equilibrium and dissociation experiments 10 uM MET significantly decreased the B_{max}, whereas the K_d and the dissociation rate constant remained essentially unchanged. The presence of cocaine protected the mazindol binding site from the action of MET. The potency of MET was greater under slightly alkaline conditions consonant with reaction between isothiocyanate and amino or sulfhydryl groups. In ex vivo experiments 5 hr after i.c.v. administration of MET, no changes were observed in the mazindol binding. MET irreversibly inhibits the the mazindol site in vitro by chemical modification. Grant NIDA DA 03025, and NYS MH

275.8

CHARACTERISATION OF A PROTON-ATPASE ACTIVITY IN RAT BRAIN SYNAPTIC VESICLES:ENERGETIC COUPLING WITH L-GLUTAMATE TRANSPORT. T. S. Sihra and S. Cidon (SPON: J. K. T. Wang). Lab. of Molec. and Cell. Neurosci., The Rockefeller University, New York, NY 10021-6399.

The ATP-dependent proton-pumping and L-glutamate transport activity of a highly purified rat brain synaptic vesicle preparation was characterised. Proton uptake was sensitive to low concentrations of NEM (IC_{50}=10uM) and NBD-Cl (IC_{50}=30nM). However, neither vanadate nor azide had any effect on the proton-pumping. Subcellular fractions obtained during the purification of synaptic vesicles were immunoassayed for their cross-reactivity with antibodies raised against the 115kDa, 72kDa and 39kDa subunits of the purified chromaffin granule proton-ATPase. Cross-reactivity increased in fractions progressively enriched in synaptic vesicles. These data suggest that the synaptic vesicle proton-ATPase belongs to the vacuolar class of ATPases rather than either the E_1/E_2 or F_1/F_0 groups. Acidification of the synaptic vesicle interior was enhanced in the presence of either permeant anions or valinomycin; conditions expected to cause the dissipation of an inside-positive membrane potential. In the absence of a pH-gradient, synaptic vesicles are therefore seen to maintain a trans-membrane potential. Importantly, this potential is seen to provide the energetic coupling of the ATP-dependent transport of L-glutamate into synaptic vesicles since uptake was greatly enhanced in the presence of impermeant anions but compromised with valinomycin. In conclusion, the results presented show the proton-ATPase found in synaptic vesicles is a vacuolar-type of ATPase. This proton-ATPase energises the ATP-dependent uptake of L-glutamate largely by virtue of the membrane potential component of the proton electrochemical gradient generated, in the absence of a significant pH-gradient.

275.9

MULTIPLE ADENOSINE TRANSPORT SYSTEMS IN RAT AND GUINEA PIG SYNAPTOSOMES. W. J. Baldy* and R. P. Shank. Dept. of Biological Research, Janssen Research Foundation, Spring House, PA 19477-0776.

We have used initial uptake conditions to study adenosine transport by synaptosomes (P_2 fraction depleted of mitochondria) from discrete regions of the rat and guinea pig brain. Based on transport kinetics and pharmacological criteria our data indicate the existence of 3 to 5 systems. In the rat two pharmacologically distinct [nitrobenzylthioinosine (NBI) sensitive and insensitive] systems exist which have apparent K_m values between 1 and 10 µM, and a third system has an apparent K_m of ∿0.4 µM. A low-affinity system with a K_m >100 µM probably exists but can not be characterized with our experimental conditions because the rate of uptake becomes non-linear within 30 s when adenosine is 100 µM or greater. Our kinetics data suggest the existence of a site with an affinity constant of ∿1 nM, which may represent binding to a receptor, rather than uptake by a carrier. Adenosine monophosphate (AMP) selectively inhibits two systems, the NBI sensitive one and the one with the K_m of ∿0.4 µM. A major difference between rat and guinea pig is that the NBI sensitive system is much more prevalent in the latter. In both rat and guinea pig, the NBI sensitive system is less prevalent in cerebellum than in cerebral cortex and diencephalon plus mesencephalon.

275.10

STIMULATION OF NOREPINEPHRINE UPTAKE BY ATPγS IN PC12 CELLS. J.E. Chaffee, Y.H. Ehrlich, and E.D. Hendley. Depts. of Physiology & Biophysics, Psychiatry, and Biochemistry, Univ. of Vermont, Burlington, VT 05405.

The potential modulation of norepinephrine (NE) uptake by extracellular ATP and divalent cations was examined in PC12 cells. An analog of ATP, ATPγS, was chosen for these studies since ATPγS can be utilized by protein kinases and the resulting thiophosphate bonds are resistant to phosphatase activity. Previous work in our laboratory has shown that both ATP and ATPγS (0.1 µM) can stimulate NE uptake. In this study, cells were incubated for 5 min at 37°C, in a Krebs-Ringer buffer conatining 1.87 mM Ca^{2+} and no added Mg^{2+}, with and without 1 µM ATPγS. The cells were then washed and NE uptake assayed 30 min later. ATPγS produced a persistent stimulation of uptake and this effect was dependent on the presence of Ca^{2+} in the pretreatment medium. Preincubation with ATP produced no increase in uptake, suggesting that there was an inactivation by dephosphorylation. Pretreatment with ATPγS and an excess of ATP resulted in the inhibition of the ATPγS-induced stimulation. The ATPγS pretreatment protocol was used to examine kinetic changes and the results showed an increase in the Vmax, with no change in the apparent Km for NE. Experiments are currently in progress to determine if inhibitors of NE uptake such as antidepressants, α-blockers, or alkylating agents, can also inhibit extracellular protein phosphorylation in PC12 cells. (Supported by NSF: R11-860679 and USAFOSR: 88-004).

275.11

HIGH AFFINITY GLUTAMINE UPTAKE IN RAT BRAIN PLASMA MEMBRANE VESICLES. R.J. Roon*, S.A. Shofner* and J.F. Koerner. Department of Biochemistry and Neuroscience Graduate Program, University of Minnesota, Minneapolis, MN 55455

Plasma membrane vesicles prepared from rat brain contain a saturable, high affinity uptake system for L-glutamine which exhibits the following characteristics: 1) The rate of L-glutamine uptake is linear up to 200 μg/ml of membrane protein. 2) The uptake rate is optimal in the range of pH 7.0 - 8.0. 3) The system exhibits a K_m for L-glutamine of ~1.7 μM and a V_{max} of ~50 pmol/min·mg protein. 4) Uptake of ^{3}H-L-glutamine is significantly reduced by lowering the assay temperature to 4^{0}C and is essentially abolished by the addition of excess unlabeled L-glutamine. 5) The system is not highly dependent upon the addition of monovalent or divalent cations. 6) Inhibitor studies reveal that the amino acid amides exhibit the highest affinity for the system and that there is a high specificity for the L-isomers. 7) Under our standard assay conditions L-glutamine uptake is significantly higher than L-glutamate uptake. Furthermore L-glutamate uptake gives a different inhibition profile than L-glutamine uptake. 8) Although no exogenous energy sources are required L-glutamine uptake activity is somewhat sensitive to cyanide and 2,4-dinitrophenol. (Supported by NIH NS 17944.)

275.12

RELEASE OF CATECHOLAMINES, [MET]ENKEPHALIN, AND NEUROPEPTIDE Y FROM EX SITU PERFUSED ADRENALS DURING HYPOGLYCEMIA. M. K. Dousa*, S. L. Chritton*, D. L. Lucas*, D. R. Roddy*, T. L. Yaksh*, and G. M. Tyce (SPON: C. L. Kornblith). Depts. of Physiology and Neurosurgery, Mayo Clinic, Rochester, MN 55905.

Hypoglycemia is a strong stimulus for adrenomedullary secretion. The present study is focused on nonneurogenic component of adrenal release of free catecholamines (CAs), [Met]enkephalin-like immunoreactive material([Met]enk), and neuropeptide Y-like immunoreactive material (NPY) under normoglycemic and hypoglycemic (20 mg % and 50 mg % glucose) conditions using ex situ isolated perfused dog adrenals. In perfusate samples taken before, during, and after 2-min stimulation with 3x10^{-5}M carbamylcholine (carbachol), norepinephrine (NE), epinephrine (E), and dopamine (DA) were determined by HPLC with electrochemical detection, and [Met]enk and NPY, by specific radioimmunoassays. Perfusion with 50 mg % glucose in the medium did not significantly change adrenomedullary secretion. At 20 mg % glucose levels, the basal effluxes of NE and DA were significantly increased, that of NPY was decreased, and no efflux changes were noted for E and [Met]enk. Evoked releases of CAs, [Met]enk, and NPY at both hypoglycemic levels were comparable to the releases in controls. These results indicate that adrenomedullary basal efflux is affected by severe hypoglycemia, whereas, carbachol-evoked secretion remains remarkably stable.

275.13

SIDEDNESS, CHEMICAL AND KINETIC PROPERTIES OF THE VESAMICOL (AH5183) RECEPTOR OF CHOLINERGIC SYNAPTIC VESICLES. W.D. Kornreich* and S.M. Parsons (SPON: K. Noremberg). Dept. of Chemistry and the Neurosciences Research Program, IES, University of California, Santa Barbara, CA 93106.

Binding of [^{3}H]vesamicol ((-)-trans-2-(4-phenylpiperidino)cyclohexanol) to its receptor in Torpedo synaptic vesicles is inhibited by the protein modification reagents 4-chloromercuriphenylsulfonate and N,N'-dicyclohexylcarbodiimide and by protease treatment of cholate solubilized receptor. Binding depends on deprotonation of a group of pK_a 6.26 ± 0.03 in the receptor. A membrane-impermeant vesamicol analogue was found to be an effective inhibitor of acetylcholine active transport with an IC_{50} value of 51 ± 8 x 10^{-9} M. Vesamicol bound the receptor with a rate constant of 1.74 ± 0.06 x 10^5 M^{-1} sec^{-1}, and unlabeled vesamicol displaced bound [^{3}H]vesamicol at 0.29 ± 0.01 min^{-1}. The dissociation process under some conditions exhibited fast and slow phases. The vesamicol receptor is a stable kinetically heterogenous protein facing the cytoplasmic compartment. It probably contains a binding site carboxylate in a hydrophobic environment and a cytoplasmically oriented sulfhydryl group.

275.14

CHOLINERGIC SYNAPTIC VESICLE HETEROGENEITY: EVIDENCE FOR REGULATION OF ACETYLCHOLINE TRANSPORT. L.M. Gracz*, W. Wang*, and S.M. Parsons (SPON: S.K. Fisher). Dept. of Chemistry and the Neurosciences Research Program, IES, University of California, Santa Barbara, CA 93106.

Classical, fully loaded VP_1 and recycling VP_2 synaptic vesicles from the electric organ of Torpedo californica were separated in an isosmotic sucrose density gradient.

The properties of the vesamicol receptor and acetylcholine (ACh) active transport system in the two vesicle types were studied. Based on a vesicle concentration standardization obtained with the SV2 antigen, characteristic of secretory granules, a typical preparation of VP_1 and VP_2 vesicles contained 237±15 and 102±3 pmol vesamicol receptor/mg of vesicle protein, respectively. The Hill coefficients, α_H, and the equilibrium dissociation constants, K, for vesamicol binding to VP_1 and VP_2 vesicles were essentially the same with α_H = 2.0 and K = 19 nM. The positive Hill coefficient suggests that the vesamicol receptor exists as a homotropic oligomeric complex. Furthermore, VP_2 vesicles transported ACh 2- to 3-fold more actively than VP_1 vesicles. The results demonstrate that VP_1 and VP_2 synaptic vesicles exhibit functional differences in the vesamicol receptor -ACh transport system, presumably as a result of regulatory phenomena. Structural differences between the two vesicle populations are currently being studied.

275.15

AN ACETYLCHOLINE BINDING SITE COPURIFIES WITH THE VESAMICOL (AH5183) RECEPTOR FROM CHOLINERGIC SYNAPTIC VESICLES. Ben A. Bahr and Stanley M. Parsons. Dept. of Chemistry and the Neuroscience Research Program (IES), University of California, Santa Barbara, California 93106.

Uptake and storage of acetylcholine (ACh) by purified Torpedo VP_1 synaptic vesicles (SV) is blocked by the potent drug l-trans-2-(4-phenylpiperidino)cyclohexanol (vesamicol, formerly AH5183). A saturable receptor is present on SVs that binds 300-400 pmoles of [^{3}H]vesamicol/mg SV protein. This receptor (VM-R) is present at 6-8 binding sites/SV and can be solubilized in cholic acid. Exogenous ACh has no effect on [^{3}H]vesamicol binding to intact vesicles but inhibits the binding to hyposmotically-shocked (lysed-SV) or solubilized (sol-SV) vesicles with an IC_{50} of 2 to 40mM, depending on the SV preparation. Increasing [^{3}H]vesamicol concentrations cause decreasing ACh effects, suggesting ACh uses a noncompetitive mechanism.

The VM-R was purified using a succession of chromatographic steps - hydroxylapatite, agarose-wheat germ lectin, DEAE ion-exchange, and size exclusion. The result was a 40-fold increase in binding specific activity and a final yield of 14%. The apparent particle M_r of the native detergent-solubilized VM-R was 230kDa, consisting of 107 and 96kDa subunits. After purification, ACh inhibited the binding activity of the VM-R with an IC_{50} of 37 ± 9mM. Therefore, an allosteric ACh binding site is present on the VM-R.

275.16

STIMULATION OF RIBOSOMAL S6 PROTEIN KINASE ACTIVITY IN PC12 PHEOCHROMOCYTOMA CELLS. A.L. Cahill and R.L. Perlman. Depts. of Peds. and Pharmacol. and Physiol. Sci. and Kennedy Mental Retard. Res. Cent., University of Chicago, Chicago, IL 60637.

Nerve growth factor (NGF), bradykinin (BK), high K^+ and phorbol dibutyrate (PDBu) increase the phosphorylation of a common tryptic peptide in tyrosine hydroxylase in PC12 cells. Because NGF, BK and high K^+ stimulate tyrosine hydroxylase phosphorylation in cells that have been depleted of protein kinase C, these agonists all appear to activate a novel protein kinase in the cells. In this study we have investigated whether this novel kinase might actually be a ribosomal S6 protein kinase. S6 kinase activity was assayed in the 100,000 x g supernatant of PC12 cells, using γ^{32}P-ATP and 40S ribosomes from rat liver as substrates. ^{32}P-labeled S6 was isolated by SDS-PAGE and ^{32}P incorporation was quantitated by Cerenkov counting. Treatment of PC12 cells with NGF, BK or high K^+ increased the activity of the S6 protein kinase. This S6 kinase in PC12 cells appears to be similar or identical to the NGF-activated protein kinase N described in PC12 cells (Rowland et al., J. Biol. Chem. 262, 7504-7513, 1987) and to the growth factor-activated S6 kinase that has been described in other cell types. This kinase may mediate the effects of NGF, BK and high K^+ on tyrosine hydroxylase phosphorylation. (Supported by NIH grants HD04583 and HL29025).

275.17

ADENOSINE UPTAKE AND METABOLISM IN MOUSE ASTROCYTES AND NEURONS IN PRIMARY CULTURES. Henry Matz and Leif Hertz, Dept. of Pharmacology, Univ. of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0, Canada (SPON: R.W. Ledeen).

We have previously reported (Trans. Am. Soc. Neurochem. 19, 90, 1987) pronounced differences in adenosine metabolism between cerebral cortical astrocytes (mainly phosphorylation) and cerebral cortical neurons (mainly deamination). In both cell types the content of adenosine itself remains remarkably low, i.e., not significantly different from the adenosine concentration in the medium (10 µM). However, in the presence of the deaminase inhibitor, deoxycoformycin, it increases 15-25-fold. The very low adenosine content under control conditions might suggest that the uptake mechanism for adenosine consists of facilitated diffusion followed by a very rapid metabolism, maintaining a low adenosine concentration. In order to test this hypothesis total uptake of radioactivity from [^{14}C]adenosine in the cells (i.e., adenosine plus all retained metabolites), was measured both under control conditions and in the presence of 10 µM deoxycoformycin. No effect was observed in astrocytes, which could be explained by the relatively low deamination rate under control conditions. However, even in neurons, where deamination is the major metabolic pathway, the total uptake was unaffected by the inhibitor. In both astrocytes and neurons large amounts of labelled inosine were formed in the medium already after "zero time" incubation. This was inhibited by deoxycoformycin.

275.18

EVIDENCE FOR A SYNAPTOSOMAL CYTOSOLIC FACTOR MODULATING VESICULAR GLUTAMATE UPTAKE. A.T. Lobur*, P.E. Kish* and T. Ueda (SPON: R. Richardson) Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Evidence accumulated over the past 26 years has led to the wide acceptance of glutamate as a major excitatory neurotransmitter in the mammalian brain. We have previously demonstrated that L-glutamate is taken up into isolated synaptic vesicles in an ATP-dependent manner. We now report that a nerve terminal cytosolic factor inhibits the ATP-dependent vesicular uptake of glutamate in a dose dependent manner. This factor appears to be a protein with a molecular weight greater than 100,000 as estimated by size exclusion chromatography, and is precipitated by ammonium sulfate (40% saturation). The inhibitory factor is inactivated by heating at 100°C. Proteolytic digestion of the ammonium sulfate fraction by trypsin and chymotrypsin did not reduce, but increased slightly the inhibition of glutamate uptake. The digest retained inhibitory activity after heating, suggesting that the proteolytic digestion may generate active fragments. The inhibition of ATP-dependent vesicular glutamate uptake is not species specific, as the factor obtained from both rat and bovine brains produced an equal degree of inhibition of glutamate uptake into vesicles from both species. The mechanism of action is not presently known. These observations suggest that vesicular uptake of glutamate may be regulated by endogenous factors *in vivo*. (Supported by NSF Grant BNS 8509679.)

275.19

A KINETIC APPROACH TOWARDS UNDERSTANDING THE LONG DURATION OF ACTION OF RESERPINE **J.D. Deupree, Department of Pharmacology, University of Nebraska Medical Center, Omaha NE 68105**

Classically the duration of action of reserpine has been known to exceed its biological half life. Reserpine acts by competitively inhibiting catecholamine transport into chromaffin granules by binding to the catecholamine transporter with an apparent Kd of 3 nM. An estimate of the rates of association (k_1) and dissociation (k_2) was obtained by analysis of specific ^{3}H-reserpine (0.4 to 3.6 nM) binding with time. The data indicated steady state binding conditions are reached in 20 min with a k_1 of 0.12 $nM^{-1}min^{-1}$ and a $t_{1/2}$ for dissociation of 9 min. However direct analysis of the rate of dissociation following the addition of 1 µM reserpine or 1 mM norepinephrine indicated that over 80% of the ^{3}H-reserpine remained bound for over 8 hours. Reserpine does not appear to be covalently bound. It is probably not being transported into the inside of the granule since reserpine is not released if the granules are made permeable with saponin. I propose that the transporter starts to transport reserpine to the inside of the granule. However, the fatty acid portion of the membrane has a high attraction for reserpine preventing both complete transport and dissociation and rendering the transporter inaccessible to catecholamines. **(Supported by NIH grant #NS 15187)**

275.20

CARBACHOL-INDUCED COSECRETION OF IMMUNOREACTIVE ATRIAL NATRIURETIC PEPTIDES WITH CATECHOLAMINES FROM CULTURED BOVINE ADRENAL MEDULLARY CELLS. M. Okazaki*[1], N. Yanagihara and F. Izumi. Dept. of Pharmacol. and [1]2nd Dept. of Int. Med. Univ. of Occupational and Environmental Health, Sch. of Med. Kitakyushu 807, Japan.

The distribution and secretion of atrial natriuretic peptides (ANPs) were investigated in bovine adrenal medulla. (1)Stimulation of nicotinic but not muscarinic acetylcholine receptor caused the secretion of α-human ANP-like immunoreactivity (α-hANP-LI) and catecholamines corresponding to the ratio of catecholamine/α-hANP-LI in cultured bovine adrenal medullary cells. (2)Secretion of α-hANP-LI by carbachol was dependent on extracellular Ca^{2+}. (3)Chromaffin granules isolated from bovine adrenal medulla contained large amounts of ANP and catecholamines with the same ratio as in cultured cells. (4)Separation on reverse phase HPLC showed two components of adrenal ANP, α-ANP and γ-ANP. These results indicate that ANPs are stored as α and γ forms in chromaffin granules, and cosecreted with catecholamines in a Ca^{2+}-dependent manner by stimulation of nicotinic acetylcholine receptor.

275.21

SODIUM / PROTON EXCHANGE SYSTEM IN CULTURED BOVINE ADRENAL MEDULLARY CELLS. N. Yanagihara, K. Yokota*, H. Kobayashi*, A. Wada* and F. Izumi. Depts. of Pharmacology and Ophthalmology, Univ. of Occupational & Environmental Health, School of Medicine, Kitakyushu 807, Fukuoka, Japan.

When cultured adrenal medullary cells were incubated in Na^+-free sucrose medium, the intracellular pH was shifted down from 7.14 to 6.56. Readdition of Na^+ to the cells caused a rapid increase in intracellular pH to 7.30. Associated with the increase in intracellular pH, $^{22}Na^+$ uptake by the cells was observed. Both the increases in intracellular pH and $^{22}Na^+$ uptake were inhibited by amiloride, an inhibitor of Na^+/H^+ exchange, in a similar concentration-dependent manner. The increase in intracellular pH by addition of Na^+ was partially inhibited by quinidine, other inhibitor of Na^+/H^+ exchange, but not by 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid, an anion exchange (Cl^-/HCO_3^-) inhibitor. Li^+ could substitute for Na^+ in this system.

These results suggest the existence of amiloride-sensitive Na^+/H^+ exchange system in cultured bovine adrenal medullary cells which may be involved in the regulation of intracellular pH.

276.1

NERVE GROWTH FACTOR (NGF) STIMULATES THE RELEASE OF ARACHIDONIC ACID (AA) FROM PC12 CELLS. D.W. Fink, Jr.,* M.L. Contreras and G. Guroff. Section on Growth Factors, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892.

Increased hydrolysis of phosphatidyl inositol (PI) is rapidly observed following treatment of PC12 cells with NGF, resulting in the formation of inositol triphosphate (IP_3) and diacylglycerol (DAG) (Contreras and Guroff, J. Neurochem. 48:1466, 1987). Involvement of IP_3 in calcium mobilization and second messenger signalling is well established, whereas DAG function remains to be clarified; it may provide a mechanism for coupling NGF-stimulated PI hydrolysis with arachidonic acid (AA) metabolism through DAG lipase-mediated formation of arachidonic acid. A second messenger role for arachidonic acid and its metabolites has been proposed. The effect of NGF on AA metabolism has been examined in PC12 cells. Addition of NGF (100 ng/ml) to PC12 cultures labelled with (^{3}H)-AA caused a 53.9 ± 6.4% increase in the release of labelled AA during a 30 minute incubation at 37°C. Both epidermal growth factor (50 ng/ml) and bradykinin (1 μM) enhanced AA release to a similar extent. NGF-stimulated AA release was associated with a 113.1 ± 7.6% increase in inositol monophosphate formation suggesting interaction between PI-hydrolytic and AA-metabolic pathways. Studies are being conducted to characterize the calcium requirement of NGF-stimulated AA release, and to determine whether antagonists of AA metabolic pathways will modulate the PC12 response to NGF.

276.2

REGULATION OF NERVE GROWTH FACTOR SYNTHESIS IN VARIOUS GLIAL CELL TYPES. J.P.Schwartz and K. Mishler*. CNB, NINCDS, NIH, Bethesda,MD 20892

Although many cell types synthesize nerve growth factor(NGF), glial cells appear to be a primary source at certain periods during development and regeneration in the PNS. We have asked two questions: 1) do CNS glial cells also synthesize NGF, thereby functioning as a source of the NGF found in the brain?; 2) what agents can regulate NGF synthesis in different glial cell types? Primary cultures of CNS astrocytes prepared from newborn rat cortex synthesize and secrete NGF, with an NGF mRNA content 7-8 fold higher than in the rat C6 glioma cell line. In both cell types, forskolin (which elevates cyclic AMP) stimulates synthesis of NGF mRNA, with a maximal increase by 2-3 hr. The β-adrenergic agonist isoproterenol(IP) can also increase NGF mRNA content in both astrocytes and C6 cells, with a time course comparable to that seen for forskolin. Since IP is known to stimulate adenylate cyclase(AC) in C6 cells, these results suggest that astrocytes contain a β-adrenergic receptor which activates AC. VIP increases the NGF mRNA content of astrocytes: whether this increase occurs as a result of increased cAMP is under investigation. No comparable effect is seen in C6 cells, suggesting the lack of a VIP receptor. These results demonstrate that the NGF gene can be regulated through a cAMP promoter element, which may provide a mechanism by which to turn on NGF synthesis in specific tissues or brain regions.

276.3

IN SITU HYBRIDIZATION OF NGF mRNA IN ADULT RAT BRAIN. R. C. Hayes, M.B. Rosenberg, G. A. Higgins, K. S. Chen, F. H. Gage, and D. M. Armstrong. Dept. of Neurosciences, University of California, San Diego, CA 92093.

Several lines of recent evidence would indicate that basal forebrain cholinergic neurons are dependent on Nerve Growth Factor (NGF) for their daily maintenance and survival. Moreover, NGF deficiencies are hypothesized to account for the dysfunctioning of cholinergic neurons in Alzheimer's disease and normal aging.

In the present study we employed in situ hybridization using ^{35}S-labeled riboprobes to localize NGF mRNA in the forebrain of Sprague-Dawley and Fischer 344 rats. In all animals the message was localized in the granule cell layer of the dentate gyrus, the pyramidal cell layer of the hippocampus, and layer II of the entorhinal cortex. Quantitation of differences in NGF and mRNA levels between behaviorally characterized young (8 months) and aged (91-24 months) animals is presently being determined.

Supported by NIH grant AG05344.

276.4

DEVELOPMENTAL REGULATION OF NGF RECEPTOR EXPRESSION IN FIBROBLASTS. S.L. Patterson*, S.J. Thompson*, G.C. Schatteman*, R Underwood*, M. Bothwell, K.A. Holbrook*, and A. Gown* (SPON: J.B. Simpson). Depts. of Physiology and Biophysics, and Biological Structure, Medicine (Dermatology), Univ. of Washington, Seattle, WA 98195.

Using immunocytochemical methods, we have investigated the role of NGF receptor in normal embryonic development, and during remodeling and repair in more mature tissues. NGF receptors were localized in human, monkey and rat tissues using monoclonal antibodies directed against human NGF receptor (Marano et al., J. Neurochem. 48: 225-232 1987) and against rat NGF receptor (Chandler et al, J. Biol. Chem. 259: 6882-6889 1984).

In addition to expected sites of expression in the nervous system, extensive immunoreactivity was detected on fibroblasts adjacent to developing epithelium, bone, muscle and vasculature. Immunoreactivity appeared to peak during periods of morphogenesis; it decreased in maturing tissues, and in normal adults, was restricted to some perineurial and perivascular cells.

Preliminary results suggest that the NGF receptor may be reexpressed in adult fibroblasts under some conditions. Immunoreactivity has been detected in stromal tissues associated with certain types of neoplasms.

It appears that fibroblastic NGF receptor expression is regulated throughout life, and that under some circumstances, adult mesenchyme may, to some extent, recapitulate developmental patterns of receptor expression.

276.5

DEDUCED AMINO ACID SEQUENCE OF THE CHICKEN NGF RECEPTOR cDNA

M. Bothwell, J. G. Heuer*, and S. Fatemi-Nainie* . Dept. of Physiology & Biophysics, University of Washington, Seattle, WA 98195.

A cDNA library representing mRNA of E10 chick head was screened using a fragment of a human NGF receptor cDNA as a probe. Two hybridizing clones were obtained containing inserts of approximately 4 kb. These were subcloned into M13mp18 for sequence analysis by the dideoxy chain termination method. Extensive sequence identity between these clones and human and rat NGF receptor indicates that these clones represent the chicken NGF receptor. Comparison of the deduced amino acid sequences of chicken with human and rat reveals that some regions are relatively poorly conserved, while others are more strongly conserved. Like human and rat NGF receptors, the extracellular domain of the chicken NGF recepor contains a 4-fold repeat of a cysteine-rich sequence motif. One highly conserved region falls within this cysteine-rich segment. We postulate that this conserved region comprises the NGF binding site. A second highly conserved region includes the membrane-spanning segment and residues at the extracellular and intracellular borders of the membrane spanning segment. We postulate that this region may be involved in lateral association in the plasma membrane with a second uncharacterized intrinsic membrane protein involved in NGF receptor signal transduction.

276.6

THE ESTABLISHMENT AND USE OF STABLE CELL LINES THAT OVEREXPRESS A TRANSFECTED β–NERVE GROWTH FACTOR (NGF) GENE: STUDIES IN VITRO, IN OCULO, AND INTRACRANIALLY. L. Olson, P. Ernfors*, T. Ebendal*, P. Mouton, I. Strömberg, H. Persson†. Dept. of Histology & Neurobiology, Karolinska Institute, S-104 01 Stockholm; *Dept. of Developmental Biology, Uppsala Univ., S-751 22 Uppsala; Dept. of Molecular Neurobiology, Karolinska Institute, S-104 01 Stockholm, Sweden.

The rat NGF gene was cloned in a mammalian expression vector under the control of the rabbit β-globin promoter containing a four tandem repeat of an 18-bp sequence which confers an inducibility to heavy metals. This contruct was cotransfected into Balb/c 3T3 cells with a plasmid containing a neomycin gene and G418-resistant cells were isolated. Conditioned medium from G418-resistant cells was then assayed for the presence of NGF in a bioassay using chick sympathetic ganglia and using ELISA for NGF. Genomic digests followed by Southern blotting of the cells producing the highest levels of NGF (3T3/3E) showed the presence of several hundred copies of the rat NGF gene per cell. RNA-blot analysis showed rat NGF hybridizing mRNA of the predicted size at levels approximately 50 times higher than the level of endogenous NGF mRNA in 3T3 cells. Medium in which 3T3-3E cells had grown contained 5- to 10-fold elevated amounts of NGF. When 3T3-3E cells were injected into the anterior chamber of the eye of mice or immunocompromized rats, the cells expressed NGF immunoreativity and caused adrenergic hyperinnervation of the host irides as compared to control eyes given similar amounts of the parent 3T3 cells. Grafting studies now in progress will further evaluate the usefulness of these cells as a source of NGF that may stimulate injured or intact NGF neurons in the host brain and peripheral nervous system, and that may support cografted NGF-dependent neurons or chromaffin cells.

276.7

High Level Expression of Recombinant Mouse β-Nerve Growth Factor (NGF) in *E. coli* with a Gene Fusion Vector.
Y. Luo* and K. E. Neet. Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106.

Nerve Growth Factor is an important neurotrophic factor required for the development and maintenance of neurons in the sympathetic, sensory, and central nervous systems. We previously reported that biologically active, recombinant NGF has been produced in *E. coli* (G.-L. Hu and K. E. Neet Gene (1988) in press), albeit in small quantity. We now report the high level expression of recombinant NGF in *E. coli* using a gene fusion vector. The 373 base pair cDNA fragment (HaeII to Pst I) encoding mature mouse β-NGF was fused to the truncated protein A gene under the control of the thermoinducible lambda P_R promoter in the vector pRIT2T , using a synthetic oligonucleotide linker. The resulting plasmid, pRIT2NGF, was selected and confirmed by restriction mapping. The protein A-NGF fusion protein was highly expressed by temperature induction in *E. coli* (strain N4830-1) containing the plasmid pRIT2NGF . Purification of this protein over an IgG affinity column yielded about 4 mg/liter of nearly homogeneous fusion protein. The recombinant NGF was cleaved from the fusion protein by CNBr treatment and purified by cation exchange chromatography. The characterization of this recombinant NGF is in progress. (Supported by an NIH grant, NS24380)

276.8

Synthesis of chimeric mouse nerve growth factor precursor and human β-nerve growth factor in Escherichia Coli : Immunological properties.

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The complete mouse prepro-nerve growth factor (NGF) cDNA was fusioned to the carboxyl terminus of the β-galactosidase (lacz) gene of E.Coli in a plasmid vector that contains a transcription stop signal at the end of the lacz gene. Similarly, a genomic fragment encoding the human βNGF comprising codons 11 to 106 (from a total of 118) was fusioned to the amino terminus of β-galactosidase. Both bacterial vectors produce high amounts of the chimeric proteins.Purification of the fusion proteins from the soluble fraction was achieved by affinity chromatography. Their antigenic similarity to the preproNGF and mouse β NGF was examined by their interaction to sera raised against synthetic peptides which reproduce sequences of the precursor protein and to sera directed against native and denatured mouse NGF using ELISA techniques. The results provide direct evidence that antisera to mouse β NGF can crossreact with the human β NGF molecule.

276.9

INTRACELLULAR APPLICATION OF GTP INCREASES THE BINDING OF ^{125}I-NERVE GROWTH FACTOR TO PC12 CELLS.
T. Koike and S. Tanaka*, Department of Natural Science, Saga Medical School, Nabeshima, Saga 840-01, Japan.

We have previously shown that the binding of ^{125}I-NGF to PC12 cells is enhanced upon membrane depolarization, suggesting the dynamic nature of NGF receptors under physiological conditions (J. Neurochem. 49: 1784-1789, 1987). Here we show that the binding is also enhanced by pulse-mediated application of GTP into cytoplasm. ^{35}S-GTPγS, a non-hydrolyzable analogue of GTP, was maximally incorporated into the cells suspended in PBS, pH 7.2 without any significant decrease in cell viability when appropriate electrical pulses (15 times at 2000 V/cm) were delivered. We found that introduction of GTPγS (30 μM) into cytoplasm caused an increase (142± 12% of control) in ^{125}I-NGF binding (100 pM at 30C for 1h). This enhancement occurred in a dose-dependent manner. GTPγS was the most potent, and less effective compounds were GTP, Gp(NH)pp and GMP. However, other nucleotides such as GDP and ATP were ineffective. Thus, the enhancement observed in this assay system suggests a possible involvement of G-protein or other GTP-binding proteins such as microtubules.

276.10

ANTIBODIES DIRECTED AGAINST NGF RECEPTOR.
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We have generated antisera targeted to the Nerve Growth Factor (NGF) receptor by immunizing rabbits with synthetic peptides. These peptides were chosen from the sequence of the cloned rat NGF receptor and represent fragments of the extracellular (NGF-binding) domain. Some of these antisera are capable of inhibiting NGF-induced neurite outgrowth from rat PC12 pheochromocytoma cells. Some antisera are also capable of inhibiting the binding of NGF to PC12 cells. Inhibiting antisera identify multiple species on immunoblots, including species corresponding in size to the NGF receptors observed in crosslinking experiments. We believe these antisera bind NGF receptors and interfere with the association of the receptor and NGF. These antisera should prove to be useful probes for molecular studies of the NGF receptor.

276.11

NGF RECEPTOR INCREASE IN THE OLFACTORY BULB AFTER EARLY ODOR DEPRIVATION IN RATS.
F. Gómez-Pinilla, K. Guthrie, M. Leon and M. Nieto-Sampedro. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Sensory experience affects development of neuronal types in the CNS and selective competition for neurotrophic factors may regulate the survival of neurons under specific environmental pressure. NGF and its receptor (NGFR) are present in the brain and appear to play a major role in the development and maintenance of normal connectivity of cholinergic neurons. The olfactory system of the rat is an interesting model to study the neurotrophic role of the NGF/NGFR system because turnover of olfactory receptors continues throughout life, and the amount of NGF detected in the olfactory bulb is the third most abundant in the brain. Here we describe the response of NGFR in the bulb after olfactory deprivation. Experimental rats received unilateral sealing of the nostril at postnatal day 1, and NGFR density was measured in the olfactory bulb 20 or 60 days later. We used an affinity purified monoclonal antibody (IgG 192 kindly provided by Dr. E. Johnson,Jr.) which was visualized by ABC immunohistochemistry or iodine-125 autoradiography (autoradiograms were quantified by computer image analysis). In normal rats NGFR immunoreactivity was located mainly in the glomerular layer along the rostrocaudal extent of the bulb. In the odor deprived rats there was an increase of NGFR density in the OB ipsilateral to the nasal occlusion. The increase of NGFR density was most dramatic in the rostral one third of the bulb, especially in the 60 days post-lesion rats. These findings suggest that NGF plays a role in the development or survival of neuronal elements in the bulb after loss of sensory input. (Supported by Grant AG 00538-09A from the NIA).

276.12

β-NGF ENDOPEPTIDASE IS A MOUSE SUBMAXILLARY KALLIKREIN (MGK 22).
J.E. Woo,* M. Fahnestock, J. Snow,* D. Walz,* and W.C. Mobley (SPON: I. Diamond). Dept. of Neurology, University of California, San Francisco, CA 94143, and SRI International, Menlo Park, CA, 94542.

β-NGF Endopeptidase (β-EP) is a serine protease which cleaves the β-subunit of NGF at the his^8- met^9 bond to release an NH_2-terminal octapeptide. The finding that this peptide has biological activity prompted further characterization of β-EP.

β-EP was purified by the method of Wilson and Shooter (J. Biol. Chem. 254:6002, 1979). In agreement with earlier studies, we found that β-EP was a single chain molecule (Mr~25 kDa) with pI = 5.6. The sequence of the NH_2-terminus was determined thru thirty amino acids . The sequence was identical to that given for EGF binding protein type A, as reported by Anundi et al (Eur. J. Biochem. 129:365, 1982); the protein is encoded by a gene designated mGK-22 (Evans et al. J. Biol. Chem. 262:8027, 1987). Contrary to its prior designation as an EGF binding protein, we found no evidence for interaction of β-EP with EGF by either kinetic or chromatographic analyses. Interestingly, the NGF octapeptide behaved as a competitive inhibitor of the enzyme; greater than 90% inhibition of β-EP activity on an artificial substrate was produced by preincubating β-EP with the octapeptide. β-EP appears to be quite specific in its interaction with growth-related factors and may have a physiological role in releasing a biologically active fragment from NGF.

276.13

HYPERALGESIC PROPERTIES OF NGF-OCTAPEPTIDE IN THE HAIRY SKIN OF THE RAT. J.D. Levine*, Y.O. Taiwo*, J.E. Woo* & W.C. Mobley. (SPON: K. Thind) Division of Neurobiology, Univ. of Calif. SF, CA 94143.

Although no biological function of the octapeptide cleaved from the NH_2-terminal end of NGF (NGF-OP) has been described, it was of interest to study its effects on peripheral nociceptive mechanisms because it has sequence homology with bradykinin (BK), NGF has recently been implicated in inflammatory mechanisms and NGF-OP may be cleaved from NGF in inflammatory lesions.

Experiments were performed in 300 g male rats in which a local irritation was induced by cutaneous application of chloroform. Hyperalgesia (HA) was quantified using the Randall-Selitto test. Drugs were injected intradermally in a volume of 2.5 ul. NGF-OP induced a potent, dose-dependent, hyperalgesia at a dose range 1/1000th that of norepinephrine (NE). This effect of NGF-OP resembles the recently described hyperalgesic effects of NE (Nature323:158, 1986) in that it is also prevented by prior sympathectomy or treatment with non-steroidal antiinflammatory analgesics. It differs, however, from the HA induced by BK in that it fails to produce hyperalgesia in normal skin. These data point to a function for a peptide found in mature NGF chains.

276.14

NGF ENHANCES THE OUTGROWTH OF NEURITES FROM AXOTOMIZED SEPTAL-BASAL FOREBRAIN NEURONS IN VITRO. M. Schinstine & C.J. Cornbrooks. Dept. of Anat. & Neurobiol., Univ. of Vermont, Burlington, VT 05405.

An in vitro model of regeneration was developed to test the ability of NGF to facilitate the outgrowth of neurites from septal-basal forebrain (SBF) neurons. Primary SBF explant cultures, prepared from fetal (E15) rats, were maintained on a collagen substrate for 5 days in either a NGF-supplemented (+NGF) or control (-NGF) media. The rate of neurite outgrowth in these cultures was not effected by NGF. Axotomized SBF neurons, prepared by severing the neuritic fields of primary SBF explants, were maintained on a collagen substrate for an additional 5 days in either the same medium used in the primary cultures or the opposite test medium. Most geurites from axotomized SBF neurons demonstrated similar growth rates (~37% slower than the rate in primary cultures). In contrast, axotomized SBF neurons exposed to NGF both before and after axotomy demonstrated an ~40% increase in neurite outgrowth as compared to control cultures. These results demonstrate that NGF can enhance the outgrowth of neurites from axotomized SBF neurons, but only if the neurons have been exposed to NGF prior to and after axotomy. Supported by NS 21811.

276.15

PROTEASE NEXIN I IS A SUBSTRATE FOR β-NERVE GROWTH FACTOR ENDOPEPTIDASE. DE Rosenblatt[1], JE Woo*[2], WC Mobley[2]. 1. Div. on Aging. Harvard Med. Sch., Boston, MA 02115. 2. Dept. Neurol., U. of C., S.F., CA 94143.

Proteolytic activity is required for processing biologically active polypeptides. β-nerve growth factor endopeptidase (β-EP) is a mouse submaxillary gland kallikrein which cleaves β-NGF releasing a biologically active N-terminal octapeptide. β-EP has little protease activity but is active on mouse plasma kininogen,its only other known substrate. We report here that protease Nexin I, a serine protease inhibitor recently shown to be identical to Glial Derived Neurite Promoting Factor is also a substrate for β-EP.

Purified β-EP and PN I formed a complex of MW 66KD that was stable on reduced SDS-PAGE. Western blot analysis with anti-PNI antibody and autoradiography for ^{125}I β-EP proved that the complex contained both PN I and β-EP. Complex formation mediated the cellular binding of β-EP to glial cells. The binding was time and temperature dependent and inhibited by heparin.

The activity of β-EP against PN I is remarkable in view of its marked substrate specificity: the data reemphasizes the potential significance of nexin's complexation of growth factor related enzymes. Biological activity may reflect enzyme inhibition or bioactive peptide formation from nexin.

276.16

IDENTIFICATION OF b-FGF AS A CHOLINERGIC NEUROTROPHIC FACTOR IN EXTRACTS OF SKELETAL MUSCLE. J. L. McManaman,* F. G. Crawford,* J. O. Richker* and R. C. Clark* (SPON: L. J. Haverkamp). Wagner ALS Research Laboratory, Dept. of Neurology and Prog. in Neurosciences, Baylor College of Med., Houston, TX 77030

Extracts of skeletal muscle contain chromatographically distinct molecules which enhance the cholinergic development of cultured embryonic rat spinal cord neurons. We have recently purified a 20-22 kDa cationic polypeptide (CDF) from rat skeletal muscle extracts which stimulates the development of CAT activity in rat spinal cord cultures (McManaman et al, J. Biol. Chem., in press). We now show that muscle extract also contains basic Fibroblast Growth Factor (bFGF) and that bFGF also stimulates CAT development in rat spinal cord cultures. Basic FGF differs from CDF in its physicochemical, chromatographic and immunological properties and by its mitogenic action on non-neuronal cells. In addition, the effects of bFGF and CDF on CAT development are additive, suggesting that their actions are mediated by different cellular mechanisms. Our findings identify two distinct polypeptides with trophic factor activities in extracts of rat skeletal muscle. These findings support the hypothesis that the embryonic development of cholinergic neurons is influenced by multiple target-derived neurotrophic factors.

276.17

CULTURED VASCULAR SMOOTH MUSCLE CELLS PROVIDE NGF-DEPENDENT NEURONAL SURVIVAL. D. J. Creedon and J. B. Tuttle, Physiology and Neuroscience, Univ. of VA Sch. of Medicine, Charlottesvl., VA 22908.

Knowledge of the regulation of NGF synthesis by the synaptic partners of NGF-dependent neurons has been elusive. The present work addresses this issue using cultures of vascular smooth muscle cells (VSM), a normal target cell of sympathetic neurons.

Primary rat aortic VSM cells were passaged as they approached confluency and plated into 24-well microplates at 10^3-10^4/cm^2. Neurons dissociated from superior cervical ganglia (SCG) of neonatal rats were plated onto the VSM. After 48 to 72 hrs, the cultures were immunohistochemically labeled with a neuron-specific cytoskeletal marker and the remaining neurons counted. Survival on VSM was always substantial compared to a general failure to survive on a collagen or laminin substrate. NGF (100ng/ml) fostered survival equivalent to the VSM. Added to VSM co-cultures the NGF enhanced neuronal survival to a variable extent, suggesting that trophic production is regulated by the VSM. Survival in all conditions could be effectively blocked by the addition of an anti-NGF monoclonal antibody (1ug/ml). These results demonstrate production and export of probable authentic NGF by normal target cells of sympathetic neurons in cell culture and suggest NGF supply by the target is under cellular control. Supported by Am. Heart Assoc (Va Affiliate) and NIH 5 T32 HL07284.

276.18

UP-REGULATION OF NERVE GROWTH FACTOR MESSENGER RNA SYNTHESIS IN ASTROCYTES. P.J. Isackson*, R. Bridges, E. Frohman, S.L. Wagner*, E.G. Jones and D.L. Benson. (SPON: H. Schwark) Dept. of Anatomy and Neurobiology, Dept. of Neurology, Dept. of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717.

Nerve growth factor (NGF) mRNA levels were quantitated in several types of cultured cells by Northern blot analysis. Human neuroblastoma and glioblastoma cell lines and primary cultures of human foreskin fibroblasts produce levels of NGF mRNA comparable to total adult mouse brain. NGF mRNA of glioblastoma cells appeared to be distinctively smaller than NGF transcripts from all other sources examined when probed with either RNA or single-stranded DNA probes. Primary cultures of astrocytes prepared from frontal cortex of neonatal rat brains and a fetal human brain produced greater than 10-fold higher levels of NGF mRNA than normal brain. This is consistent with high levels of NGF protein reported to be present in the media of cultured rat astrocytes (Furukawa et al.,BBRC,136:57,1986). The level of NGF mRNA synthesis was found to be related with the extent of confluency of the astrocytes. These results suggest that glial cells may produce NGF in the brain normally and are capable of producing higher levels in certain trauma or disease states. Supported by Grants from NIH NS 24747 and ADRDA IIRG-87-100.

276.19

INTRASPINAL SYNAPTOGENESIS OF PRIMARY AFFERENT FIBERS INVOLVES NGF. C.E. Hulsebosch and S.M. Carlton. Dept. of Anat. and Neurosci. and Marine Biomed. Inst., Univ. Tex. Med. Br., Galveston, TX 77550.

We previously reported an increase in the number of unmyelinated sensory fibers in the dorsal root and Lissauer's tract in adult rats after daily treatment with rabbit antibodies for β-Nerve Growth Factor (Anti-NGF, 3 µl/gm body weight undiluted sera) from birth for 1 month. We interpret this increase to be sprouting of the unmyelinated population in response to NGF deprivation. To test for synaptogenesis, we immunostained for calcitonin gene-related peptide (CGRP), a marker for unmyelinated and small myelinated primary afferent terminals in laminae I and II. We report a 103% increase in CGRP immunoreactivity as determined by computer assisted image analysis, in the Anti-NGF treated rats compared to controls. The increased immunoreactivity diminishes after a unilateral dorsal rhizotomy of 12 segments. This supports the hypothesis of synaptogenesis of CGRP afferent terminals after Anti-NGF treatment. (Supported by Spinal Cord Research Foundation, NIH grants NS11255 and NS25450, Marie Hall Foundation and Bristol-Meyers.)

NEURAL PLASTICITY IN ADULT ANIMALS: NEUROMUSCULAR AND AUTONOMIC

277.1

LONG-TERM UNCOUPLING OF MUSCLE AND MOTONEURON: EFFECTS ON IA SYNAPTIC FUNCTION. S. Vanden-Noven and M. J. Pinter. Dept. Anatomy, Medical College of PA at EPPI, Philadelphia, PA 19129.

In deeply anesthetized cats, we studied heteronymous Ia synaptic function in axotomized medial gastrocnemius (MG) motoneurons that were denied the possibility to reinnervate muscle. The severed MG nerve was sutured onto the lateral gastrocnemius (LG) muscle and the MG muscle excised. At various days post-operative (DPO, up to 150), we recorded composite Ia EPSPs evoked by electrical stimulation of the undamaged LG-soleus nerve. At 44 DPO, significant changes were found in average EPSP amplitude (decrease) and time-to-peak (increase). Between 60, 90 and 120 DPO, however, there was a clear trend for the values of these parameters to return to control levels. Detailed analysis, however, indicates that this does not represent a restoration of normal synaptic function. For example, at several DPOs, post-tetanic depression of Ia EPSPs was observed. Moreover, the apparent recovery of EPSP amplitude was not evident at 150 DPO.

Supported by NIH grants NS24000 and NS24707.

277.2

LONG-TERM UNCOUPLING OF MUSCLE AND MOTONEURON: EFFECTS ON MOTONEURON ELECTRICAL PROPERTIES. M.J. Pinter and S. Vanden-Noven (SPON:F. Haun). Dept. Anatomy, Medical College of PA at EPPI, Philadelphia, PA 19129.

We studied the extent to which changes in motoneuron (MN) electrical properties provoked by axotomy persist when reinnervation of muscle is prevented. In deeply anesthetized cats, axotomized medial gastrocnemius (MG) MNs were studied by intracellular recording at various days post-operative (DPO). Reinnervation by MG MNs was prevented by suturing the severed MG nerve onto the normally-innervated lateral gastrocnemius (LG) muscle and excising the MG muscle. For DPOs up to 150, we found no trends for recovery of control values among the variety of MN electrical properties studied. Significant correlations observed among control values of certain parameters could be found at all post-operative intervals, but the occurrence of significant correlations decreased over time. Although disruption of connections between muscle and MNs markedly affects MN electrical properties, these connections are not required for the expression of correlations among motoneuron properties.

Supported by NIH grants NS24000 and NS24707.

277.3

ATROPHY OF THORACIC MUSCLES FOLLOWS HINDLIMB AUTOTOMY IN GRASSHOPPERS. EA Arbas ARL Div. of Neurobiol. Univ. of Ariz., Tucson AZ 85721.

We have reported earlier (Weidner & Arbas, 1985 Soc. Neurosci. Abstr. 11, 959.) that undamaged, fully innervated muscles intrinsic to the thorax of grasshoppers Barytettix psolus atrophy to <15% of their normal mass after hindlimb autotomy. Experimental cutting of nerves and muscle loading/unloading indicated that it is the severing of the leg nerve (N5) during autotomy that induces muscle atrophy. We have now also studied the grasshoppers (Schistocerca americana) and (Melanoplus differentialis), and find significant differences among these taxa. Grasshoppers were made to autotomize a leg in the first days of adult life and certain of their thoracic muscles were individually weighed 35-70 days later. All 6 muscles studied in Barytettix atrophied significantly, to 3-16% of control (n=10 insects). In Schistocerca (n=15) only two muscles atrophied significantly to 70-80% of control. In Melanoplus (n=10) 5 muscles atrophied to 58-90% of control. For example, the mass of muscle #133 was reduced as follows by 35-70 days after autotomy: In Barytettix to 3 +/- 0.6% of control; Melanoplus, 58 +/- 3%; Schistocerca, 72 +/- 5%. Thus, hoppers of these taxa show different sensitivities to autotomy-induced nerve damage as adults. If Schistocerca (n=10) were made to autotomize a leg earlier in development (2nd instar), muscle atrophy increased significantly. Weighed individually on the 1st day of adulthood (26 days post-autotomy), 5 muscles showed significant atrophy, weighing 39-93 % of control. In this group, muscle #133 weighed 39 +/- 4% of control, representing significantly greater atrophy than in the adult-treated group above. It appears that Schistocerca is quite sensitive to nerve damage early in its development, and loses this sensitivity as an adult. In Barytettix, sensitivity to nerve damage persists throughout the entire life cycle. [Supp. by the Univ. of Ariz. Foundation]

277.4

STRUCTURE OF MOTORNEURONS TO MUSCLES THAT ATROPHY FOLLOWING HINDLIMB AUTOTOMY IN GRASSHOPPERS. AR Villalobos*, EA Arbas (SPON: T Kingan), Dept Physiol & ARL Div of Neurobiol, Univ of Ariz, Tucson AZ 85721

Grasshoppers often autotomize a hindleg to escape predators or to shed it when damaged. This severs the leg nerve (N5) and causes undamaged, separately innervated thoracic muscles to atrophy (e.g. Weidner & Arbas, 1985 Soc. Neurosci. Abstr. 11; 959). Atrophy could be initiated if damage to axons in N5 caused regression of motorneurons (MNs) innervating these muscles. MNs might degenerate or withdraw their axons from target muscles or atrophy in the CNS. We have tested for this by cobalt backfilling MNs of tergo- trochanteral muscle (M #133 b,c), which atrophies to <5% of normal mass after hindlimb autotomy in grasshoppers Barytettix psolus. MNs were stained 4-6 weeks post-autotomy, or in controls of the same age. Different fills in controls revealed up to 3 ventral neurons that could always be identified as either a large (ca. 47-61 um diam.) anterior soma; or one of pair of smaller (ca.32-50 um diam.) neurons. All 3 MNs could be identified in backfills of atrophied M #133 in treated insects. Clearly, muscle atrophy is not due to degeneration of the MNs. Dendrites of MNs in both groups showed variability but no differences indicating atrophy in treated insects were apparent. Somata were traced on a digitizing tablet and profile areas calculated. Comparison of 5 larger anterior somata from normals with 10 from the treated group showed no significant differences. Comparison of 9 smaller somata from normals with 16 from the treated group showed the latter to be ca. 46% smaller (t-test $P<0.001$). Thus, soma shrinkage in the smaller pair accompanies muscle atrophy. Shrinkage of somata may indicate reduced biosynthetic activity. The relationship of this change to muscle atrophy is a subject for future research. [Supp. by Univ. of Ariz. Foundation].

277.5

THE RELATIONSHIP BETWEEN MOTORNEURON CELL SIZE & THE SIZE OF THE PERIPHERAL FIELD. H.Tissenbaum*& D.J. Parry* (SPON: J.Thakar), Dept. Physiol., Univ. Ottawa, Ottawa, ON K1H 8M5.

To elucidate the relationship between motorneuron soma size and the size of the peripheral field we examined changes in the motorneuron soma size using horseradish peroxidase (HRP) retrograde labelling in both control (C57BL) and dystrophic (dy2j/dy2j) mice. After partial denervation of the anterior tibialis (TA) muscle, mean soma size of the surviving motorneurons was significantly increased when compared to the unoperated side. In 12 C57 mice, mean soma area was 245.1±18.5μm^2 on the control side and 298.5±15.9μm^2 on the partially denervated side. In 12 dy2j mice, mean soma area on the control side was 235.4±17.6μm^2 and 291.8±17.6μm^2 on the operated side. It is possible that the increased motorneuron size may be associated with the increased motor unit size known to occur with partial denervation. To prevent this increase, experiments were performed involving partial denervation accompanied by partial extirpation of TA. Following recovery, in 12 C57 mice, cell soma size was 250.7±14.5μm^2 on the control side and 255.7±19.1μm^2 on the operated side. In 12 dy2j mice, mean area on the control side was 257.3±16.3μm^2 and 247.3±10.5μm^2 on the operated side. We conclude the size of the motorneuron in the spinal cord is related to the total number of muscle fibers innervated (ie. motor unit size). Supported by Medical Research Council of Canada.

277.6

CENTRAL AND PERIPHERAL NEURONAL ALTERATIONS IN EXPERIMENTAL DIABETES. A.M.Di Giulio, B. Tenconi*, R. La Croix*, M.P. Abbracchio*, F. Cattabeni, and A.Gorio. Inst. Med. Pharmacol., Faculty of Med., Via Vanvitelli 32, and Inst. Pharmacol. Sci., Via Balzaretti 9, Milano, Italy

It is known that diabetes is correlated with sensory and motor peripheral degenerative atrophy. We now report that alterations are present also in CNS and in neurons innervation the gut. The caudate is hyperinnervated by met-enkephalinergic axons with a correlated decrease in the efficiency of the inhibitory transduction signal of the dopaminergic receptor, clearly suggesting an alteration of the Gi protein system. 5-HT containing neurons display a proximal-distal atrophy, as suggested by the significant 5-HT decrease in the pons-medulla oblongata and by lack of changes distally. The gut shows a typical hyperinnervation by the NA system in the duodenum, while the ileum shows a degeneration of the enteric substance P system.

277.7

DIMINISHED SPINAL CORD CONDUCTION VELOCITY IN DIABETIC RATS. L.R. Whalen*, R.E. Carsten*, and D.N. Ishii (SPON: N. Chaudhari) Dept. Anatomy and Neurobiology, and Dept. Physiology, Colorado State Univ., Ft. Collins, CO 80523.

Insulin can support the survival of sensory and sympathetic neurons and enhance neurite formation at physiological concentrations (reviewed in Ishii and Mill, Curr. Topics Membranes Transport 31: 31, 1987). Because similar effects are observed in cultured spinal cord neurons (see Glazner and Ishii, this meeting), we predicted that a decrease in insulin activity may cause functional disturbances in the cord. A novel procedure to measure evoked spinal cord potentials was devised. The sciatic nerve was stimulated, recording electrodes were placed at interarcuate levels C2-3 and T8-9, and the distance between the recording electrodes measured. The difference in latency of the compound action potentials recorded at the two sites permitted calculation of the conduction velocity. This is the first demonstration that conduction velocity is decreased in the spinal cord of diabetic rats ($P < 0.05$). A common mechanism is suggested because the kinetics and magnitude of the decreases were similar in the spinal cord, common peroneal nerve, and saphenous nerve following the induction of diabetes with streptozotocin. The results suggest that insulin may help maintain conduction velocity through direct effects on central and peripheral neurons. (Supported by NIH grant R01 NS24327).

277.8

PROLONGED AXONAL SURVIVAL FOLLOWING AXOTOMY. E.R.Lunn*, V.H.Perry*, M.C.Brown, H.Rosen*, S.Gordon*. University Laboratory of Physiology, Dept. of Experimental Psychology and Sir William Dunn School of Pathology, Oxford University, Oxford, England

Myelomonocytic cells are recruited into the distal half of sectioned mammalian peripheral nerves. The following experiments suggest that they do not simply phagocytose debris but actively bring about axonal destruction and stimulate Schwann cell mitosis. i) In one strain of C57BL mouse, sciatic nerve section is not followed by the usual rapid loss of form and function in the distal half: nearly all axons appear normal 7 days after axotomy at both light and EM levels and most conduct action potentials. In this strain myelomonocytic cell recruitment and Schwann cell mitosis are absent at 7 days. ii) In all other strains of mice examined the ability to conduct action potentials is lost by day 2 after axotomy and all axons have disintegrated by 3 days. When cell recruitment is prevented in these strains by intravenous injection of a monoclonal antibody that hinders leucocyte migration across the blood vessel wall (Rosen,H. & Gordon,S., J.Exp.Med., 166:1685, 1987) axonal integrity was preserved for up to 5 days in 14 out of 22 treated mice.

277.9

A QUANTITATIVE STUDY OF NEURONAL DEGENERATION INDUCED BY RICINUS TOXIN IN THE CILIARY GANGLION OF QUAIL. §P. Paggi, ◊A. Ciofi Luzzatto*, #M.E. De Stefano*, #D. Guidolin*, ◊G. Mangano* and ◊G. Toschi*. §Università de L'Aquila, ◊Università "La Sapienza" Roma, #Fidia Laboratories, Abano Terme. Italy.

One useful approach to the study of synaptic contacts formation and maintenance consists of target cells removal. The experimental model chosen for this purpose was the quail ciliary ganglion, where we can compare two independent cell populations, showing different types of preganglionic terminals. Ricinus toxin was applied to crushed postganglionic nerves and after a 3 to 40 day period the ganglia were examined by light microscopy, for changes in neuron number, volume and morphology. Normal and crush-treated controls were also analyzed. At day 40 a 40 to 50% loss of both the ciliary and choroid cells was observed in the toxin-treated ganglia. However, the two cell populations showed different degeneration patterns: in fact, at day 3 the degenerating ciliary neurons showed a massive nuclear pycnosis and nucleolar fragmentation, a phenomenon never observed at any stages in the choroid cells. Moreover, the surviving ciliary cells, unlike the choroid cells, revealed an increased cellular volume.

These observed behavioral differences between the two cell populations may affect the survival of their respective preganglionic terminals differently, and/or may be attributed to the diversity between the two types of terminals themselves.

277.10

DISUSE AND FUNCTIONAL OVERLOAD INDUCED BY TOOTH EXTRACTION ALTERS NMJ MORPHOLOGY IN C57BL/6J MICE. M.K. El Kerdany, M.H. Andonian, and M.A. Fahim, USC Andrus Gerontology Center, Los Angeles, CA 90089-0191.

The effects of functional disuse and overload on the morphology of neuromuscular junctions (NMJ) in masseter muscles of 12 month old C57BL/6J mice were studied. In order to simulate disuse associated with tooth loss, teeth were extracted from one upper quadrant of 10 mice. The side where teeth were removed was functionally disused (D), since no occlusion could be achieved. Similarly, the intact contralateral (CL) side experienced overload. Morphological measurements were taken from zinc iodide osmium (ZIO) stained NMJs from control animals, and treated animals at 7 days (7D) and 4 months (4M) post extraction. Data was analyzed with repeated measures multivariate analysis of covariance (MANCOVA).

Test animals did not loose weight, indicating sufficient food was consumed after tooth extraction. Fiber diameters from the 7D group were smaller than the controls, while those from the 4M animals were larger. In both the 7D and 4M animals, the CL fibers were larger than the D fibers. There was a trend toward larger NMJs in the 4M animals when compared with control animals of the same age. NMJs from the CL side of the 4M animals had 14% larger nerve terminal areas ($P < .01$), while the 4M D side was 6% larger ($P < .10$). NMJs from the 4M D group had 5% longer nerve terminals ($P < .07$); while the 4M CL NMJs were 6% longer ($P < .05$). NMJs from the D side of the 7D animals were 5% smaller areas while the CL side were 11% smaller ($P < .01$). Nerve terminals from 7D disused were 6% longer ($P < .05$) than controls.

Changes in NMJ morphology following tooth extraction were probably due to differences in degeneration - regeneration of nerve terminals. Acute disuse and overload probably produced greater nerve terminal retraction, whereas chronic changes consisted of a recovery characterized by NMJ elaboration similar to that observed in exercise, disuse and aging studies. M.A.F. supported by NIH AG04755

277.11

EFFECTS OF SPINAL CORD TRANSECTION ON SYNAPTOLOGY OF PREGANGLIONIC NEURONS IN THE SACRAL PARASYMPATHETIC NUCLEUS OF CATS. M.G. Leedy, M.S. Beattie, and J.C. Bresnahan. Depts. of Anatomy and Neurosurgery, Ohio State University, Columbus, OH 43210.

The preganglionic neurons (PGNs) of the sacral parasympathetic nucleus (SPN) were labeled by placing HRP onto the pelvic nerve in cats which received: 1) no spinal cord transection (Normal), 2) transection four days prior to sacrifice (Acute TX) or 3) transection 10 weeks prior to sacrifice (Chronic TX). Ultrastructural analysis indicated that removal of the supraspinal inputs, while not affecting the number of terminals per 100 um of membrane perimeter, significantly decreased the terminal coverage of the labeled somata and proximal dendrites in the Chronic TX animals. Changes in terminal types were also seen, with the Normal group exhibiting a greater proportion of terminals containing dense cored vesicles than did the transection groups. In addition, terminal size was significantly reduced in the Chronic but not the Acute TX animals. These results provide evidence of alterations in synaptic inputs to the preganglionic neurons of the SPN in response to spinal cord transection.

277.12

CHANGES IN IN VITRO MOUSE SPINAL CORD FOLLOWING NERVE CRUSH AND HIND LIMB UNLOADING.
C.J. Somps and M.W. Luttges. Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309.

Rapid (days) changes in electrophysiological indices of spinal cord function have been observed following peripheral nerve injury. These changes include time dependent depressions/elevations in primary afferent depolarizations and interneuron responses, as well as injury dependent somatotopic reorganizations of afferent specificity. Recent histological and in vitro biochemical studies have revealed post-injury changes in neuronal peptide concentrations and synaptic uptake of neurotransmitter. These chemical changes occur in the same general time-frame, and commonly in the same direction, as electrophysiological changes. In order to best correlate post-trauma chemical and electrical changes, present tests were done on an in vitro, hemisected, adult mouse spinal cord preparation. Alterations in afferent input were induced, in vivo, by crushing the sciatic nerve 3,6,9, & 12 days prior to testing, or by unloading the animal's hind legs for 30-40 days prior to testing. Stable (>8 hrs), multi-component, electrophysiological responses (latencies of 1-10 msec) were obtained from dorsal horn laminae using both dorsal root and dorsal tract stimulations. These responses showed clear sensitivities to temperature, ion concentrations, and drug concentrations, as well as experimental manipulations of afferent inputs. Mechanisms underlying these sensitivities are postulated.

277.13

NORMAL SUCCINATE DEHYDROGENASE ACTIVITY IN MOTONEURONS SIX MONTHS AFTER SPINAL ISOLATION
G. R. Chalmers, R. R. Roy, and V. R. Edgerton. Kinesiology Department and Brain Research Institute. UCLA, Los Angeles, CA 90024.

Five female adult cats underwent surgical spinal isolation (SI), i.e., complete spinal cord transection at T12-T13 and at L7-S1 with all dorsal roots between transection sites severed, to eliminate electrical activity in the motoneurons of the lumbar spinal cord. The cats were maintained in excellent health for 6 months. The right leg muscles received cyclic passive stretching for 30 min/day, 5 days/week. An almost total lack of neuromuscular activity was verified by EMG recordings. Succinate dehydrogenase activity (SDH), an aerobic marker enzyme, of a population of motoneurons in the lumbosacral enlargement was determined using the technique described by Chalmers and Edgerton (Soc. Neurosci. Abstr., 13:1416, 1987). Six normal cats served as controls (C). In the SI cats, 289 and 319 cells were sampled in the left and right ventral horns respectively. There was no significant difference in mean SDH between the two sides, thus the data were pooled for subsequent analyses. There was no significant difference between the SI and C animals in motoneuron mean SDH ±SD (SI=0.0155 ±0.048 OD/min, n=608; C=0.0164 ±0.0047 OD/min, n=720). The soma cross-sectional area was determined for cells in which there was a visible nucleus. There was no significant difference in mean soma area ±SD between the two groups (SI=3754 ±1295 um^2, n=214; C=3850 ±1192 um^2, n=289). A plot of SDH activity vs. soma size (for cells with nuclei) showed that small cells had a wide range of SDH activities (i.e., low to high) while larger cells had only low SDH activities. These data indicate that soma size and SDH activity of lumbar motoneurons are not affected by a large reduction in number of action potentials. *Supported by NIH Grant NS16333*

277.14

LONG-TERM ADAPTATION OF CRAYFISH EXTENSOR MOTONEURONS. A.J. Mercier and H.L. Atwood, Dept. of Physiol., Univ. of Toronto, Toronto, Ont. M5S 1A8

Previous studies with the crayfish claw (eg. Lnenicka et al, J. Neurosci. 6:2252, 1986) have shown that conditioning the phasic closer excitor motoneuron in situ for 3 or more days (at 5 Hz for 2 hrs/day) changes its synaptic properties to more closely resemble those of more active, tonic neurons. This "long-term adaptation" involves a reduction in EPSP size and a decrease in the rate of fatigue of EPSP's. The present study shows that similar conditioning (2.5 Hz, 4 hrs/day for 3 days) applied in situ to phasic abdominal extensor motoneurons causes a marked reduction in EPSP size, apparent 1 day after cessation of conditioning. Mean quantal content, determined using a focal extracellular electrode to record excitatory synaptic currents, was found to decrease in response to conditioning. Calculated values for binomial parameters n and p also decreased, suggesting a reduction in the number of active release sites and in release probability. The results show that long-term adaptation occurs in several crustacean phasic neurons and provide direct evidence that the reduction in EPSP size involves presynaptic mechanisms.

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NEUROETHOLOGY IV

278.1

MULTI-UNIT ANALYSIS OF A NEURAL ENSEMBLE CONTROLLING DIRECTED MOVEMENT. J.P. Dowd[1*], S.R. Smith[2*], C.M. Comer[1] and B.C. Wheeler[2]. [1]Dept. Biological Sciences & Committee on Neuroscience, Univ. of Illinois-Chicago. [2]Dept. Electrical and Computer Engineering & Bioengineering Program, Univ. of Illinois-Urbana.

Intracellular recordings from the giant interneurons (GIs) of the cockroach (P. americana) have shown that these cells respond selectively to winds from certain directions. Evidence suggests that GI information orients the animal's escape turning response to wind. We sought to understand group properties of the GIs, and their ensemble coding, using multi-unit recording techniques.

Simultaneous intracellular and extracellular recordings from the cockroach nerve cord showed that single units could reliably be separated from multi-unit records using computerized signal analysis. Identification of these separated cells was accomplished through template matching with our intracellularly-derived GI receptive fields. This approach has discerned the relative timing of neural activity of cells within one connective and between cells on the two sides of the nerve cord. It also enabled analysis of the covariance between specific units in their responses to winds. We are currently using this method of signal analysis to determine the ensemble coding of GIs recorded from chronically implanted electrodes in an intact animal.

(Supported in part by NSF grant #BNS-8617393 to C.M.C.)

278.2

COMPARISON OF WIND-ELICITED EVASIVE BEHAVIOR MEDIATED BY GIANT INTERNEURON (GI) AND NON-GI PATHWAYS IN THE COCKROACH, Periplaneta americana.
I.E. Stierle*, M.E. Getman* and C.M. Comer (SPON: E. Polley). Dept. Biol. Sciences, Univ. of Illinois, Chicago, IL 60680

Giant interneurons (GIs) in the nerve cord of the cockroach receive cercal sensory input and play a role in triggering the animal's wind-elicited escape response; a turn away from the wind and then a run. However, we recently found that when GI input to thoracic ganglia was blocked by section of the abdominal portion of the cord, evasive responses to wind persisted. Animals with this lesion responded at a low rate (30%) to a standard wind stimulus, and their responses were about 3 times longer in latency than normal. This behavior was supported by non-GI pathways which apparently receive input from head sensory structures, such as the antennae (Brain Res. 445:370-375, 1988).

We have now characterized non-GI mediated responses to wind in animals with the cerci removed or with all the GIs ablated from the cord by electrocautery. Tested under free-ranging conditions, these animals responded to wind on 50-75% of all trials, and the average latency of responses was approximately doubled. The directionality of these responses was roughly normal: they were oriented away from the wind source, but with a poorer correlation between turn angle and wind stimulus angle than is normally seen.

In animals with either of these lesions, subsequent removal of the antennae significantly reduced responsiveness to wind and further elevated latency. Antennal removal alone does not affect response rate or latency.

(Supported by NSF grant #BNS-8617393 to C.M.C.)

278.3

RESPONSE INCONSTANCY IN THE FLASH COMMUNICATION SYSTEM OF FEMALE PHOTURIS VERSICOLOR FIREFLIES. Jonathan Copeland, Kathleen King*, and Albert D. Carlson. Dept. of Biol., Swarthmore Coll., Swarthmore, PA 19081 and Dept. Neurobiol. and Beh., SUNY Stony Brook, Stony Brook, NY 11794.

Prior to mating, a female Photuris versicolor firefly readily responds to the triple pulse flashes of its conspecific male. After mating, a female versicolor undergoes a behavioral shift, becoming predatory. A mated female responds to a wide range of different flash patterns. This mating-induced behavioral switch appears to involve the modulation of neural circuits in the firefly's brain.

Is this behavioral switch permanent or does female versicolor response behavior change as the animal ages? Comparisons between predatory females captured early in the summer and those captured 4 weeks later reveal that older versicolor females become less discriminating in their flash responses. Whereas younger versicolor females respond mostly to double flashes, older ones respond equally well to single, double, and triple flashes.

Anatomical examination of older field-captured versicolor females reveals that they possess significantly fewer motile sperm than those captured earlier in the summer. Although the nutritional state of these fireflies could not be determined, our earlier experiments indicate that fed versicolor females live longer than unfed controls. They also mature and lay more eggs than unfed controls.

Multiple internal and external factors may shape this firefly's flash communication system.

278.4

BEHAVIORAL CHOICE IN THE LEECH; CHARACTERIZATION OF THE SHORTENING MOTOR PATTERN. G. Wittenberg and W.B. Kristan, Jr. Dept. of Biology, University of California at San Diego, La Jolla, CA 92093-0322.

The leech, *Hirudo medicinalis*, may react variably to repetitions of a constant stereotyped mechanical stimulus, producing at least two different behaviors: swimming, an undulatory movement, or shortening, a withdrawal involving several segments near the stimulated site. We addressed the issue of whether this choice is a choice between sets of motor neurons (MNs), that is, whether the two behaviors use the same set of MNs or use two different sets.

Because shortening and swimming both use longitudinal muscles, we studied the responses of the motor neurons innervating them. These muscles are innervated by a set of 16 pairs of MNs in each segmental ganglion. Of these MNs, the L-cell pair excites the entire cylinder of longitudinal muscle in its segment, while the others excite or inhibit more restricted bands. Although L cells have the appropriate motor output to produce shortening and receive excitatory input directly from mechanosensory neurons within their own ganglion, most of them are hyperpolarized in segments that are producing the shortening motor pattern. The exception is in the stimulated segment and the adjacent ones, in which the direct excitation from mechanosensory cells predominates.

By contrast, the responses of the other longitudinal excitatory MNs (eg. cell 3) during shortening are pure depolarizations, are larger, and have a segmental extent that correlates well with the extent of the shortening behavior. Thus, the shortening behavior uses the same set of MNs as does swimming. The difference between the two behaviors is in the pattern of MN activity producing them. These data imply that some of the interneurons mediating the shortening response make interganglionic excitatory connections onto this set of longitudinal MNs. We will search for these interneurons and determine whether they also inhibit both the swimming behavior and the L-cells.

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278.5

HINDSIGHT AND RAPID ESCAPE IN AN AQUATIC OLIGOCHAETE. C.D. Drewes* and C.R. Fourtner. Zool. Dept. Iowa State Univ., Ames, IA 50011 and Bio. Sci. SUNY Buffalo, NY 14260.

Mechanosensory stimuli applied to posterior segments are effective in evoking lateral giant nerve fiber (LGF) spikes and rapid posterior shortening in a wide variety of aquatic and terrestrial oligochaete worms (*Can. J. Zool.* 1983, 6:2688-2694; *J. Com. Physiol.* 1987, 161:729-738). We report the first instance of an oligochaete (Lumbriculus variegatus) in which LGF spikes and posterior shortening are elicited by decreased light intensity or moving shadows. Experiments from freely behaving intact worms showed that abrupt cessation of background illumination triggered multiple LGF spiking (latency, 321 ms ± 41 SD; n=11) and rapid shortening (latency, 389 ms ± 64 SD; n=16). Nearly identical responses to cessation of light were observed in amputated tail-piece preparations (latency to LGF spiking = 365 ms ± 87 SD; n=13). Since responses to light cessation and moving shadows never occurred in amputated pieces from mid-body or anterior regions, we conclude that LGF photosensitivity is resident in posterior segments. Electron microscopic observations of tail segments suggest that the photoreceptor candidates are isolated epidermal cells invaginated distally to form a cavity (phaosome) filled with microvilli. These cells closely resemble the known photoreceptors of anterior segments in earthworms and leeches. We suggest that the adaptive significance of this LGF-mediated escape response to shadow is related to the worm's habitat and lifestyle, which involve living in shallow margins of ponds and conspicuously positioning tail segments at air-water interface.

278.6

ORGANIZATION OF EGG LAYING BEHAVIORS IN THE POND SNAIL, *LYMNAEA STAGNALIS*. G.P. Ferguson, A.W. Pieneman* and A. Ter Maat. Department of Biology, Free University, Postbus 7161, 1007 MC Amsterdam, The Netherlands.

The egg laying behavior of *Lymnaea* is induced by the electrical discharge of the neuroendocrine Caudo Dorsal Cells (CDCs) and then continues until the eggs are deposited on the substrate. Animals show three distinct phases of egg laying behavior; Resting, Turning and Oviposition. These phases are characterized by specific postures and by the temporal patterns of locomotion and rasping movements of the buccal mass.

Immediately after the CDC discharge the animal enters the Resting phase, and locomotory and rasping activity decrease. The reduction in rasping still occurs when animals are exposed to a chemical stimulus (high sucrose in the water) that would normally stimulate rasping behavior. This suggests that the peptides released by the CDCs have an inhibitory effect on the buccal system of *Lymnaea*.

During the subsequent phases of egg laying (Turning and Oviposition) rasping increases in frequency and animals show slow locomotion around the area of the tank where the eggs will be deposited. Lesions disrupting specific neuronal pathways between the reproductive tract and the ganglia of the CNS that control locomotory and buccal activity abolish these behaviors, demonstrating that sensory input from the reproductive tract is essential for coordinated egg laying behaviors.

Chronic recordings from the muscles of the buccal mass showed that the rasps made during egg laying were characterized by a different pattern and duration of activity to those made during feeding. This suggests that in addition to the inhibitory effects that the CDC peptides have on the buccal system in the Resting phase, they also have a modulatory effect on buccal activity during the later phases of egg laying.

278.7

THE NEUROETHOLOGY OF THE EYE-BAR RESPONSE: THE MOTOR CONTROL. M.M. Hagedorn and R.D. Fernald. Institute for Neuroscience, University of Oregon, Eugene, OR 97403.

We are investigating the eye-bar response in the African cichlid, *Haplochromis burtoni*, as a neuroethological model for visual processing. The eye-bar response is a visually mediated chromatic color change in the melanocytes below the eye, displayed between territorial males.

Attached to the modified scale of the eye-bar are two fine nerves which branch from the facial nerve, and appear to be under sympathetic control (Muske and Fernald, 1986). Methylene blue dissections of these nerves show that they follow the mandibular branch of the facial nerve into the trigeminal ganglion complex. In our current investigation, we have focused upon the anatomy and physiology of the peripheral nerves, henceforth called the eye-bar nerves.

Stimulation of the eye-bar nerves with hook-electrodes causes an immediate contraction of the melanocytes in the eye-bar region, as previously reported. These nerves were severed and injected with horseradish peroxidase. Filled ganglion cells were found in the trigeminal ganglion complex with some fibers projecting into the brain. These direct projections suggest that the two nerves are not both of sympathetic origin, perhaps, with only one directly controlling the melanocytes. Tyrosine hydroxylase immunocytochemistry is currently being used to resolve this issue. During immobilization with curare, the animal maintains its eye-bar in a pale state, severing the eye-bar nerves at this time causes an immediate darkening of this region. This suggests that there is some spontaneuos activity in these nerves. When the eye-bar is dark, however, recordings from these nerves shows no spontaneous activity.

278.8

OPERCULAR DILATOR MUSCLE INNERVATION IN AN "AGGRESSIVE" AND A "NON-AGGRESSIVE" FISH. Dennis L. Gorlick, Department of Biological Sciences, Columbia University, New York, N.Y. 10027.

Respiratory movements of the operculum are produced by the adductor operculi and dilator operculi muscles. In the Siamese fighting fish, *Betta splendens*, the dilator operculi is also the effector muscle for aggressive gill-cover erection behavior. In carp (*Cyprinus carpio*), which do not perform the gill-cover erection display, the dilator operculi is exclusively a respiratory muscle whose motor neurons are located in cranial nerve motor nucleus V (trigeminal) (Luiten, J. Comp. Neurol. 1976). To see whether differences in the function of this muscle were reflected in the organization of the CNS, I used retrograde transport with HRP to identify motor neurons that innervate the dilator operculi in *Betta*.

Although neurons that innervate the dilator operculi are located in cranial nerve motor nucleus V in both carp and *Betta*, the topographical organization of the nucleus and the distribution of motor neurons within the nucleus differ in the two species. While the rostral nucleus of n.V (Vr) has distinct medial and lateral subnuclei in carp, it consists of only a medial part in *Betta*; the caudal nucleus of n.V (Vc) consists only of a medial part in carp, but has a medial subnucleus with large cells and a lateral subnucleus with smaller cells in *Betta*. Dilator operculi motor neurons in *Betta* are located in both the lateral and medial subnuclei of Vc. The medial subnucleus of Vc does not exist in carp, and all dilator operculi motor neurons are found in the lateral subnucleus of Vc in this species.

The presence of a separate Vc subnucleus in *Betta*, but not in carp, may be related to the use of the dilator operculi muscle for aggressive display behavior in *Betta*. (Supported by BNS-84-11437).

278.9

AUTORADIOGRAPHIC LOCALIZATION OF ESTROGEN-CONCENTRATING CELLS IN THE BRAIN AND PITUITARY OF THE OYSTER TOADFISH. M.L. Fine, H. Russel-Mergenthal* and D.A. Keefer*. Depts. of Biology, Virginia Commonwealth University, Richmond, VA 23284 and Loyola College, Baltimore, MD 21210.

We examined the distribution of ^{3}H estradiol concentrating neurons in gonadectomized male and female oyster toadfish (Opsanus tau) by thaw-mount autoradiography. Compared to other teleosts studied in this fashion, the toadfish is of interest because of its reliance on male sound production in courtship. Labelled cells were found in periventricular midline regions of the forebrain. These included the ventral telencephalon pars ventralis, thalamus, nucleus preopticus parvocellularis and magnocellularis, ventral hypothalamus (equivalent to the nucleus lateralis tuberis), and dorsal hypothalamus, including portions lining the lateral recess of the third ventricle in the inferior lobes. Labelled cells were also present in large areas of the pars distalis of the pituitary. In contrast to a previous study with testosterone, the brainstem and rostral spinal cord were negative for labelled cells. The distribution of estrogen-concentrating neurons conformed to the general pattern seen in other teleost fishes.

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278.10

5HT DISTRIBUTION IN APTERONOTUS LEPTORHYNCHUS BRAIN AND RELATION TO MALE SOCIAL BEHAVIOR. S. Johnston, L. Maler and B. Tinner*. Dept. of Anat, U. of Ottawa, Canada K1H 8M5

We investigated the effect of 5HT agonist DMT 2mg/kg and antagonists methysergide 1.7mg/kg, citalopram and fluoxetine 0.1-5mg/kg (all IM) on the social responses of male fish under rest conditions (spontaneous chirps(sc)) and during simulated social encounters with a conspecific (evoked chirps (ec) and jamming avoidance response (JAR)). Immunohistochemical brain mapping of 5HT was undertaken with attention to the electro-sensory-motor pathway. DMT which inhibits the firing of dorsal raphe neurons increased sc and ec for 35 min. Methysergide, a 5HT receptor antagonist and fluoxetine and citalopram 5HT uptake inhibitors (0.5mg/kg and higher) significantly increased sc and ec for 2 hr. No consistent effects on JAR were evident. 5HT was located in cell bodies at the preoptic area, posterior periventricular nucleus, hypothalamus and raphe nuclei. Terminations were evident in ventral telencephalon, hypothalamus and electroreceptive areas. Specifically, there were dense terminations at the lateral inferior olive (IOl), and medium terminations at the prepacemaker nucleus. The terminations indicated a role for 5HT of modulation of the electro-sensory-motor pathway. Notable questions are: **1.** the possible electroceptive role of 5HT in reference to the dense terminations at the IOl and **2.** the neuroendocrine role of dense cells, fibers and terminations at the nucleus tuberis lateralis in relation to these social behaviors.

278.11

INTRASEXUAL DIMORPHISM IN A SOUND PRODUCING FISH: ALTERNATIVE REPRODUCTIVE MORPHS ? R. K. Brantley and A. H. Bass. Sect. Neurobiology and Behavior, Cornell University, Ithaca, NY, 14853.

Two classes of sexually mature males are found in the sound-producing ("sonic") teleost fish, the plainfin midshipman (*Porichthys notatus*). They are distinguishable by a number of traits including: (1) body length, (2) body coloration, (3) relative gonad size, (4) sonic muscle size, (5) vocalization behavior, and (6) nest occupancy during the breeding season. Nesting *Porichthys* larger than 11 cm standard length (SL) were designated "Type I" males, while those smaller than 11 cm were designated "Type II" males. Type I males have a dark, gray-olive ventral coloration, while Type II males have a female-like yellow coloration ventrally. Type II males have an average gonad to body weight ratio of 8.3% (N=21) , which is several fold larger than that of Type I males (N=23, 1.2%). There are also significant differences between the two male morphs in the size of the sound-generating "drum" muscles which are attached to the swimbladder walls. Muscles of the Type I males (N=19) are on average 62% of total bladder weight. By contrast, muscles of the Type II males (N=10) and females (N=8) average only 11% and 7.5% of total bladder weight, respectively.

Type I males produce several types of "vocalizations", unlike Type II males and females which have not been found to vocalize. Additionally, Type II males were *never* found to nest independently; instead they were *always* found in the nests of Type I males, frequently coincident with spawning females.

We suggest the smaller size class, Type II males, possessing female-like coloration and sonic muscles, but with greatly enlarged testes, pursues an alternative reproductive tactic of cuckoldry of the larger size class of mate-calling, nest and egg guarding, Type I males. Supported by NIH, NSF and Hatch Grants and a Rosenblatt Fellowship.

278.12

SEXUAL POLYMORPHISMS IN CENTRAL AND PERIPHERAL ELEMENTS OF A SONIC MOTOR SYSTEM IN A TELEOST FISH (*PORICTHYS NOTATUS*). M. Marchaterre, A.H. Bass and J. Olvarchuk*. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

In the sound-producing teleost fish, *Porichthys notatus*, there are two classes of sexually mature males: "Type I" and "Type II". Type II males resemble females in a number of morphological and behavioral traits. This sexual polymorphism extends to the level of peripheral sonic muscle fibers and central motoneurons that underlie the production of sounds. The sonic muscle fibers of Type I males differ significantly from those of Type II males and females (which resemble each other) in several ways: 1) They are 3-5 times larger and have a characteristically large volume of sarcoplasm that is densely filled with mitochondria. 2) The average Z-line length (1.2 um) of their myofibrils is 20 times greater. 3) The sarcoplasmic reticulum which lies between adjacent myofibrils is more highly branched. The mean diameter of sonic motoneurons is also significantly greater in Type I males (45um) compared to Type II males (27um) and females (30 um) which are significantly different from each other.

The peripheral and central elements of juvenile males and females are monomorphic and resemble those of adult females. Preliminary data indicate that gonadal steroid hormones (testosterone) can induce Z-line expansion in juvenile males and females and adult females; their effects on other traits of peripheral and central elements are being studied.

The distinct features of the sonic motor system in Type I males are considered adaptations related to their known role in sound production and the unique long duration "hums" generated during the breeding season. The similarity in the sonic motor system between females and Type II males, which are not known to "vocalize", are considered adaptations related to alternative reproductive tactics adopted by Type II males. Supported by NSF, NIH and Hatch Grants.

278.13

SPATIAL AND TEMPORAL INTEGRATION OF TASTE AND TACTILE INFORMATION IN THE RETICULAR FORMATION OF A TELEOST. J. S. Kanwal & T. E. Finger. Dept. of Cell. & Struct. Biology, Univ. of Colorado Sch. Med., Denver, CO 80262.

Ictalurid catfish possess an elaborate and highly sensitive extraoral taste system, innervated by the facial nerve, which enables them to search for and acquire food in the aquatic environment. Neuroanatomical and electrophysiological results obtained previously suggested the involvement of the reticular formation (RF) during the target-tracking (homing) phase of food search.

The gross organization of reticular neurons was examined by injecting horseradish peroxidase in the spinal cord. This labelled clusters of medium (20 um) and large (30 um) sized reticulospinal neurons arranged in a rostrocaudal segmental fashion. The location of a subset of these neurons coincided with the previously specified anteroposterior level of projections from the facial lobe to the RF. The existence of a facio-reticulo-spinal pathway was confirmed by electrophysiological experiments where a reticular neuron sensitive to tactile stimulation of the extraoral surface also responded to electrical stimulation of the spinal cord. Electrophysiological recordings also indicated that reticular neurons had either small (restricted to the snout region) or large bilateral receptive fields. The large receptive field units exhibited different response characteristics (inhibition versus excitation) to gustatory stimulation of the ipsi- and contralateral flank regions, respectively. In contrast to the response of the facial nerve, the activity of reticular neurons was adaptive to continuous tactile stimulation of the receptive field. Thus, reticulospinal neurons within the medullary region integrate spatial as well as temporal aspects of taste and tactile information and may accordingly modulate locomotion via spinal motoneurons.

278.14

2-DEOXYGLUCOSE ACCUMULATION FOLLOWING OPTOKINETIC STIMULATION IN THE RED-SIDED GARTER SNAKE (THAMNOPHIS SIRTALIS PARIETALIS). E.E. Allen and D. Crews, Zoology Dept., Univ. Texas, Austin, TX 78712.

In addition to using chemical cues in foraging and sexual behaviors, the Canadian red-sided garter snake (Thamnophis sirtalis parietalis) uses visual cues such as motion for orientation to relevant stimuli. As a preliminary stage in examining the functional neurology of sexual behaviors in this species, unilateral visual stimulation was combined with injection of ^{14}C-2-deoxyglucose (2DG) in order to establish the suitability of this method for delineating the extent of CNS activity. Subjects with unilateral visual blockade were injected with 2DG (0.2 uCi/g body weight) and restrained in the center of a drum patterned with alternating vertical black and white stripes (1.3 cm width). This drum was rotated about the subject for 45 - 85 minutes such that the stripe pattern moved at 7.5 cps. Drum rotation for 5 minutes was alternated with 1 minute periods during which it was stationary in order to reduce the probability of visual system habituation. Immediately after the period of stimulation the brain was removed, frozen in liquid freon-12 (-20°C), and sectioned at 20 u in a cryostat. Sections were mounted on cover slips, quickly dried, and exposed with X-ray film (Kodak SB-5) for one week. Examination of the autoradiograms revealed differential accumulation of 2DG ipsilateral to the stimulated (uncovered) eye distal to the optic chiasm in optic nerve. Proximal to the chiasm, accumulation was predominantly contralateral to visual stimulation and was greatest in optic tracts, thalamus, and superficial tectum.

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278.15

NEURAL MECHANISMS OF SHORT-TERM MEMORY IN FROGS. D. Ingle and K. vS. Hoff*. Marine Science Center, Northeastern University, Nahant, MA 01908.

Earlier studies in our lab showed that integrity of the uncrossed tectomedullary projections is essential to the frog's ability to turn away from approaching visual threat. This generalization is complicated by a recent finding that unilateral lesions within the ventromedial tegmentum (which do not interrupt tectal efferent fibers) cause frogs to turn toward threat approaching within the opposite visual field. The lesion also causes frogs escaping from pinching the dorsum to turn toward and collide with striped barriers placed within the hemifield opposite the lesion. However, a second lesion in ventromedial tegmentum of the opposite side will immediately "cure" wrong-way turns produced initially by unilateral lesions. We hypothesize that the tegmentum plays a role in establishing "preparatory response sets" for escape routes in the frog's natural environment, but is not necessary for fleeing from an immediate threat.

In an attempt to test this idea, we devised a task in which frogs maintain (for at least 10s) a tendency to avoid an area where a barrier has recently been seen while escaping from a looming disk. Frogs with tegmental lesions are deficient in maintaining this response set, as are frogs with ablations of the striatum, although neither lesion reduces efficiency of avoiding seen barriers (see abstract by Hoff and Ingle). We propose that a system involving the frog's striatal-tegmental projections mediates this kind of memory.

278.16

FROGS HAVE SHORT-TERM MEMORY OF OBSTACLES. K. vS. Hoff* and D. J. Ingle. (SPON: J.N. Evanoff) Marine Science Center, Northeastern University, Nahant, MA 01908.

We have previously proposed that the biasing effects of tegmentum on the frog's escape routes from threat reflects a mechanism for "preparatory response set" - a kind of short-term memory (see Ingle and Hoff abstract). Here we have demonstrated the operation of such response sets using the following procedure: (1) a striped barrier is positioned near the frog, (2) the barrier is removed after 10s, and (3) after a further 10s delay in an empty arena a black disk is loomed at the frog from a constant retinotopic direction. The difference between the distributions of jump directions elicited by threat with previously seen barriers and without previously seen barriers shows that normal frogs avoid only those areas where obstacles were recently seen.

In a second test, each animal was rotated on a turntable by 45^{o} clockwise or counterclockwise after the barrier was removed. The direction of avoidance jumps elicited by the subsequent visual threat showed that frogs remembered the real-world barrier location rather than the retinotopic location of the barrier. This kind of memory is, therefore, not analagous to an eidedic image with retinotopic coordinates.

In a third experiment, a dark obstacle was set 90^{o} from the frog's midline and the threat approached from the front. In this case as well, frogs did not jump directly toward the remembered position of the object. Rather, they preferentially jumped into the rear quadrant behind the obstacle's former location, as if hiding from the threat. We propose that this robust kind of memory for obstacles and for hiding places would be useful during foraging when a frog is focussed on potential prey, but must retain some representation of the environment in order to guide rapid escape from suddenly appearing threat.

278.17

A MULTIVARIATE ANALYSIS OF PUTATIVE MEASURES OF EMOTIONALITY IN THE RAT. S.E.Maier, D.P.Crowne*& K.A.Dawson. Dept. of Psychology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

In the study of cortical specialization and its lateralization in non-human species,many different measures of emotionality have been used,often interchangeably.In a study examining the various dimensions of rodent emotionality,71 rats were given a battery of tests including exploration,timidity,fear-induced defecation, passive avoidance and reactivity to handling.The tests yielded 217 separate measures that were systematically reduced by aggregation and factor analysis within each test situation to a final set of 14.A factor analysis of these final 14 variables resulted in 4 well defined orthogonal factors identified as elimination,exploration, cautiousness and reactivity to handling. Behaviours such as defecation and exploration seem to be generalizable to different test situations,while behaviours such as cautiousness and reactivity to handling are situation specific.The emergence of 4 independent dimensions of rodent emotionality stresses the need for researchers to represent them in studies of the underlying brain substrate of emotional behaviour.

278.18

NEURAL SUBSTRATE FOR VOCALIZATION AS DETERMINED BY STIMULATION OF PAG CALL SITES WITH 2DG AND HRP METHODS. F. F. Lang* and N. C. de Lanerolle. Section of Neurosurgery, Yale University School of Medicine, New Haven, CT 06510.

In order to define the neural substrate of vocalization, electrical brain stimulation of the periaqueductal grey (PAG) for the evocation of vocalizations in the domestic cat was combined with the 2-deoxyglucose (2DG) method and horseradish peroxidase (HRP) tract tracing method.

Stimulation of the PAG yielded pure vocalizations which included growls, hisses, and long and short meows. Quantitative analysis of 2DG data showed: 1) increased metabolic activity in the PAG both around and beyond the electrode tip, 2) increased activity in the lateral and ventrolateral tegmentum extending from the PAG and continuing caudally just beyond the inferior colliculus, 3) an altered pattern of activity in the deep layer of the superior colliculus, 4) bilateral reduced activity in the facial nucleus, vestibular/cochlear nucleus, and superior olive, 5) bilateral decreased activity in the caudate, and bilateral increased activity in the visual cortex (area 17) and in a well demarcated band in cortical area 7. HRP studies with injections into ventrolateral pontine call sites demonstrated that the number of retrogradely labelled cells was highest in the PAG.

From these studies it can be concluded that a descending pathway from the PAG into the ventrolateral tegmentum may represent a common pathway of vocalization and that such a pathway may be used in human speech and emotional utterances. Further, attention mechanisms may be critical for vocalization since all of the rostral brain areas, namely the superior colliculus, caudate, visual cortex and cortical area 7, have been demonstrated to play an important role in the generation of orienting movements associated with attentional mechanisms.

278.19

ACOUSTIC DIFFERENCES IN SEPARATION CALLS OF RHESUS MONKEYS FOLLOWING NEONATAL ABLATION OF TEMPORAL LOBE LIMBIC AREAS. J.D. Newman and J. Bachevalier. Lab. of Comparative Ethology, NICHD and Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

Eight rhesus macaques receiving bilateral ablations to the amygdala or hippocampal formation in the first week of life were briefly separated from familiar conspecifics at 1 year of age in order to assess the acoustic characteristics of the species-typical isolation call ("coo"). Sound spectrograms were subjected to quantitative analysis and compared with isolation calls from 5 normal controls. Significant differences from the control group were found in the acoustic structure of the calls from animals with amygdalar ablations. The clearest difference was in the reduction of the peak frequency of the calls. Animals with hippocampal lesions exhibited less clear and more variable differences in their isolation calls. These findings support the view that the amygdala plays an important role in the development of normal affective vocal responses, and suggest for the first time that the hippocampal gyrus may be involved in the development of this aspect of nonhuman primate behavior, as well.

278.20

CYTOCHEMICAL TRACING OF CEREBRAL CONNECTIONS OF MIDLINE FRONTAL CORTEX IN SAIMIRI MONKEYS. P.D. MacLean. SCSBB/Lab. Clin. Sci., NIMH, Poolesville, MD 20837.

The separation cry ranks as a basic mammalian vocalization. Ablation of midline frontolimbic cortex entailing areas 24, 25, and 12 eliminates the spontaneous cry in squirrel monkeys (MacLean and Newman, Brain Res., in press). With focus on area 25, the present study provides a comparison of the cerebral connections of this infragenual area with those of other midline areas. Site injections of 0.2 µl of 4% WGA-HRP are made in Saimiri monkeys (survival time 72 h; TMB as chromogen).

WGA-HRP applied to area 25 results in retrograde thalamic labeling of cells in certain parts of N. ventralis anterior et lateralis; N. anterior ventralis et medialis; N. centralis medialis; N. paraventricularis; N. parataenialis; N. centralis superior lateralis; dorsal and caudal N. medialis dorsalis; N. pulvinar medialis; N. limitans; and N. parafascicularis. Elsewhere, cellular labeling appears in posterior cingulate and retrosplenial cortex; posterior parahippocampal gyrus; claustrum; and in anterior and basal nuclei of amygdala.

Together with clinical data, the thalamic findings on area 25 and neighboring midline frontal areas suggest a linkup of frontal lobe and striopallidonigral mechanisms implicated in both the affect and expression of crying and laughter. Cerebellar mechanisms are also implicated.

279.1

SOMATOTOPIC ORGANIZATION OF HINDLIMB MUSCLE AFFERENT PROJECTIONS TO THE COLUMN OF CLARKE OF THE RAT STUDIED WITH CHOLERAGENOID HORSERADISH PEROXIDASE CONJUGATE

C. Rivero-Melian and G. Grant (SPON: F, Valverde), Karolinska Institutet, Dept. of Anatomy, Stockholm, Sweden

The aim of this study has been to investigate hindlimb muscle afferent projections to the column of Clarke (CC) in the rat. The binding subunit of cholera toxin conjugated to horseradish peroxidase (B-HRP) was used for the labeling of primary afferent fibers. Injections of B-HRP were made into either the tibial, peroneal, femoral, obturator, superior gluteal nerves or into the dorsal ramus at the L1 and L2 levels. Following 2 days survival the animals were perfused with an aldehyde mixture. Frozen 60µm thick sections were cut from the upper thoracic to the lower sacral spinal cord. The sections were processed using TMB as the chromogen. In the CC, the main subject of this study, the projections from the injected nerves were somatotopically organized, even though an overlap between different nerves was found to exist. The tibial nerve projected to the medial part of the CC and the projections from the other nerves, in the order given above, were shifted toward successively more lateral parts. Furthermore, for each nerve, a lateral to medial shift in the rostral direction was seen. This study indicates that the projections from distal hindlimb muscle afferent fibers are located medial to those from proximal hindlimb muscle afferent fibers in the CC in the rat.

279.2

SOMATOTOPY OF CUTANEOUS AXONS IN CAT DORSAL HORN LAMINAE III - IV: QUALITATIVE.

P. Brown, R. Sonty*, R. Millecchia, W. Gladfelter, and J. Culberson. Anatomy and Physiology Depts., WV Univ. Med. Ctr., Morgantown, WV 26506.

Injection of HRP into identified hindlimb cutaneous axons on both sides of the spinal cord was used to analyze somatotopy of projections to laminae III and IV. Terminal fields of axons injected in the same animal always had relative positions in laminae III-IV in accord with the somatotopy of dorsal horn cells and projections of dorsal roots and cutaneous nerves, described in previous studies: The mediolateral III-IV axis mapped a distoproximal trajectory on the leg, and the rostrocaudal axis mapped a preaxial-postaxial trajectory. Axon projections were subsets of their parent cutaneous nerve projections, with shapes in the horizontal plane which varied in accord with somatotopy similar to shape variations of cutaneous nerves and dorsal roots: lateral projections from hip were long and thin, and medial projections from toes were short and fat, as expected from the shapes of the map representations. Curvatures of projections could also be accounted for by somatotopy.

Supported by NIH grants NS12061 and NS25238.

279.3

SOMATOTOPY OF CUTANEOUS AXONS IN CAT DORSAL HORN LAMINAE III - IV: QUANTITATIVE.

R. Sonty*, P. Brown, R. Millecchia, W. Gladfelter, and J. Culberson. Anatomy and Physiology Depts., WV Univ. Med. Ctr., Morgantown, WV 26506.

Terminal fields of HRP-filled hindlimb cutaneous axons in laminae III and IV were analyzed to determine precision of somatotopy. Approximately 20 receptive fields could be lined up along the length of the leg, but only 5 - 6 terminal fields could be lined up across III-IV. Similar smearing occurred in the projections of toes and glabrous pads to III-IV, and for rostrocaudal distributions of axon terminals. Good correspondence existed between the average map locations of receptive field centers for primary afferents and dorsal horn cells, and for the widths and lengths of primary afferent projections and the distributions of responding dorsal horn cells. These calculations were from averages across animals; agreement may not be as good within individual animals. Given widths and lengths of dorsal horn cell dendrites, passive sampling of the presynaptic terminals by dendrites would produce larger cell receptive fields; either more peripheral connections to dorsal horn cells are inactive or axodendritic contacts are selective.

Supported by NIH grants NS12061 and NS25238.

279.4

CUTANEOUS AFFERENT FIBER PROJECTIONS TO DEEP DORSAL HORN LAMINAE (V - VI).

J. Culberson, R. Sonty*, R. Millecchia, W. Gladfelter, and P. Brown, Anatomy and Physiology Depts., WV Univ. Med. Ctr., Morgantown, WV 26506.

Low threshold mechanoreceptor axons send collaterals to terminate, usually with well-defined spatial resolution, in laminae III-IV of the dorsal horn. Some collaterals reach deeper laminae; this study examines these with respect to afferent fiber types of origin and relationship to dorsal projections of the same axon. Deep collaterals are always a minor part of an axon's projection field. In our sample, hair afferents have occasional small inputs to lamina V, typically in mediolateral register with the dorsal projection field. Pacinian corpuscles have numerous deep endings; they are located in medial V-VI, not aligned with the dorsal field. Slowly adapting fibers generate wider deep fields, again not in exact register with dorsal projections. These data suggest that all collaterals of these mechanoreceptive afferents are not necessarily aligned in the dorsoventral axis; precision of alignment appears to vary with afferent fiber type.

Supported by NIH grants NS12061 and NS25238.

279.5

ORIGIN OF S-7B8 IMMUNOREACTIVITY IN THE SPINAL CORD.

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The S-7B8 monoclonal antibody binds to an integral membrane protein in selected nerve terminals in the CNS. In the spinal cord, S-7B8 immunoreactivity is concentrated in laminae I and IIi of the dorsal horn. Less dense staining extends into lamina V and sparse staining is present in the ventral horn. Most of the S-7B8 immunoreactivity in the dorsal horn arises from primary afferent nerve terminals. However, descending projections also give rise to S-7B8 immuno-staining in the spinal cord. Double-labelling studies employing retrograde tracers indicate that neurons of origin of the corticospinal, rubrospinal, vestibulospinal and reticulospinal tracts contain the S-7B8 antigen. Also, thoracic spinal transections result in accumulation of the S-7B8 antigen in axons rostral to lesions, in the corticospinal tract and in the lateral and ventral spinal funiculi, indicating that axonal transport of the S-7B8 antigen occurs in spinal descending systems. Studies are in progress to identify the neurotransmitter content of the neurons of origin of spinal cord S-7B8 immunoreactivity. Supported by NIH grant NS23783.

279.6

A MONOCLONAL ANTIBODY THAT IDENTIFIES LARGE DIAMETER PRIMARY AFFERENTS IN THE SPINAL CORD.

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One aim of our laboratory has been to generate reagents that identify specific neurons and pathways. We have generated a monoclonal antibody (Rat-102) which identifies large diameter primary afferents. Rat-102 identifies a cytoplasmic protein of 200 kd in apparent molecular weight. Rat-102 -labeled axons include the Ia and Ib primary afferents which are seen to ramify extensively and distribute terminals in laminae VII through IX, including a dense arborization in Clarke's column. Antibody staining completely excludes the substantia gelatinosa. Rat-102 identifies primary afferent axons throughout both their central and peripheral processes. In normal rats the antibody does not label cell bodies in the dorsal root ganglia (DRG). However, blocking transport of the antigen recognized by Rat-102 by siatic nerve ligation has allowed us to visualize DRG cell bodies labeled with the antibody. To determine the contribution of primary afferents to labeling in the spinal cord, we have performed chronic dorsal rhizotomies. The reduction of Rat-102 in target regions of lesioned dorsal root axons suggests that the majority, if not all, the Rat-102 staining derives from primary afferents.

We have also studied Rat-102 staining during development in the rat. Rat-102 identifies primary afferents very early in development. Antibody positive axons can be seen in the dorsal root by embryonic day 12. Examination of rat DRG neurons in culture has confirmed that the DRG is a source of the early detected Rat-102 antigen.

Supported by the NSF.

279.7

CHANGES IN SPINAL CORD PEPTIDERGIC NEURONAL NUMBERS FOLLOWING PERIPHERAL NERVE LESIONS

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To determine whether there are changes in peptidergic neurones in the spinal cord following peripheral nerve damage the sciatic nerve was ligated and cut distally in 6 anaesthetised rats. After three weeks colchicine was administered to the lumbar cord. After 48 hr the animals were perfused, 50 µmsections of L4-L5 cord immunocytochemically processed and examined for the numbers of DYN, ENK, SOM, NPY, NT, VIP and GAL neurones. There was an increase in the numbers of all types of peptidergic neurones except NPY on the side of the spinal cord with the sciatic nerve lesioned. Different peptidergic neurones showed differing increases, which depended on the neurones location. The most marked (50-120%) were for DYN, ENK, NT and VIP. SS and GAL showed smaller (~20%) increases. Sciatic nerve section thus produces alterations in the numbers of several types of spinal peptidergic neurones. It may be part of a generalized one where the numbers of most peptidergic neurones are altered following perturbations to sensory nerves, mediating the long-term changes in spinal physiology seen following peripheral trauma.
Supported by the DFG.

279.8

GLUTAMATE DEHYDROGENASE IMMUNOREACTIVITY IN THE RAT SPINAL CORD AND DORSAL ROOT GANGLIA.

K.E. Miller, J.A. Bigness, J.E. Madl, and A.A. Larson. Dept. Neurol. Surgery, Univ. Miami, Miami, FL 33136 and Dept. Vet. Biol., Univ. Minn., St. Paul, MN 55108.

Glutamate dehydrogenase (GDH) is an enzyme involved in glutamate metabolism. Because of the role glutamate plays in spinal synaptic mechanisms, the distribution of glutamate dehydrogenase was examined with immunohistochemistry in the rat spinal cord and dorsal root ganglion (DRG). Male rats were anesthetized and perfused transcardially with a 2% glutaraldehyde, 0.2% picric acid solution. Spinal cords and DRG's were removed, frozen or vibratome sectioned and processed for GDH-immunoreactivity (IR) by the avidin-biotin peroxidase procedure. With light microscopy, no GDH-IR was observed in the DRG's. In the spinal cord, small immunoreactive puncta were observed throughout the white and gray matter. The heaviest GDH-IR occurred in laminae I-II of the dorsal horn. With electron microscopy in dorsal horn, GDH-IR was located within mitochondria. Most immunoreactive mitochondria were located in astrocytes that contacted terminals containig large dense core vesicles and/or small clear vesicles. Many terminals were glomerular in morphology, indicative of primary afferent terminations. Occasionally, glomerular terminals contained GDH-immunoreactive mitochondria. These results support the hypothesis that glutamate dehydrogenase may play a role in the metabolism of neurotransmitter glutamate.
Supported by NS01105, DA 04090, & The Miami Project.

279.9

SEROTONIN FIBERS APPOSE ENKEPHALIN- and NEUROTENSIN-IMMUNOREACTIVE NEURONS IN THE RAT SPINAL CORD.

A.T. SALVATIERRA and K.E. MILLER. Dept. Neurological Surgery, University of Miami, Miami, FL 33136.

Recent studies have shown immunohistochemical localization of enkephalin (ENK) and neurotensin (NRT) immunoreactive (IR) cell bodies in the sixth lumbar (L6) and first sacral (S1) spinal cord segments in the rat. Other studies have shown brainstem nuclei giving rise to descending serotonergic (5HT) projections to the spinal cord. The extent and density of 5HT innervation is prominent in areas overlapping the locations of ENK and NRT neurons, namely marginal zone, substantia gelatinosa, nucleus proprius, and dorsal gray commissure. The present study examines the close apposition of 5HT fibers with ENK- and NRT-IR cells in the lumbosacral region of the rat spinal cord.

Male Sprague-Dawley rats, weighing 250-300 g, were anesthetized and intrathecal cannulae were introduced to the level of L5-L6. Colchicine (2.5 mg/ml, 10 ul volume) was administered through the cannulae at 0 and 24 hours after cannulation. After 48 hours animals were perfused with buffered paraformaldehyde and the segments of the spinal cord that corresponded with the cannulae tip were dissected. Cryostat sections were cut at 10 um and processed for simultaneous double immunofluorescence. Examination of lumbosacral segments indicated 5HT-IR fibers in close apposition to ENK and NRT immunofluorescent cells. Many of these cells were found in the substantia gelatinosa layer of the dorsal horn and in the dorsal gray commissure. A few cells were also found in the marginal zone and intermediate gray. These results further implicate serotonin as a modulator of nociceptive sensory input via interaction with enkephalin and neurotensin neurons in the spinal cord.
Supported by The Miami Project to Cure Paralysis.

279.10

IMMUNOHISTOCHEMICAL DEMONSTRATION OF CALCITONIN GENE-RELATED PEPTIDE AXONS CONTACTING DYNORPHIN A 1-8 DORSAL HORN NEURONS IN A RAT MODEL OF PERIPHERAL INFLAMMATION AND HYPERALGESIA.

O. Takahashi, R.J. Traub and M.A. Ruda. NAB, NIDR, NIH, Bethesda, MD 20892.

Spinal cord dynorphin peptide (DYN) and preprodynorphin m-RNA have been shown to increase following unilateral experimental acute inflammation. Calcitonin gene-related peptide (CGRP) in the spinal dorsal horn likely originates exclusively from small diameter primary afferents and may play a role in the processing of nociceptive information. This study examines potential interactions between DYN-like immunoreactive (DYN-LI) neurons which demonstrate an upregulation in response to acute inflammation and hyperalgesia, and small diameter CGRP-like immunoreactive (CGRP-LI) primary afferent axons.

Inflammation of one hindpaw in 11 male rats was induced by injection of 150 µl complete Freund's adjuvant/saline (1:1; s.c.). Three or four days later, the animals received an intrathecal injection of colchicine and 24 hours later were perfused with 4% paraformaldehyde. The lumbar spinal cord was cut into 24 µm transverse or parasaggital cryostat sections and processed for two-color PAP immunocytochemistry using first an antiserum to human CGRP (black chromogen) and second, an antiserum to DYN A 1-8 (brown chromogen).

CGRP-LI labeled varicosities were observed contacting approximately half of the DYN neurons in laminae I, II and V of the dorsal horn. The contacts were located on both the cell soma and proximal dendrites and typically numbered greater than 25 per cell. DYN-LI neurons in other laminae rarely received CGRP-LI contacts.

These observation suggest that there are direct contacts between presumptive nociceptive small diameter primary afferents and opioid neurons which exhibit an upregulation of DYN as a result of peripheral inflammation and hyperalgesia.

279.11

THE PROJECTION OF SMALL DIAMETER PRIMARY AFFERENTS CONTAINING CALCITONIN GENE-RELATED PEPTIDE-LIKE IMMUNOREACTIVITY IN THE CAT LUMBOSACRAL SPINAL CORD: A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS.

R.J. Traub, A. Solodkin* and M.A. Ruda. NAB, NIDR, NIH, Bethesda, MD 20892.

Previous studies have shown that some small diameter primary afferents project the length of the lumbosacral enlargement in Lissauer's tract (LT). Additionally, it has been reported that a subpopulation of small diameter primary afferents contain calcitonin gene-related peptide-like immunoreactivity (CGRP-LI). We examined the projection of CGRP-like immunoreactive (CGRP-IR) axons in the lumbosacral spinal cord following dorsal rhizotomies.

Unilateral dorsal rhizotomies were performed on 5 consecutive dorsal roots (L4-S1 or L5-S2) under sterile surgical conditions in 5 cats. In order to eliminate caudal projections, an ipsilateral hemisection was made in the most rostral rhizotomized segment in 2 cats. Two weeks later the animals were sacrificed and sections from segments L2-S3 were treated immunocytochemically for CGRP-LI using standard PAP methodology.

CGRP-LI was greatly reduced in all rhizotomized segments compared to the intact contralateral side. The hemisections did not appreciably alter the location or density of the CGRP-LI in the ipsilateral segments. Most of the remaining CGRP-LI was observed in LT, the dorsal surface of the dorsal columns, and the lateral part of laminae I, IIo, and V. A few CGRP-IR axons were also observed in medial laminae I, IIo, and the central canal region. At the electron microscopic level, unmyelinated CGRP-IR axons were observed in LT throughout the length of the rhizotomized region. In laminae I and V, CGRP-IR varicosities containing oval agranular vesicles and a few dense core vesicles were observed to form asymmetrical synaptic contacts on unlabeled dendrites.

These results demonstrate CGRP-IR axons and synaptic terminals 4-5 segments rostral to the closest ipsilateral intact dorsal root. Ascending branches of small diameter primary afferents originating in these distal intact dorsal roots are the most likely source of the CGRP-LI.

279.12

EFFECTS OF CAPSAICIN ON SPINAL CORD AXONS THAT COLOCALIZE SUBSTANCE P AND CALCITONIN GENE-RELATED PEPTIDE.

Solodkin, A.* & M.A. Ruda. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Previous studies have shown a time-dependent return of thermal sensitivity and partial recovery of spinal calcitonin gene-related peptide (CGRP) after neonatal capsaicin (CAP) treatment. Recovery of primary afferent substance P (SP) could not be determined since spinal SP originates from several sources. However, CGRP in the dorsal horn likely originates exclusively from small diameter primary afferent axons and can be utilized to mark them. This study used the colocalization of immunoreactive SP and CGRP (SP-IR/CGRP-IR) to examine the effect of neonatal CAP on SP primary afferent axons.

Two day old rats were treated with CAP (50 mg/kg; sc) or vehicle. At 10 days and 3, 4, 6, 8, 12 & 16 weeks, animals were perfused with 4% paraformaldehyde. Transverse and sagittal 18 µm cryostat sections of spinal cord were processed for species-specific double immunofluorescence staining using rat monoclonal antiserum to SP and rabbit polyclonal antiserum to CGRP.

At 10 days, few SP-IR/CGRP-IR axons remained in Lissauer's tract (LT), and laminae I, II and V compared to controls. No SP-IR/CGRP-IR axons were visible in the lamina X ependymal bundle. At 6-8 weeks, the density of SP-IR/CGRP-IR axons was similar to that at 10 days. However, traversing laminae III and IV, there was a reappearance of some CGRP-IR axons which did not colocalize SP-IR. Most of these were of the non-varicose type. At 12-16 weeks, more SP-IR/CGRP-IR axons in LT and laminae I and II were apparent in CAP animals than were observed at earlier time points, although laminae V and X were still depleted.

These data suggest that neonatal CAP treatment significantly depletes primary afferent SP but does not destroy all SP primary afferent axons in either the superficial or deep dorsal horn laminae. Also, there appears to be a partial return of SP primary afferents more than two months after CAP treatment.

279.13

SPONTANEOUS ACTIVITY OF CAT DORSAL ROOT GANGLION NEURONS. M.J. Hoffert, G.W. Lu, and V.Miletic. Spalding Hospital, Denver, CO 80218, and University of Wisconsin, Madison, WI 53706.

The intraganglionic portion of dorsal root ganglion (DRG) cells is not silent under pathologic conditions. This study investigated the characteristics of the spontaneous discharges of DRG neurons under both normal and abnormal situations.

Spontaneous discharges were recorded from 50 of 130 sampled neurons in the L5-S1 ganglia of anesthetized cats. The spontaneous activity occurred in animals in which: (1) the preparation was left intact, (2) both dorsal and ventral roots were cut, and (3) ventral roots were cut and dorsal roots were locally anesthetized. Of the spontaneously active units, 40 discharged without intentional stimulation of their peripheral or central branches, or their receptive field. In the remaining 10 neurons spontaneous discharges were stimulus-related. In 1/5 of the spontaneously active neurons no collision of orthodromic and antidromic activity could be attained, although such collision was easily achieved in the remaining 4/5.

These results indicate that DRG neurons possess the ability to spontaneously discharge under both normal and abnormal conditions. It is possible that at least some of this activity is due to the presence of active synaptic and/or other types of junctional linkages within the DRG.

279.14

EPSP-LIKE ACTIVITY RECORDED FROM CAT DORSAL ROOT GANGLION NEURONS. V. Miletic and G.W. Lu. Depts. of Comp. Biosci. and Neurology, Univ. of Wisconsin, Madison, WI 53706.

The frequency dependence of dorsal root ganglion (DRG) neurons suggests the presence of synaptic and/or other types of junctional contacts in the DRG. This study investigated the existence of potentials that might be generated from the activation of these postulated contacts.

Intracellular recordings were made from 70 neurons in the L5-S1 spinal ganglia of anesthetized cats. The ganglia had their dorsal and ventral roots sectioned. Two types of action potentials were observed. In 56 of the sampled neurons, action potentials exhibited sharp rise-times and the absence of detectable prepotentials. In 14 neurons, however, individual spikes showed distinct EPSP-like components, variable latencies, inflections on their rising and/or falling phase, and a poor ability to follow electrical stimulation even at frequencies of 1-2Hz. These two very different spikes were recorded even in cases in which a single neuron was activated from both the dorsal root and the sciatic nerve.

These results demonstrate the occurrence of EPSP-like activity in DRG neurons, and imply the presence of synaptic and/or other types of junctional contacts in the DRG.

279.15

FREQUENCY DEPENDENCE OF EVOKED ACTIVITY IN CAT DORSAL ROOT GANGLION NEURONS. G.W. Lu and V. Miletic. Depts. of Neurology and Comp. Biosci., Univ. of Wisconsin, Madison, WI 53706.

The intraganglionic portion of dorsal root ganglion (DRG) neurons might play an important role in primary afferent impulse propagation. This study systematically investigated the responses of DRG neurons to repetitive electrical stimulation of their central and peripheral processes.

Extra- and intracellular recordings were made from 50 neurons in L5-S1 ganglia of anesthetized cats while the sciatic nerve and relevant dorsal root were stimulated at different frequencies. Twenty of the sampled neurons were able to follow stimulus frequencies of 100-300Hz, and an identical number could follow frequencies of 5-25Hz. The remaining 10 DRG units exhibited jitter in their response latency and an inability to follow even very low (1-2Hz) frequencies. As frequency of stimulation would increase, the shape and size of the action potential of these units would degrade into its IS and/or M components. The ability to follow high frequency stimulation decreased in the order of M > IS > S.

These results indicate that somata of DRG neurons are highly susceptible to high frequency volleys, and imply the possible involvement of synaptic and/or other types of junctional contacts in the DRG.

279.16

RAT DORSAL ROOT GANGLION (DRG) CELLS DIFFER ACCORDING TO THEIR PERIPHERAL RECEPTOR. A.M. Ritter* and L.M. Mendell Dept. Neurobiol. and Beh., SUNY, Stony Brook, NY, 11794

To establish the generality of previous findings in cat that DRG somal spikes[1] and functional central projections[2] differ according to peripheral receptor type, intracellular recordings were made in DRG cells of anesthetized female Sprague Dawley rats aged 5-8 weeks. An inverse correlation was noted between baseline AP duration and peripheral fiber conduction velocity (PFCV). However, within each PFCV group, high threshold mechanoreceptors (HTMRs) had broader spikes than low threshold mechanoreceptors (LTMRs) due to an inflection on the falling phase of their AP, observed in no LTMR. AP amplitude was larger in HTMR somata than in LTMRs, independent of PFCV. Afterhyperpolarization (AHP) of HTMRs tended to be of long duration, but a few LTMRs also had long AHPs. The second of 2 cord dorsum potentials (CDP) evoked by paired stimulation (50 ms ISI) of single HTMRs was facilitated; LTMR CDPs facilitated to a small extent or depressed. The CDP evoked by the second of 2 shocks to rapidly-adapting units depressed more than the response to paired stimulation of slowly-adapting units. These results, similar to those reported in the cat[1,2], suggest that the rat, which offers technical advantages for pharmacological studies, can be used to extend results obtained in the cat.

[1]Rose et al., (1986),Neurosci.Lett.63; [2]Koerber,H.R., Mendell, L.M., (1987) Soc. Neurosci. Abst. 13. Supported by PO1 NS14899, RO1 NS16996, MH 18010 and NS 2420601

279.17

EFFECT OF ERGOTAMINE ON SPINAL CORD PROCESSING OF SENSORY INFORMATION FROM THE CRANIAL VASCULATURE. G.A.Lambert*, A.S.Zagami* and J.W.Lance* (SPON: B.Walmsley) Dept. of Medicine, University of New South Wales, NSW, 2031. Australia

Electrical stimulation of cranial blood vessels activates neurons in the trigeminal nucleus and upper spinal cord, an effect which can be blocked by peripheral administration of the antimigraine drugs ergotamine and DHE. In these experiments we have examined whether the site of action is at the spinal or, (as suggested by Saito et al; Neurosci. Abstr. 13,1669) in the periphery. Cats anesthetised with chloralose/urethane were used. A length of the dura and falx containing the sagittal sinus (SS) was isolated, placed on platinum hook electrodes and stimulated with supra-maximal shocks (40-120V, 250 usec, 1 per 5 sec). Multi-barrelled glass/tungsten electrodes were used to record single unit activity and to apply drugs. Electrical or chemical (bradykinin) stimulation of the SS evoked field potentials and activation of single neural elements in the C2 spinal cord. These responses were blocked by application of xylocaine to the sinus, demonstrating that they arose from direct stimulation of SS. Responsive units were accelerated by the iontophoretic application of homocysteic acid, and were therefore probably cell bodies, rather than fibers. IV injection of ergotamine and DHE (4 and 40 ug/kg) reduced or abolished the responses to electrical stimulation. Iontophoretic application of DHE (10 to 30 nA) to responsive units reversibly blocked the responses, but local application of DHE or ergotamine solutions (1 mg/ml) to SS did not. We conclude that the effects of ergotamine and DHE are primarily at the central and not the peripheral ends of the trigeminal nerve.

279.18

STIMULATION OF THE SUPERIOR SAGITTAL SINUS INCREASES LOCAL GLUCOSE UTILIZATION IN THE DORSOLATERAL AREA OF THE CERVICAL SPINAL CORD OF THE CAT *P.J. Goadsby, A.S. Zagami* and G.A. Lambert** Department of Neurology, The Prince Henry Hospital, and School of Medicine, University of New South Wales, Sydney, Australia.

The pain of migraine and other vascular headache involves regions innervated by the trigeminal and cervical sensory systems and is usually accompanied by changes in cerebral blood flow (Lance, J.W. et al., *Headache*, 23:258,1983). In this series of experiments a pain sensitive intracranial vascular structure was stimulated electrically and local glucose utilization (lCGU) measured in the cervical cord.

Cats were anesthetised with α-chloralose (60mgkg^{-1}) and prepared for monitoring. The animals were paralysed and ventilated with continuous observation of blood pressure, core temperature, FI_{O2}% and end-expiratory CO_2. The superior sagittal sinus (SSS) was stimulated with platinum hook electrodes that delivered stimulus-isolated single shocks (40-120 V ≡ 500 μA - 1.5 mA, 250 μsec duration) and lCGU was determined using [^{14}C]-2-deoxy-D-glucose, tissue autoradiography and computerised densitometry.

Metabolic activity (lCGU) in the dorsolateral (DLA) cervical cord at the C_1 level was 8.5 μmol/100g/min in control and 8.0 μmol/100g/min in stimulated animals. It was, however, significantly increased to 108 μmol/100g/min in the DLA of the C_2 level of the cord in stimulated but unchanged in the corresponding area in unstimulated animals (11 μmol/100g/min). The activation of a discrete area of the DLA of the C_2 cervical cord corresponds with single cell activity that is linked to SSS activation that we have previously reported (Lambert et al., *Soc. Neurosci. Abstr.* 13:115,1987). These results suggest that there may be a region in the upper cervical cord of the cat more caudal than that of the classical spinal trigeminal nucleus that mediates head pain in conditions such as migraine and cluster headache.

279.19

CAPSAICIN RELEASES PURINES FROM PRIMARY AFFERENT NERVE TERMINALS IN THE SPINAL CORD. J. Sawynok, T.D. White and M.I.Sweeney. Dept. of Pharmacology, Dalhousie Univ., Halifax, Nova Scotia, Canada. B3H 4H7.

Morphine and K^+ release adenosine, while serotonin releases a nucleotide, from capsaicin-sensitive primary afferent terminals in the spinal cord. Capsaicin depolarizes specific primary afferent neurons and releases peptides such as substance P. The purpose of the present study was to determine whether capsaicin releases purines from primary afferent nerve terminals in the spinal cord. Release of adenosine and ATP by capsaicin from rat spinal cord synaptosomes was determined by HPLC of etheno-adenosine (JPET 243:657, 1987) or by the firefly luciferin-luciferase assay (J.Neurochem.30:329, 1978), respectively. In some cases, rats were pretreated with capsaicin either as neonates or adults and used in release studies 17-20 weeks or 1 week later, respectively. Capsaicin (1-100 μM) produced a dose- and Ca^{2+}-dependent increase in the release of endogenous adenosine from dorsal, but not ventral, spinal cord synaptosomes which was reduced by both methods of capsaicin pretreatment and inhibition of ecto-5'-nucleotidase. Release of endogenous ATP from dorsal spinal cord synaptosomes was observed with the highest concentrations of capsaicin. These results indicate that purines in primary afferent nerve terminals in the spinal cord are released by depolarization with capsaicin. (Supported by MRC of Canada)

279.20

USE OF A RAT SPINAL CORD SLICE-DORSAL ROOT GANGLION IN VITRO PREPARATION TO STUDY SYNAPTIC TRANSMISSION. S. Jeftinija. Dept. Vet. Anatomy, Iowa State University, Ames, IA 50011.

To study the transfer of information from the sensory neuron to the dorsal horn (DH) neuron, a horizontal spinal cord slice (400-500um thick) and the functionally connected dorsal root with the dorsal root ganglion (DRG) were prepared from 18-28 day old rats. Conventional intracellular recording from DH and DRG neurons using 3M K-acetate-filled electrodes was employed. Dorsal roots were electrically isolated from the spinal cord and stimulated with pulses of different intensity and duration to evoke afferent volleys monitored intracellularly from DRG neurons. Results are based on the recordings from 55 DH neurons located in the three most superficial laminae of the spinal DH. Low intensity stimulation of the DR elicited excitatory post-synaptic potentials (e.p.s.p.) in all of the DH neurons tested. In 24% of the neurons this initial e.p.s.p. was followed by a hyperpolarizing potential. High intensity stimulation of the DR sufficient to activate small DRG neurons resulted in a large and more complex e.p.s.p. Thus, the initial burst of action potentials was followed by a prolonged depolarization with (27% of neurons) or without (49% of neurons) subsequent firing of action potentials. Neurons that were hyperpolarized in response to the low intensity stimulus maintained the same response to the high intensity stimulus, but with increase in the amplitude and duration. Supported by DHHS/NIH and USDA.

279.21

EFFECTS OF KYNURENIC ACID ON ACTIVATION OF DORSAL HORN NEURONS BY NATURAL STIMULI *IN VITRO*. S.P. Schneider and E.R. Perl. Department of Physiology, Univ. of N. Carolina, Chapel Hill, NC 27514

An *in vitro* preparation of mammalian spinal cord and a patch of skin with intact intervening innervation has been used to study synaptic transmission between identified primary afferent units and the spinal neurons upon which they synapse.

Neurons were synaptically excited by electrical volleys in a cutaneous nerve and by a variety of mechanical stimuli applied to the skin surface.

Kynurenic acid (KYN; 1 mM) reversibly blocked the synaptic activation and responses to cutaneous stimuli for 6 of 8 of the neurons excited selectively by Aδ fibers from high threshold mechanoreceptors (nociceptors) and for 7 of 9 of the neurons excited by field-type afferents. KYN was much less effective in antagonizing the responses to skin manipulation and nerve volleys of neurons excited by tactile Type II (0/3) and hair afferent sense organs (1/3).

These results suggest that at least two functional classes of myelinated, cutaneous afferent fibers, high threshold mechanoreceptors and field receptors, utilize a glutamate-like amino acid to excite second order cells and that Type II and hair receptors appear to use an excitatory transmitter that is not blocked by KYN.

Supported by grant NS 10321 from the NINCDS.

279.22

SUBLIMINAL FRINGES AND THE PLASTICITY OF DORSAL HORN NEURONS' RECEPTIVE FIELD PROPERTIES. C.J. Woolf* and A.E. King* (SPON:ENA) Dept. of Anatomy, University College London, London WC1E 6BT, UK.

Because of their large polysynaptic input, the size of the cutaneous receptive fields of dorsal horn neurones is not related in a direct way to their dendritic spread (Woolf, C.J. & King, A.E. J. Neurophysiol., 58: 105, 1987). Brief C-afferent fibre conditioning stimuli can in fact produce increases both in the size and in the responsiveness of these neurons' receptive fields (Cook, A.J., Woolf, C.J., Wall, P.D. & McMahon, S.B., Nature 325: 151, 1987). We have now investigated the contribution of subliminal zones in determining the capacity of dorsal horn neurons to dynamically alter their response properties.

Intracellular recordings have been made from deep dorsal horn neurons with cutaneous mechanoreceptive fields in decerebrate-spinal rats. Mechanical stimulation of the centre of the receptive field generated both supra and subthreshold excitatory responses. As stimuli are applied more peripherally the subthreshold responses predominate. Application of the chemical irritant mustard oil to the skin results in the firing zone of the receptive field expanding to incorporate and often extend beyond the preconditioning subliminal zone. The subliminal fringe of dorsal horn neurons enables small changes in synaptic efficacy or excitability to generate a considerable plasticity of receptive field properties.

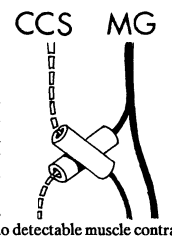
279.23

COMPARISON OF SPINAL CORD POTENTIALS ELICITED BY CUTANEOUS VERSUS MIXED NERVE STIMULATION IN HUMANS. A. Beric, J. Halter. Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030.

We have been interested in the characteristics of spinal cord evoked potentials elicited by different types of peripheral input, in particular the differences between cutaneous and mixed nerve. Similar to animal studies, two major post-synaptic events can be recorded. In humans the generators of the initial negative potential followed by lower amplitude longer duration positivity have yet to be confirmed. We recorded spinal cord potentials from percutaneously introduced 4-contact epidural electrodes placed over the conus medullaris region after stimulation of the tibial or sural nerve at the ankle. These electrodes were being used for the evaluation of the effectiveness of spinal cord stimulation on pain and spasticity in two incomplete cervical spinal cord injury patients. The positive potential was of very low amplitude or absent during stimulation of the sural (cutaneous) nerve compared to the tibial (mixed) nerve resulting in a higher value of the negative versus positive potential ratio (N/P). These observations suggest differences in the generators of these spinal cord potentials.

279.24

PROPERTIES OF NEURONS CROSS-INNERVATED IN SENSORY AND MOTOR NERVES. H. Nishimura*, D. Mosier, G. Sypert and J. Munson. Univ of FL Coll of Med, Gainesville FL 32610.



We are interested in the ability of foreign targets to sustain or alter the properties of peripheral neurons. In cats, the caudal cutaneous sural (CCS: commonly "sural") and half of the MG muscle nerve were cut. The proximal portion of cut MG was cross-connected (x-conn) with the distal portion of CCS. Proximal CCS was x-conn with the distal portion of cut MG. Properties of cross-innervated (x-inn) sensory and motor neurons were tested 1 year later. MG motoneurons (Mns) x-inn into the CCS nerve had electrical properties (I_{rh}, CV, R_N) intermediate between those of normal and those of axotomized MG Mns (Foehring et al. JNp 55), but their stimulation produced no detectable muscle contraction. Stimulation of MG muscle afferents, x-inn into CCS nerve, gave EPSPs of normal configuration in uncrossed MG Mns, as did uncrossed MG afferents in crossed MG Mns. Stimulation of CCS afferents, x-inn into the distal MG nerve, gave PSP complexes with normal EPSP/IPSP configuration (Burke et al. JNp 39), but of reduced amplitude (mV ms), in both crossed and uncrossed MG Mns, as did stimulation of the presumably unaltered lateral cutaneous sural nerve.

This suggests: 1) intra-spinal connections of sensory nerves were not respecified following cross-innervation in peripheral nerves; 2) normal motor and sensory nerve functions were at least partly sustained following such cross-innervation.

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279.25

CENTER-PERIPHERY RELATIONSHIPS OF SINGLE FIBERS AFTER REGENERATION OF TRANSECTED PERIPHERAL NERVES. H.R. Koerber, A.W. Seymour* and L.M. Mendell. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY, 11794.

Individual neurons projecting through regenerated tibial nerves were impaled in the dorsal columns (HRP (15%) filled electrodes) or the L7 DRG (3M KCl) in chloralose anesthetized cats. After establishing its receptive field (RF) the single afferent was stimulated while simultaneously recording the cord dorsum potentials (CDPs) at 4 locations. HRP was then injected into selected fibers. Afferents were studied at various intervals after transection (3, 8-14 months). Unlike fibers in intact nerves, many fibers conducting centrally with cutaneous RFs produced no CDP whereas others with muscle RFs did. This, combined with the finding that many such afferents evoked CDPs in spinal cord regions out of register with their RF location, suggests that afferents failed to reinnervate the correct target. CDPs whose properties and distributions were consistent with the peripheral target (location & modality) were largest. Otherwise CDPs from the regenerated fibers were smaller than those from intact ones. Fibers with no RF could evoke CDPs but the number of such fibers as well as this ability decreased with time. Anatomical results confirmed that some fibers had reinnervated the wrong peripheral receptors, e.g., a) a group Ia fiber (C.V. = 88 m/s; boutons in laminae VI, VII & IX) innervating a slowly adapting cutaneous receptor; b) a nociceptive afferent (boutons in laminae II & V) innervating a rapidly adapting, low threshold cutaneous receptor. Supported by NS 23725(HRK); NS 14899 & NS 16996(LMM).

279.26

SIGNIFICANT TONIC SEROTONERGIC INHIBITION OF SOME SPINAL DORSAL HORN NEURONS EXISTS IN INTACT, AWAKE, DRUG-FREE CATS. Y. Saito,* J.G. Collins, H. Iwasaki.* Dept. of Anesthesiology, Yale Univ. School of Medicine, New Haven, CT 06510

The presence of descending modulation of spinal dorsal horn neuronal activity has been well established in many acute preparations. Information about the influence and pharmacology of tonic systems in intact preparations has been lacking. The systemic administration of methysergide to intact, awake, drug-free cats while recording from single spinal dorsal horn neurons has provided evidence of significant tonic serotonergic modulation of stimulus response functions of some neurons. Methysergide (0.05-2.0 mg/kg) may produce a dose dependent increase in the area of the receptive field activated by non-noxious stimuli, an enhanced response to noxious pinch and heat, and an increased correspondence between stimulus intensity and neuronal firing frequency. These data demonstrate that some spinal dorsal horn neurons have their stimulus response functions significantly modulated by serotonergic systems. This modulation may provide a mechanism to open additional channels of spinal sensory transmission.

Supported by NIH NS 23033

279.27

IN VIVO INTRACELLULAR INVESTIGATION OF THE VIBRATON-INDUCED DEPRESSION OF NOCICEPTIVE DORSAL HORN NEURONES IN THE CAT SPINAL CORD. Y. De Koninck and J.L. Henry. Depts. Physiol. and Anæsthesia Res., McGill Univ., Montréal, Qué. Canada, H3G 1Y6.

In a previous extracellular study, we described an adenosine mediated vibration-induced depression of nociceptive dorsal horn neurones (Salter & Henry, *Neurosci.*, 22:631,1987). To further investigate the mechanism of this depression, we undertook intracellular recording from dorsal horn neurones. Cats were anæsthetised with α-chloralose, paralysed and artificially ventilated. Glass microelectrodes were prepared from single capillary tubes (filled with K-acetate; resistances of 20-50 MΩ in saline) and, in some cases, combined with an extracellular iontophoretic electrode. A feed back controlled mechanical stimulator was used to apply a vibration to the glabrous skin of the receptive field. Vibration induced a reversible hyperpolarization of the membrane potential (10mV; slowly decaying over 2-3s) associated with a decrease in membrane resistance. It was also possible to abolish this IPSP by hyperpolarizing the membrane potential. Thus, the results implicate a direct postsynaptic component in the vibration-induced depression of dorsal horn neurones in the cat spinal cord. (Supported by the Canadian MRC; YDK is a stagiaire de recherche of the FRSQ.)

279.28

DYNAMICS OF, AND INFLUENCE OF WHOLE BODY SINUSOIDAL TILT ON, NECK MUSCLE SPINDLE ACTIVITY IN THE DECEREBRATE, UNPARALYZED CAT. J. Kasper, B.J. Yates and V.J. Wilson. The Rockefeller University, N.Y., NY 10021.

We recorded the activity of presumed neck muscle spindle afferents from the C2 ganglion of decerebrate, unparalyzed cats. In contrast to findings in paralyzed preparations (Chan et al., J. Neurophysiol. 57: 1716, 1987), few afferents show high gain, non-linear responses to muscle stretch during neck rotation (body rotation on a fixed head): 2/16 vs. 24/51. This may be because of static fusimotor tone. In view of previous suggestions that the vestibular system acts on neck spindles via fusimotor neurons (Ezure et al., J. Neurophysiol. 41: 459, 1978) we tested the effect of sinusoidal whole body tilt in vertical planes on spindle activity. Wobble stimuli at 0.05 and 0.5 Hz, with amplitudes of 5 and 10 deg., were typically used. Firing of only a few afferents was weakly modulated by these stimuli, and similar modulation could be observed in labyrinthectomized animals. These results suggest that during moderate amplitude, passive, vertical head movements, the vestibular system exerts little or no effect on neck spindle activity. Supported by NIH (NS02619), DFG fellowship KA694/1-2 (J.K.), and NIH fellowship NS08050 (B.J.Y.).

279.29

EFFERENT PROJECTIONS TO RAT SPINAL CORD FROM TRIGEMINAL NUCLEUS ORALIS UTILIZING THE METHOD OF PHA-L. L. A. Smith*, W. M. Falls and J. I. McMillan*, Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Rat trigeminal nucleus oralis (Vo) contains ventrolateral (VL), dorsomedial (DM), and border zone (BZ) subdivisions. Utilizing retrograde and anterograde techniques efferent projections from all three subdivisions terminate in cranial nerve nuclei. In addition, projections from VL terminate in the medullary dorsal horn while DM and BZ innervate the cerebellum. In this study the anterograde transport of Phaseolus vulgaris leucoagglutinin lectin (PHA-L) was used to determine the overall pattern and organization of efferent projections from each Vo subdivision to the spinal cord (SC). Injection sites were localized within the boundaries of each Vo subdivision. Neurons in DM and VL innervate cervical, thoracic and lumbosacral levels of SC bilaterally. Cells in BZ project only as far as upper cervical segments bilaterally. Axons from neurons in each subdivision arborize in dorsal, ventral and intermediate horns. Based on these findings an extensive Vo-SC projection may contribute to the modulation of sensory projection neurons in the dorsal horn; influence the output of motor neurons involved in withdrawal reflexes (e.g., trigemino-neck reflex); or contribute to the expression of other reflexes involving the integration of trigeminal and spinal somatic and autonomic systems. (Supported by N.I.H. Grant DE06725)

279.30

COMPARISON OF SPINAL RESPONSES FOLLOWING CORTICAL STIMULATION TO CORTICAL RESPONSES FOLLOWING SPINAL STIMULATION. JB Myklebust, JF Cusick, DJ Maiman. Zablocki VA Medical Center and Medical College of Wisconsin, Milwaukee, WI 53295

With spinal cord stimulation, a short latency spike is recorded anterior to the central sulcus of the cerebral cortex. The latency of this potential is consistent with a conduction velocity of approximately 50-60 m/s and is similar to that of the corresponding efferent response (recorded from the spinal cord secondary to cortical stimulation). In global ischemia, it is obliterated at approximately the same time (2-3 minutes) as are the post synaptic responses recorded from the dorsal root entry zone of the spinal cord, from the thalamus, and the cortex-to-cord potential. Both the short latency cord-to-cortex and the cortex-to-cord potentials areunaffected by increases in stimulus frequency up to 300 to 400 Hz. They follow at reduced amplitude up to 700-900 Hz. The latency, waveform, and frequency response imply that the short latency response is monosynaptic. The behavior in ischemia suggests that it is dependent upon the integrity of a cell body. Although the spinal stimulation is applied over the dorsal columns, the pyramidal tract is in the dorsal half of the cord and has relatively large poopulation of large, low-threshold fibers. It is reasonable to hypothesize that spinal stimulation would activate the pyramidal tract. While direct spino-cortical afferent connections have been reported, this response is probably due to antidromic conduction in the pyramidal tract.

This work has been supported by funds from VA Rehabilitation R&D and VA Medical Research.

279.31

MEASUREMENT OF MONOAMINES AND METABOLITES IN DORSAL HORN WITH MULTIPLE DIALYSIS FIBERS IN URETHANE-ANESTHETIZED RATS. JL Steinman, DB Masters & BR Komisaruk. Rutgers Univ, Inst. Animal Behav. Newark NJ 07102.

We have shown previously that vaginal stimulation (VS) evokes increases in NE & 5HT measured in spinal superfusates and that VS-induced analgesia is blocked by monoamine (MA) receptor antagonists. Using dialysis of extracellular fluid within unilateral dorsal horn, the present study analyzed the VS- or KCl-induced efflux of MA and metabolites. Two dialysis tubes (DF: 168 u dia, Cuprophan), coated with epoxy except for a 0.5 mm segment, were inserted transversely across the cord at S1 after laminectomy. DF(A) was placed 1 mm caudal to DF(B). Sample periods were 30 min. After a 2 hr baseline period, VS (400 g, every 30 sec) was administered; KCl was infused more than 90 min later. At the end of each experiment, each DF was infused with fast green dye and the spinal cord prepared for histological verification. NE levels during VS were four-tenfold greater than those in the pre-VS interval; NE levels also were elevated by KCl. In a preliminary study, KCl was infused first through DF(A): NE levels measured from this DF rose from 2.7 to 4.7 pg/ul whereas the NE levels measured in DF(B) were unaffected by the KCl infusion 1 mm away (2.7 & 2.5 pg/ul). When KCl was added to DF(B), the NE level rose to 4.5 pg/ul. Similar findings were observed for 5HIAA levels measured in the same samples.

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279.32

Nd:YAG LASER EFFECTS ON NERVOUS TISSUE IN THE RAT. U. Wesselmann, S.-F. Lin* and W. Z. Rymer, Dept. of Physiology and Biomedical Engineering, Northwestern University Medical School, Chicago, IL 60611.

This study was designed to evaluate the effects of Q-switched Nd:YAG laser light (1064 nm) on spinal cord dorsal column white matter in rats (n=14). Nd:YAG laser light penetrates biological tissue for several millimeters because of weak absorption by water and high scattering. To evoke a synchronous sensory input the sciatic nerve was stimulated electrically at supramaximal intensities. The resulting spinal cord potential was recorded from the dorsal columns of the ipsilateral side and consisted of three components: An early positive peak (P1), representing the responses of the most rapidly conducting fibers, followed by two negative waves (N1, N2), which are mainly due to synaptic effects of small afferent fibers on dorsal horn cells. Laser pulses at 50 mJ/pulse and above caused a severe reduction in the amplitudes of N1 and N2. In contrast, the amplitude of P1 was not significantly altered.

The observed selective loss of the synaptic field in the spinal cord might be due to preferential impairment of high threshold fiber input to dorsal horn neurones by laser irradiation, since studies on compound action potentials of dorsal roots (n=7) and the sciatic nerve (n=13) showed that irradiation with Q-switched Nd:YAG laser light caused a progressive reduction of the late component of the electrically evoked compound action potential, implying a differential effect on slow versus fast conducting fibers. Alternatively, the selective impairment of the synaptic field in the spinal cord could be due to differential effects of laser irradiation on synaptic transmission. In either event it is likely that the mechanism of the observed effects of laser irradiation on electrically evoked potentials in the spinal cord is at least in part photothermally mediated, since intracord temperatures during laser application were above 60° C (denaturation point).

Supported by the Medical FEL Program ONR/SDIO N00014-86-K-0188, and by VA Merit Review (W. Z. R.).

279.33

MEMORY SUBSTRATES IN PRIMATE SPINAL CORD PRODUCED BY OPERANT CONDITIONING OF H-REFLEX: ANATOMIC METHODS DEVELOPMENT. C.L. Lee, J.S. Carp, and J.R. Wolpaw. Wadsworth Labs, NYS Dpt Hlth, Albany, NY 12201.

Monkeys can increase or decrease the largely monosynaptic triceps surae (TS) H-reflex in one leg when reward depends on reflex amplitude (J. Neurophysiol. 57:443-458, 1987). Conditioned reflex asymmetries persist after the animal is anesthetized and thoracic cord transection isolates the lumbosacral cord (Wolpaw & Lee, this vol.). A probable site of plasticity is the Ia afferent synapse on the alpha motoneuron.

To define this cord plasticity, conditioned animals are anesthetized, transected, studied physiologically for 2-3 days under deep anesthesia, and then sacrificed by overdose and perfused. The anatomic goal is to identify and compare TS motoneurons and their Ia synapses on conditioned and control sides of cord.

We have labeled TS motoneurons with horseradish peroxidase (HRP) by muscular injection, peripheral nerve crush, and intracellular injection. Muscle or nerve application 2-7 days before perfusion does not give sufficiently consistent or complete labeling, possibly due to the 2-3 days of anesthesia and the chronic TS nerve cuffs. Intracellular injection provides much more complete and reliable labeling of soma and dendrites. Primary afferent fibers are labeled by crushing HRP onto L6-S1 dorsal roots 15-24 hr prior to perfusion. Labeling of afferents in ventral horn begins within 5 hr, and synapses on TS motoneurons may be counted and measured.

Intracellular TS motoneuron labeling combined with primary afferent labeling by dorsal root crush is allowing identification and assessment of Ia synapses and TS motoneurons. The crucial control is furnished by the unconditioned side of the spinal cord.

(Supported by NIH NS22189 and United Cerebral Palsy.)

OPIATES, ENDORPHINS AND ENKEPHALINS: ANATOMY AND CHEMISTRY II

280.1

MORPHINE WITHDRAWAL FOLLOWING INTRACEREBROVENTRICULAR ADMINISTRATION: FUNCTIONAL ANATOMY AND BEHAVIOR. R.E. Adams and G.F. Wooten. Dept. of Neurology, Univ. of Virginia, Charlottesville, Va 22908.

Regional cerebral glucose utilization (RCGU) has been studied using [^{14}C]-2-deoxyglucose (^{14}C-2-DG) autoradiography during morphine (MS) withdrawal in rats following chronic systemic administration of MS (Brain Res. 332: 69-78, 1985). In the current study, rats were made dependent on MS administered by intracerebroventricular (ICV) infusion to determine whether any of the behaviors or RCGU changes in withdrawal result from peripheral rather than central MS effects. MS was administered into the right lateral ventricle (75µg/µL/hr) for 5 days using an Alzet osmotic minipump. Two hrs after the MS infusion, naloxone (5mg/kg) and ^{14}C-2-DG (10µCi/100g) were administered intravenously. Morphine withdrawal syndrome (MWS) behaviors were recorded for the next 50 min, the animals then killed,and brains prepared for autoradiography. RCGU increased by 27% in the lateral hypothalamus, 58% in the central nucleus of the amygdala, and 29% in the lateral habenular nucleus, changes similar to those previously described for the MWS. Behavioral signs of the MWS (jumps, wet dog shakes, weight loss and autonomic signs, including diarrhea) were present in all rats and indistinguishable from rats treated with systemic MS. Our results suggest that the behavioral characteristics of, and RCGU changes during, the MWS are similar whether dependence is produced by systemic or ICV MS administration.

280.2

MU OPIOID RECEPTORS IN THE DORSAL RAPHE ARE AN IMPORTANT SITE OF ACTION FOR SYSTEMICALLY ADMINISTERED OPIOIDS. J.K. Belknap, S.E. Laursen* and K.E. Sampson*, Dept. of Pharmacology, School of Med., Univ. No. Dakota, Grand Forks, ND 58202.

One line (strain) of mouse has been selectively bred in our laboratory for 15 generations to exhibit a very high sensitivity to levorphanol-induced analgesia on the hot plate assay (HAR or high antinociceptive response line). Concurrently, a second line (LAR or low antinociceptive response line) has been bred in the opposite direction, i.e., to exhibit a very low sensitivity to i.p. levorphanol, a morphine-like opioid, under the same conditions. A 7-fold difference in sensitivity between HAR and LAR mice has been bred into these animals through changes in gene frequency. Receptor autoradiographic studies were carried out using ^{3}H-DAGO to find receptor populations differing greatly in density between HAR and LAR mice to parallel their in vivo sensitivity differences: such receptors would then be implicated in mediating in vivo analgesia. The caudal portions of the dorsal raphe showed about two-fold differences in density of mu sites, while the periaqueductal gray showed no significant difference, suggesting that the latter is not involved at the mu receptor level.

280.3

EFFECTS OF OPIOID PEPTIDES ON THE EXCITABILITY OF CA3 HIPPOCAMPAL PYRAMIDAL CELLS IN VIVO AND IN VITRO. W. Liu*, M. Washburn* and H.C. Moises (SPON.: S. R. Barry). Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109

Opiates and various opioid peptides have been shown to increase the excitability of hippocampal pyramidal cells (HPCs) in CA1. These actions have been attributed to a disinhibitory mechanism mediated via the activation of mu (u) and delta (∂) opiate receptors. The issue examined here was whether dynorphin, an endogenous opioid localized in the mossy fiber (MF) projection from dentate granule cells, exerts similar electrophysiological actions on HPCs in CA3 and if such actions involve the activation of kappa (K) rather than u- or ∂-receptors.

In acute experiments *in vivo*, 5-barrel micropipettes were used to record extracellularly from single CA3 HPCs in halothane-anesthetized rats and to apply peptides by iontophoresis or pressure ejection. Dynorphin A_{1-8}, Dynorphin A_{1-17} and Leu-enkephalin had consistent, naloxone(NX)-reversible excitatory effects on spontaneous activity. The u-receptor agonists morphine and morphiceptin had similar but less robust facilitating effects on HPC firing. In contrast, the K agonist U50488H consistently produced NX-insensitive inhibitions of HPC discharge. Inhibitory responses to DYN A_{1-17} were observed in a small percentage of HPCs and these effects were also insensitive to NX blockade.

Superfusion of peptides in the hippocampal slice revealed a similar pharmacological profile of peptidergic effects on CA3 population spike responses evoked by MF stimulation. Dynorphin A_{1-8} and a stabilized analogue of DYN A_{1-13},DAFPHEDYN, generally increased the amplitude of the population spike and NX typically blocked this effect. However, U50488H routinely attenuated population spike amplitude both in the absence and presence of the antagonist (1-10 uM).

These results clearly distinguish the electrophysiological actions of dynorphin in CA3 from that of a prototypical K-receptor agonist. This raises the possibility that endogenous dynorphins might influence HPC excitability in this region of hippocampus via interaction at u and/or ∂ receptors, as occurs in CA1. (Supported by NIDA grant DA-03365)

280.4

USE OF BIOTINYLATED β-h-ENDORPHIN IN PURIFICATION OF SOLUBILIZED CALF BRAIN OPIATE RECEPTORS. D. Helmeste. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada M5T 1R8.

The high affinity between avidin and biotin (K_d=10^{-15} M) makes possible the isolation of numerous biological materials via derivatization with either biotin or avidin and subsequent affinity chromatography. This method has been applied to the isolation of the opiate receptor, by derivatizing βh-endorphin (β-EP), a potent opiate peptide, which maintains high affinity for the opiate receptor throughout the solubilization procedure. β-EP was derivatized with sulfosuccinimidyl 2-(biotinamido) ethyl-1,3'-dithiopropionate (NHS-SS-Biotin) using 2 to 1 molar ratio of biotin to peptide. With 0.05 M potassium phosphate (pH 7) as the incubation buffer, 90% of the peptide precipitated during the 4 h, 22°C incubation. HPLC analysis revealed that this precipitate represented biotinylated β-EP (BBE) only. Underivatized (free) β-EP (<10% of total peptide) remained in the supernatant. The precipitated BBE was then redissolved in dimethyl sulfoxide and characterized in terms of: (1) radioligand binding to calf brain membrane opiate receptors; (2) binding to solubilized calf brain opiate receptors; (3) HPLC analysis of tryptic digests and native peptide. Affinity of BBE for opiate receptors is only slightly reduced compared to β-EP alone. Use of BBE for affinity purification (avidin-sepharose) of solubilized opiate receptor appears to be useful as an initial step in purification of the opiate receptor.

Supported by the Medical Research Council of Canada (MA-10179).

280.5

SOLUBILIZATION OF AN OPIATE RECEPTOR COMPLEX FROM CALF BRAIN. L.R. Murthy*, M.A. Gistrak*, M. Price*, and G.W. Pasternak (Spon: W.R. Shapiro). The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Cornell U. Medical College, New York, NY 10021.

^{3}H-NalBzoH, the benzoylhydrazido derivative of naloxone, is a unique opiate ligand. In binding studies it labels mu receptors and a non-mu, non-delta site which may represent a novel kappa receptor subtype. Binding to mu receptors is unique in that the half-life of dissociation at 25°C is approximately 24 hours. Despite this slow rate of dissociation, binding is not covalent and can be reversed readily by lowering the pH of the buffer or adding GTP or stable GTP analogs. In view of the stability of binding, we attempted to solubilize receptors prebound with ^{3}H-NalBzoH. CHAPS (10 mM) solubilzed approximately 20-35% of specific ^{3}H-NalBzoH binding. The solubilized binding remained sensitive to both lowered pH and GTP analogs, implying that the solubilized binding was not covalent. Attempts to identify residual binding in the membrane pellet have been unsuccessful, but we cannot be certain whether all sites have been solubilized. Preliminary exclusion chromatography suggests a molecular weight of greater then 200 kdaltons, which probably reflects a complex of the receptor and other associated proteins.

280.6

SOLUBILIZATION AND CHARACTERIZATION OF SIGMA RECEPTOR FROM GUINEA PIG BRAIN MEMBRANES. M. P. Kavanaugh*§, J. Parker*§, D. H. Bobker*§, J. F. W. Keana*¶, and E. Weber§. § Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201 and ¶Department of Chemistry, University of Oregon, Eugene, OR 97403

The sigma receptor, a distinct binding site in brain tissue which may mediate some of the psychotomimetic properties of benzomorphan opiates and phencyclidine, has been solubilized using the ionic detergent sodium cholate. The solubilized receptor has been biochemically and pharmacologically characterized using gel permeation chromatography, isoelectric focusing, and binding studies with tritiated sigma-selective ligands. Brain membrane suspensions were treated with 20 mM cholate followed by ultracentifugation at 105,000 x g and filtration through a 0.45u filter. Binding assays were performed with the solubilized receptor using vacuum filtration over polyethyleneimine-treated glass fiber filters. The pharmacological specificity of the solubilized binding site for sigma receptor ligands is very similar to the membrane-bound form of the receptor, with the order of potencies for displacement of the selective sigma ligand [^{3}H]-di-o-tolylguanidine ([^{3}H]DTG) well correlated (r=.88). The stereoselectivity for (+) benzomorphan opiate enantiomers was retained by the solubilized receptor. The soluble receptor retained high affinity for binding of [^{3}H]DTG (Kd = 31 ± 4 nM) and [^{3}H] (+)-3-(3-hydroxyphenyl)- N-(1-propyl) piperidine ([^{3}H](+)-3-PPP) (Kd = 33 ± 7 nM). Estimation of the Stokes radius of the [^{3}H]DTG binding site was obtained by Sepharose Cl-6B chromatography in the presence of 20 mM cholate and calculated to be 8.7Å. This value was identical to the molecular size of the binding sites of the sigma-selective ligands [^{3}H](+)-3-PPP and [^{3}H](+)-SKF-10,047. These results demonstrate that the sigma receptor is a membrane-bound complex which can be solubilized in a form that retains the pharmacological characteristics of the native form, providing a basis for further biochemical characterization and purification of the receptor.

280.7

PURIFICATION AND PHARMACOLOGICAL CHARACTERIZATION OF THE SIGMA RECEPTOR FROM RAT AND BOVINE CEREBELLUM. F.J Arnold, D.I. Schuster and R.B. Murphy. Dept. of Chemistry and Center for Neural Science, New York University, New York, N.Y. 10003

We have purified to apparent homogeniety and pharmacologically characterized the sigma receptor from both rat and bovine cerebellum. Tissue was solubilized with the detergent CHAPS and recirculated through an affinity column derivatized with a 3-PPP analog previously prepared in our laboratories. The column was eluted with 20 uM haloperidol. Purified material was desalted, subjected to SDS-PAGE, and pharmacologically characterized. The material was purified ~6,000 fold based upon biological activity. It consisted of two major bands on SDS-PAGE of M_r 63 and 65 KD. In order to examine the pharmacology of the purified receptor it was necessary to reconstitute into bimolecular lipid vesicles. Reconstituted material possessed appropriate pharmacology of the sigma receptor; enantiospecificity with both (±) SKF 10,047 and (±) butaclamol, high affinity toward haloperidol, and the newly described sigma ligand 1,3-di-o-tolyl-guanidine. Spiperone, ketanserin, prazosin, phencyclidine, and etorphine possessed little or no activity. Current studies are directed toward the molecular sequencing and structural determination of the isolated proteins.

280.8

PHOTOAFFINITY LABELING OF AFFINITY PURIFIED RAT AND BOVINE CERRBELLAR SIGMA RECEPTOR WITH [^{3}H]-AZIDO-1,3-DI-O-TOLYL-GUANIDINE (DTG). D.I. Schuster, F.J. Arnold, and R.B. Murphy. Dept. of Chemistry and Center for Neural Science, New York University, New York, N.Y. 10003

We have recently purified to apparent homogeniety the sigma receptor from rat and bovine cerebellum (Arnold et al Soc. Neurosci. Abstr., this meeting). The receptor consists of two bands on SDS-PAGE of M_r 63 and 65 KD. Recent studies of the sigma receptor in guinea pig brain homogenates using the highly selective photoaffinity label [^{3}H]-Azido-DTG indicate that a single component on SDS-PAGE of 29 KD is covalently labeled (Kavanaugh et al PNAS in press). In order to elucidate the relationship between these observations we photolysed [^{3}H]-Azido-DTG with the affinity-purified sigma receptor. Under non-denaturing conditions, a high molecular mass complex (300-400 KD) was covalently labeled. Incorporated activity was displaced in the presence of the selective sigma ligand haloperidol. A similar fraction was labeled in crude tissue homogenates. These results suggest that the 29 KD photoaffinity labeled protein is a component of the affinity purified sigma receptor. We extend our sincere thanks to M.P. Kavanaugh and E. Weber for their gift of [^{3}H]-Azido-DTG.

280.9

CHARACTERIZATION OF LOW AFFINITY DELTA OPIOID RECEPTORS COUPLED TO ADENYLATE CYCLASE IN RAT BRAIN MEMBRANES. S. R. Childers, J. Harris and J. King, Dept. of Pharmacology, Univ. Florida Coll. Med., Gainesville, FL 32610.

Since receptor-mediated inhibition of adenylate cyclase (AC) requires NaCl and GTP, μM concentrations of opioid agonists are required to inhibit AC in brain membranes. Our previous studies showed that blockade of high affinity (nM) opiate receptors with alkylating agents or phospholipase A (100 ng/ml) had no effect on opioid inhibition of AC. To determine whether AC is coupled to low affinity receptor sites, we performed binding studies in brain membranes with [3H]naloxone in buffer containing both NaCl and GTP. The affinity of naloxone in blocking opiate-inhibited AC is 30-50 nM, so we used 50 nM [3H]naloxone to label low affinity binding sites, and displaced naloxone binding with various unlabeled agonists. Under these conditions, IC_{50} values of both mu and delta agonists were μM, with the most potent being mu agonists. However, the delta agonists DPDPE and DSTLE exhibited a biphasic displacement of [3H]naloxone binding, with a distinct plateau at 1 μM agonist concentration. This plateau was approx. 25% of total [3H]naloxone binding, and was defined as low affinity delta sites. These sites had an identical distribution to opiate-inhibited AC in rat brain, with highest levels in striatum and frontal cortex. Like opiate-inhibited AC, the low affinity delta sites were not sensitive to naloxonazine or ß-FNA, but were blocked by ß-CNA. Compared to high affinity delta sites ([3H]DPDPE binding without NaCl/GTP), the low affinity delta sites were significantly less sensitive to phospholipase A and ß-CNA. Protection experiments, in which the effects of ß-CNA were blocked by pre-incubation with specific ligands, revealed clear differences between high and low affinity delta sites, since high affinity delta sites were protected by 200 nM DPDPE but not 200 nM naloxone, while low affinity delta sites, and opiate-inhibited AC, were protected by equal concentrations of both naloxone and DPDPE. These studies show that properties of opiate-inhibited AC correlate with those of low affinity delta receptors, and suggest that these two states of delta sites have different binding properties.

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280.10

OPIATE REGULATION OF ADENYLATE CYCLASE IN C-6 GLIOMA CELLS AND NEONATAL RAT PRIMARY NEURONAL CULTURES. D.R. Marckel and S.R. Childers. Dept. of Pharmacology, Univ. of Florida Coll. Med., Gainesville, FL 32610.

Opiates regulate adenylate cyclase activity in various cell lines (i.e. NG108-15) and mammalian brain. C-6 glioma cells express opiate receptors when treated with desmethylimipramine, but supposedly lack opiate receptors when left untreated. We now report that opiates have both inhibitory and stimulatory actions on adenylate cyclase in untreated C-6 cells, and we confirm some of these actions in primary neuronal cell cultures from neonatal rat brain.

Suspensions of C-6 glioma cells in EGTA buffer were incubated with theophylline and various agents for 10 min at 37°. cAMP levels were assayed by the protein binding method. D-ala-met-enkephalinamide (D-ala enk) produced a biphasic response with inhibition predominant at low (nM) concentrations and stimulation at higher (μM) concentrations. The inhibitory component was best observed in the presence of 0.1-1.0 μM forskolin, where D-ala enk inhibited 20-30% of forskolin stimulated cAMP levels. Preincubation with phorbol 12-myristate 13-acetate (PMA) eliminated the inhibition and shifted the stimulation to slightly lower concentrations. In PMA-treated cells, basal cAMP levels were decreased, while the % stimulation of cAMP by isoproterenol was increased. Similarly, stimulation by D-ala enk was increased to 2-3 fold. To confirm that these effects of opiates were mediated through adenylate cyclase, identical experiments were conducted with C-6 cells permeabilized with saponin, with adenylate cyclase assayed in the presence of [3H]ATP, GTP, and opiate agonists. Results were identical to those seen in intact cells.

Primary neuronal cultures were made from 1 day old rats in which the whole brain was removed, cleaned of pia, triturated, treated with trypsin and DNAse I, and plated. Cultures were treated with cytosine arabinoside for 3 days to decrease the glial population and obtain cultures of 80-85% neurons. After 10 days of culture, cAMP levels were determined in cells incubated for 5 min at 37° with isobutylmethylxanthine (IBMX) and various agents. D-Ala enk produced a marked stimulation (≈5 fold) of cyclic AMP levels, whereas isoproterenol was able to stimulate cyclic AMP levels 10-20 fold. These results suggest that in C-6 glioma cells, as well as primary neuronal cultures, opiate receptors have dual effects on adenylate cyclase activity.

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280.11

INHIBITION OF MEMBRANE PROTEIN PHOSPHORYLATION BY OPIATE-INHIBITED ADENYLATE CYCLASE IN RAT BRAIN MEMBRANES. L. M. Fleming, G. Ponjee and S. R. Childers, Dept. of Pharmacology, Univ. Florida Coll. Med., Gainesville, FL 32610.

Opiate-inhibited adenylate cyclase (AC) results in decreased protein phosphorylation by cAMP-dependent protein kinase. Pretreatment of rat brain membranes at pH 4.5 maximizes opiate-inhibited AC. In order to discern which proteins are ultimately affected by this opiate inhibition, rat striatal membranes were pretreated at pH 4.5 and resuspended in a Mg++/Tris buffer containing IBMX, GTP, NaCl, and EGTA. Membranes were preincubated with 0.5 mM App(NH)p for 5 min at 30°C before addition of 10 μCi (0.1 μM) of γ-[32P]ATP for 20 sec. The reaction was terminated with SDS sample buffer, and protein samples were subjected to gradient SDS-PAGE and autoradiography. Incubation with EGTA blocked calcium and calmodulin-dependent kinases and provided for maximum effects of cAMP-dependent phosphorylation. App(NH)p is a substrate for AC, but is not a good substrate for most ATP-kinases and therefore did not block phosphorylation of most proteins.

In the presence of App(NH)p, proteins stimulated directly by cAMP were also stimulated by forskolin. Forskolin had no effect on protein phosphorylation in the absence of App(NH)p. Since forskolin stimulates AC, it should also increase the signal for opiate inhibition and aid in identifying opioid-inhibited phosphoproteins. Addition of D-ala-met-enkephalinamide (D-ala-enk) decreased phosphorylation of two protein bands of MW 63 kDa and 85 kDa. Both bands were stimulated by forskolin. D-Ala enk-inhibited phosphorylation was observed in both basal as well as forskolin-stimulated conditions, as long as forskolin concentration was <1 μM. Consistent with the idea that D-Ala enk-inhibited phosphorylation was mediated by opiate-inhibited AC, μM concentrations of agonist were required for inhibition, and inhibition was more pronounced in low pH pretreated membranes than in untreated membranes. The inhibition of phosphorylation by D-ala enk was blocked by naloxone. Inhibition by D-Ala enk was maximal in striatum, while inhibition of the same protein bands by the mu agonist DAGO was observed in membranes from thalamus. These results suggest that two principal targets of opiate-inhibited AC in brain membranes are cyclic AMP-dependent membrane phosphoproteins of 63 kDa and 85 kDa.

Supported by PHS grant DA-02904 from the National Institute on Drug Abuse.

280.12

RAT BRAIN MICROSOMES CONTAIN G-PROTEIN UNCOUPLED OPIOID RECEPTORS. M. Sztics* and C.J. Coscia, Dept. of Biochemistry, St. Louis Univ. Sch. Med., St. Louis MO 63104.

Using subcellular fractionation techniques developed in our laboratory we have separated two discrete populations of opioid receptors from rat brain. One is associated with a synaptic plasma membrane (SPM) enriched fraction, and the other with smooth microsomes. The relative distribution of high affinity agonist μ, δ and κ receptor binding and its modulation by guanyl nucleotides, Na+ and divalent cations were examined using highly selective radioligands. Mn++ (0.2-50 mM) elicited a concentration-dependent inhibition of μ and κ binding, but increased δ binding in both fractions. Microsomal sites were more sensitive to divalent cations and the inhibitory effect of Na+ than SPMs, and they also lacked the sensitivity to the dose-dependent guanyl nucleotide inhibition displayed by SPMs and crude membrane fractions. To determine whether GTP-binding (G) proteins were present in microsomes, they were treated with [32P]NAD in the presence of pertussis toxin. A doublet with molecular weights of 39 and 41 kDa was labeled in both microsomes and SPMs. Thus, microsomes also possess the α-subunit of pertussis toxin-sensitive G protein. Combined with independent binding kinetics data, these results suggest that opioid receptors in rat brain microsomes are not coupled to G-protein. Supported by NSF grant BNS 85-08933.

280.13

PRENATAL OPIOID RECEPTOR BINDING IN RAT BRAIN: [125I]BETA-ENDORPHIN AUTORADIOGRAPHY . H.I. Kornblum, S.E. Loughlin and F.M. Leslie. , U.C. Irvine, Irvine,CA 92717.

Previous studies have demonstrated the presence of opioid receptors in the developing rat brain. These studies have shown that the different receptor subtypes change in both distribution and density during development. However, the majority of these studies have been performed using postnatal rats. In the present study we have used [125I]Beta-endorphin (BE) to localize opioid receptors in the embryonic and postnatal rat brain. The development of these receptors is correlated with the ontogeny of opioid peptides in selected brain areas. Rat brains from embryonic day 13 (E13) to adulthood were frozen and prepared for in vitro autoradiography using 50 pM [125I]BE in the presence or absence of a variety of unlabelled competing ligands. In the adult, binding was to mu and delta receptors, with the mu receptor component being completely inhibited by 300 nM DAGO. All binding was inhibited by 1 uM levallorphan. In the embryonic rat, binding was present by E16. Binding was present almost entirely within the developing neostriatum and was completely inhibited by DAGO. By E19 binding sites were more extensively distributed. Binding within the neostriatum was homogeneous. Binding was also present within the brainstem, the epithalamus, and the olfactory bulb, as well as within the germinal zone bordering the lateral ventricles. By P0, binding was present in many more brain regions, taking on a distribution more similar to the adult. Within the neostriatum the ontogeny of opioid receptors correlates with that of enkephalin-immunoreactive striato-pallidal fibers which are present by E17. The presence of binding sites within the germinal zone suggests that opioid receptors are present on dividing or migrating cells, and is consistent with previous observations of Beta-endorphin immunoreactive cells within that region. The present data and previous studies demonstrate the presence of opioid peptides and receptors early during brain development, indicating that endogenous opioid systems are functional in the developing brain.

Supported by NIH grant NS 19319.

280.14

NEONATAL RAT BRAIN δ OPIOID RECEPTORS. C.J. Coscia and M. Szücs*, (SPON. S. McLean) E.A. Doisy Dept. of Biochemistry St. Louis Univ. Sch. Med., St. Louis MO 63104.

Rat brain contains at least three classes of opioid receptors, μ, δ, and κ, which undergo differential postnatal development. While both μ and κ opioid receptor binding are detectable in brain of new-born rats, high affinity δ binding is absent. During the first two weeks after birth δ receptor binding rapidly emerges. By analyses of homologous displacement curves with the LIGAND computer program, we have compared the binding parameters, Kd and Bmax, of the δ selective enkephalin analog, ^{3}H(D-Pen^2,D-Pen^5) enkephalin (^{3}H-DPDPE) for opioid binding sites in 5-day-old neonates and adult rats. Although binding affinity did not change appreciably, >5-fold increases in Bmax were observed. Heterologous displacement of ^{3}H-DPDPE with μ and δ selective ligands gave the same rank order of potency in neonates and adults. Pertussis toxin-mediated ADP ribosylation experiments revealed the presence of the α subunit of guanine nucleotide binding proteins, Go and Gi, in brain as early as 0.5 h after birth. Inhibition of ^{3}H-DPDPE binding by Gpp(NH)p did not change significantly between day 5 and adulthood. The authentic δ character of the sites recognized by ^{3}H-DPDPE in the neonate was further evidenced by divalent cation stimulation of its binding. Supported by NIH grant DA05412.

280.15

WITHDRAWN

280.16

CYCLIC [D-PEN^2,D-PEN^5]ENKEPHALIN (DPDPE) ANALOGUES WITH INCREASED AFFINITY AND SELECTIVITY FOR DELTA OPIOID RECEPTORS. G.K. Lui*, G. Toth*,R.J. Knapp*, V.J. Hruby*, and H.I. Yamamura.(SPON: P. Deshmukh).Departments of Pharmacology and Chemistry, University of Arizona, Tucson, Arizona 85724.

[D-Pen^2,D-Pen^5]enkephalin (DPDPE) is a cyclic, conformationally constrained enkephalin analogue with high delta opioid receptor selectivity. DPDPE was modified by halogenation of the phenylalanine residue . A series of modified DPDPE analogues were evaluated by radioligand binding studies using rat brain (minus cerebellum) membranes in Tris-buffer (50mM). Incubation was for 2 hr at 25C. The binding affinity and selectivity of four DPDPE analogues were the following:

	[^{3}H]DPDPE	[^{3}H]CTOP	RATIO
Analogue	IC_{50}(nM)		μ/δ
DPDPE	5.25	609	116
pF-Phe^4-DPDPE	2.50	623	249
pCl-Phe^4-DPDPE	1.57	901	574
pI-Phe^4-DPDPE	4.74	964	203
pBr-Phe^4-DPDPE	1.73	418	242

Halogenation in the para position of the DPDPE phenylalanine residue increased both its affinity and selectivity for the delta opioid receptor.

280.17

COMPARATIVE SATURATION STUDIES ON TWO NON-SELECTIVE OPIOID LIGANDS IN GUINEA-PIG BRAIN. J. Magnan and M. Tiberi. Dép. de pharmacologie, Univ. de Montréal, Montréal H3C 3J7 Canada

In order to ascertain whether [^{3}H]-bremazocine and [^{3}H]-ethylketazocine are interchangeable universal opioid ligands, we have used these and selective μ-, δ- and κ-opioid ligands and measured their relative equilibrium dissociation constant (K_D) and respective maximum binding capacity. Results show that μ-, δ- and κ-selective ligands respectively label 7.22 $\pm$ 0.66, 3.33 $\pm$ 0.10 and 4.47 $\pm$ 0.20 pmoles/g tissue for a total opioid binding capacity of 15.0 pmoles/g tissue. The saturation curve for [^{3}H]-ethylketazocine is best-fitted to a two-sites model with a high-affinity K_D of 0.19 $\pm$ 0.02 nM and a capacity of 5.32 $\pm$ 1.11 pmoles/g tissue and a low-affinity component with a K_D of 2.38 $\pm$ 0.83 nM for a capacity of 9.34 $\pm$ 0.72 pmoles/g tissue. Total binding capacity for [^{3}H]-ethylketazocine is 14.7 $\pm$ 1.11 pmoles/g tissue which corresponds with the total (μ + δ + κ) opioid capacity. [^{3}H]-bremazocine binding can also be resolved into high and low affinity components of respective K_D 0.09 $\pm$ 0.01 nM and 0.90 $\pm$ 0.14 nM and capacities of 11.0 $\pm$ 1.34 and 16.2 $\pm$ 1.69 pmoles/g tissue. Thus its total binding capacity is 27.2 $\pm$ 0.86 pmoles/g tissue, a value significantly higher than that of [^{3}H]-ethylketazocine. It seems that these two cannot be used indiscriminately as non-selective opioids.

280.18

EVIDENCE FOR KAPPA OPIOID RECEPTOR HETEROGENEITY, D.E. Hurlbut, R.S. Broide* and F.M. Leslie, University of California, Irvine, Ca. 92717, U.S.A.

Using membrane binding techniques, we have compared the binding properties of [^{3}H]ethylketocyclazocine (EKC) and [^{3}H]diprenorphine (DIP) binding in guinea pig and rat brain. In all experiments, selective blockers were included within the assay buffer to preclude ligand binding to mu and delta receptor types. Specific binding was defined as the difference in radioactivity in the absence and presence of levallorphan (1uM). In guinea pig brain, saturation curves for both radioligands best fit a one-site model. However, there were significantly fewer [^{3}H]EKC than [^{3}H]DIP binding sites. Dose-response curves for inhibition of [^{3}H]DIP binding by several competing ligands were biphasic, and could be best fit to a two-site model; Dynorphin A and U50,488H exhibited over 1000-fold differences in affinity at these two sites. In contrast, dose-response curves for inhibition of [^{3}H]EKC binding were monophasic and best fit a single site model. K_i values for inhibition of [^{3}H]EKC binding were highly correlated with K_i values for inhibition of the U50488H-sensitive [^{3}H]DIP binding site. These data are consistent with the hypothesis that [^{3}H]DIP labels two sites with equal affinity in guinea pig brain, one of which is selectively labeled by [^{3}H]EKC. Preliminary membrane binding and autoradiography data have indicated a differential regional distribution of U50-sensitive and -insensitive sites throughout guinea pig brain. In rat brain, dose-response curves for inhibition of [^{3}H]DIP binding were consistently monophasic. There was a high correlation between K_i values for inhibition of [^{3}H]DIP binding in rat brain and K_i values for inhibition of the U50488H-insensitive [^{3}H]DIP binding site in guinea pig brain. These data suggest that guinea-pig brain contains two kappa-like [^{3}H]DIP binding sites while rat brain contains only one.

This work was supported by NIH grant NS 19319.

280.19

NEUROANATOMICAL DISTRIBUTION OF RAT AND GUINEA PIG BRAIN KAPPA OPIOID RECEPTORS: EVIDENCE FOR MULTIPLE KAPPA RECEPTOR SUBTYPES. E.M. Unterwald and R.S. Zukin. Dept. Neuroscience, Albert Einstein Col. of Med., Bronx, NY 10461.

Recent data from our laboratory have suggested the presence of two populations of κ opioid receptors in brain. The κ_1 site predominates in guinea pig, is of high affinity and U-69,593-sensitive, and the κ_2 site predominates in rat brain, is of lower affinity and U-69,593-insensitive. This study investigates the neuroanatomical distribution of κ receptor subtypes in rat and guinea pig brain using quantitative *in vitro* autoradiography. Total κ receptors were labelled with [^{3}H](-)bremazocine or [^{3}H](-)ethylketocyclazocine (EKC) in the presence of an excess of DAGO and DADLE, κ_1 receptors with [^{3}H]U-69,593 and κ_2 sites with [^{3}H](-)bremazocine or [^{3}H]EKC in the presence of DAGO, DADLE and U-69,593. Guinea pig κ_1 sites were most dense in layers I and V-VI of the neocortex, striatum, endopiriform nucleus, cerebellum and primary olfactory cortex. κ_2 sites were found in guinea pig striatum, nucleus accumbens, hippocampus, thalamus, and inferior colliculus. Distribution of κ_1 sites in rat brain was similar to that of guinea pig in the striatum, nucleus accumbens and endopiriform nucleus but differed in the neocortex, cerebellum and hippocampus and overall, were of much lower density in rat brain. κ_2 receptors in rat brain were of higher density and more widely distributed than in guinea pig brain. Results from this study provide evidence for heterogenous neuroanatomical distributions of κ_1 and κ_2 opioid receptors.

280.20

UNEXPECTED BINDING PROFILE OF [^{3}H]-BREMAZOCINE IN GUINEA-PIG BRAIN. M. Tiberi and J. Magnan, Dép. de pharmacol., Univ. de Montréal, Montréal H3C 3J7 Canada

We have previously demonstrated that the non-selective ligand [^{3}H]-bremazocine labels more than μ-, δ- and κ-opioid binding sites in guinea-pig brain. The pharmacological characterisation of this additional opioids site was studied using a series of opioid compounds known to be μ-, δ- and κ-selective and also non-selective opioid ligands. Competition assays were performed with [^{3}H]-bremazocine in the presence of unlabelled μ-, δ- and κ-selective opioids to suppress its binding at those sites. The relative equilibrium dissociation constants (K_d) were calculated from the displacement curve using a computerized curve fitting program. The results show that μ- and δ-selective compounds have a very low affinity (K_d > 5 μM) for this binding site. Opioids such as diprenorphine, cyclazocine, ethylketazocine, levallorphan, U-50,488H and nalorphine have higher affinity with K_D values varying between 5 and 125 nM. Furthermore, (-) SKF 10,047 and (+) SKF 10,047 have respective K_d values of 45 nM and 7227 nM demonstrating the stereoselectivity of this non-conventional opioid site. We have established a correlation between the affinities of the various opioids at this binding site and their effects in different bioassays. The results suggest that this unexpected opioid site labelled with [^{3}H]-bremazocine might be a κ-related site.

280.21

COMPARATIVE DISTRIBUTION OF OPIOID RECEPTOR SUB-TYPES IN HUMAN STRIATAL TISSUES. C. Pilapil, J. Magnan and R. Quirion. Douglas Hosp. Res. Centre and Dept. of Pharmacology, Univ. of Montreal, Quebec, Canada H4H 1R3.

In the rat striatum, mu opioid receptors are concentrated in "patches" or "striosomes" associated to acetylcholinesterase-poor areas. We report here on the comparative localization of mu, delta and kappa opioid binding sites in human striatal tissues. Post-mortem human brain brains were processed as recently described (Quirion et al, Synapse 1, 448-454, 1987). Brain sections were then incubated under appropriate conditions (Pilapil et al, Brain Res. Bull. 19, 611-615, 1987) in presence of [^{3}H]DAGO, [^{3}H]DTLET and [^{3}H]bremazocine (with mu and delta blockers) or [^{3}H]U-69,593 to label mu, delta and kappa sites, respectively. Our results clearly demonstrate the differential distribution of mu, delta and kappa sites in human striatal tissues. In contrast to rat brain, mu sites are densely but diffusely distributed in rat striatum. Interestingly, kappa binding sites are heterogeneously distributed in the human striatum. This is especially evident in the caudate nucleus where kappa binding sites are clearly associated to acetylcholinesterase-poor striosomes. Thus, important species differences exist in regard to the association between striosomes and opioid receptor sub-types in striatal tissues.

280.22

OPIATE RECEPTORS IN THE SUBSTANTIA NIGRA EXIST PRIMARILY ON STRIATO-NIGRAL AFFERENTS. N.L. Ostrowski and A. Pert. LCM and BPB, NIMH, Bethesda, MD 20892.

Autoradiographic studies were conducted to examine opiate receptor binding relative to dopamine neurons. Afferent and efferent connections to the substantia nigra were eliminated in male rats with 6-hydroxydopamine lesions of the medial forebrain bundle or electrolytic lesions of the internal capsule. Internal capsule lesions produced significant decreases in the dorsal (pars compacta) and ventral (pars reticulata) substantia nigra, the ventral tegmental area and the medial terminal nucleus of the accessory optic tract. In contrast, elimination of the dopamine cells produced minimal change in [^{3}H]naloxone binding in the same regions. These data suggest that the primary site of opiate receptor binding is on nigral afferents, and not on dopamine neurons.

280.23

MEDIOBASAL AND PREOPTIC AREA OPIOID BINDING SITES ARE DECREASED FOLLOWING TRANSECTION OF THE ASCENDING NORADRENERGIC PATHWAY. M.G. Dube*, A. Sahu*, S.P. Kalra, and W. Jacobson* (SPON: M.W. HEFT). Department of Obstetrics and Gynecology, University of Florida, Gainesville, FL 32610

LH secretion can be influenced both by endogenous opioid peptidergic (EOP) as well as adrenergic systems. However, uncertainty persists as to the nature of the anatomical relationship between the two. Using intact brain slices (Life Sciences 39: 2037, 1986), we have investigated the effect of interrupting the ascending noradrenergic fibre pathway (ANP) on [^{3}H]-Naloxone (NAL) binding sites in the mediobasal hypothalamus (MBH) and the preoptic area (POA). Two weeks following transection of the ANP in intact male rats, MBH levels of norepinephrine were decreased by 54.4%, and epinephrine levels were undetectable. Neither dopamine nor serotonin systems were affected by the transection. Similar changes were noted in the POA. Saturation binding curve analysis indicated that this loss of catecholamines was accompanied by a parallel loss in the number of NAL binding sites in slices of MBH (B_{max}: sham=45.9±2.5; cut=27.1±9.5 fm/mg tissue) and of POA (B_{max}: sham=58.7±3.6; cut=36.6±2.3 fm/mg tissue). The affinity of the receptor for the ligand was unaffected by the transection (MBH: K_D: sham=3.8±0.4 nM; cut=3.0±1.9 nM; POA: K_D: sham=3.0±0.3 nM; cut=2.2±0.3 nM). Cortical NAL binding sites were unaffected by ANP transection. The receptor population was further investigated in the MBH at 5 and 8 weeks following ANP transection. The amount of NAL bound remained depressed in the transected group and did not recover with time (7.0 nM NAL; 2 wk: sham= 28.7±1.7, cut= 22.2±1.5; 5 wk sham= 24.7±2.0, cut= 19.6±1.3; 8 wk sham= 25.8±1.7, cut= 20.1±0.9 fm/mg). These data indicate the presence of opioid binding sites presynaptically on noradrenergic nerve fibers innervating the preoptic area and the mediobasal hypothalamus. Supported by NIH HD11362.

280.24

DISTRIBUTION OF SPECIFIC OPIOID RECEPTOR SUBTYPES AND THEIR CORRELATION WITH ENKEPHALIN IMMUNOREACTIVITY IN THE DORSOLATERAL PONS. Robert M. Bowker and Roger P. Dilts, Dept. of VCAPP, Washington State Univ., Pullman, WA. 99164

The nuclei of the dorsolateral pons--the medial and lateral parabrachial nuclei and the locus coeruleus--are known to have wide spread connectivities. In the present study the localization of the specific opioid receptor subtypes were ascertained using in vitro autoradiography and were correlated with the enkephalin immunoreactivity.

In vitro receptor autoradiographic studies were performed with the selective ligands DAGO and DPEN 2,5-enkephalin. After incubations these two receptor subtypes were evident in the dorsolateral pons. Differential distributions of the receptors were seen within a subregion of these nuclei. Immunocytochemistry was performed using the peroxidase reaction which revealed dense patterns in both the lateral and medial parabrachial nuclei, as well as the locus coeruleus nucleus. Differential distributions of the immunoreactive fibers and neurons were also evident. The opioid receptor subtypes and the enkephalin immunoreactivity are being correlated. These findings suggest that these nuclei probably have sub regions mediating different functions and support the notion that opioid neurotransmission is quite complex and entails many subtleties necessary to carry out their actions. Supported by NIH grants NS22321 and NS24388.

280.25

CHRONIC ADMINISTRATION OF MORPHINE AND NALTREXONE UP-REGULATE MU OPIOID BINDING SITES LABELED BY ^{3}H-DAGO: FURTHER EVIDENCE FOR TWO MU BINDING SITES. R.B. Rothman[1], V. Bykov[1], J.B. Long[2], L.S. Brady[3], M. Little[3], M. Herkenham[3], A.E. Jacobson[4], K.C. Rice[4] and J.W. Holaday[2]. [1]LCS and [3]Unit on Functional Neuroanatomy, NIMH, and [4]Section on Drug Design and Synthesis, NIDDK, Bethesda, MD, 20892. [2]Neuropharmacology Branch, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

In this study we examined the hypothesis of two mu binding sites (mu_{CX} and mu_{nCX}) by studying the effect of chronic administration of morphine and naltrexone to rats (via subcutaneous pellet implantation) on mu binding sites labeled by ^{3}H-DAGO. We additionally measured kappa binding sites labeled by ^{3}H-bremazocine, and conducted receptor autoradiography studies at the level of the thalamus to localize which brain areas were up-regulated by chronic morphine.. Mu opioid binding sites were labeled with ^{3}H-DAGO, using membranes preincubated in the absence (CNT-P2) or presence of 1 uM beta-funaltrexamine (FNA-P2). Kappa binding sites were labeled using membranes pretreated with the site-directed acylating agents BIT (mu-selective) and FIT (delta-selective). ^{3}H-DAGO results:

	CNT-P2		FNA-P2	
	Bmax	Kd	Bmax	Kd
PLACB.	74±2.5	2.9±0.04	69.7±4.3	7.5±0.1
MORPH.	150±5.0*	6.1±0.12*	75±6.0	8.0±0.20
NALTRX	130±2.2*	2.7±0.05	94±3.7*	5.8±0.12*

These data indicate that up-regulated mu binding sites are completely (chronic morphine) or partially (chronic naltrexone) sensitive to FNA. Chronic naltrexone, but not chronic morphine, increased the Bmax of kappa binding sites by 26%. Receptor autoradiography of mu binding sites at the level of the thalamus localized the areas of greatest increase to the hypothalamus, amygdala and striatum. The major finding of this study is that chronic morphine selectively up-regulates the mu_{CX} (FNA-sensitive) binding site, while chronic naltrexone up-regulates both the mu_{CX} and the the mu_{nCX} (FNA-insensitive) mu binding site.

280.26

OPIOID RECEPTOR UPREGULATION IN SPECIFIC BRAIN REGIONS SHOWS CONCOMITANT CHANGES IN BRAIN FUNCTIONAL ACTIVITY. A. Tempel[1] and L. Brown[2]. Dept. Neuroscience[1] and Neurology[2], Albert Einstein College of Medicine, Bronx, NY 10461.

The phenomenon of naltrexone-induced upregulation of brain opioid receptors is well documented (Tempel et al., 1984; 1985). However, the functional neuroanatomical changes associated with this phenomenon remain unclear. In order to identify brain regions with changes in functional neuronal activity following opioid receptor blockade, 2-deoxy-D-[^{14}C]glucose (2DG) autoradiography was carried out on brain tissue from chronic naltrexone treated and control rats (Sokoloff 1977). Analysis of selected brain regions revealed significant increases in glucose utilization in several thalamic nuclei (including the lateral geniculate, lateral ventroposterior, ventrolateral and rhomboid nuclei) basolateral nucleus of the amygdala and the molecular layer of the hippocampus. Decreases in glucose utilization were seen in the anterior ventral thalamic nucleus. Each of these regions also showed upregulation of opioid receptors in our previous study (Tempel et al., 1984). At least two of the thalamic areas are involved in somatosensory processing, but it is clear that multiple systems are affected. These results suggest that naltrexone-induced upregulation of opioid receptors in a specific brain region can have an impact on functional neural activity in that region, in a quiet, awake animal.

280.27

[^{125}I]β-ENDORPHIN BINDING TO δ AND μ OPIOID RECEPTORS IN NG108-15 AND SK-N-SH CELL MEMBRANES: REGULATION BY GUANINE NUCEOTIDES AND CATIONS D.E. Selley and J.M. Bidlack. Department of Pharmacology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

The binding of opioid agonists to μ and δ opioid receptors is inhibited by μM concentrations of GTP. However, we have previously shown that the binding of the opioid agonist, [^{125}I]β$_h$-endorphin ([^{125}I]β$_h$-EP), to rat brain membranes is not inhibited by GTP unless monovalent cations are present. In the present study, regulation of [^{125}I]β$_h$-EP binding by guanine nucleotides was examined in membrane preparations of two opioid receptor-containing cell lines, NG108-15 cells, which contain only δ opioid receptors, and SK-N-SH cells, which contain predominantly μ receptors. In the absence of cations, the binding of [^{125}I]β$_h$-EP to NG108-15 cell membranes was not altered by GTP or Gpp(NH)p. However, a significant, concentration-dependent inhibition of [^{125}I]β$_h$-EP binding by GTP was observed in the presence of NaCl, but not LiCl, KCl, $MgCl_2$, $CaCl_2$, or $MnCl_2$. This inhibitory effect was shared by GDP, but not GMP or any of the non-guanine nucleotides tested. [^{125}I]β$_h$-EP binding to SK-N-SH cell membranes was also not altered by GTP in the absence of cations. As with the NG108-15 cells, GTP did inhibit [^{125}I]β$_h$-EP binding to SK-N-SH cell membranes in the presence of NaCl. Experiments are underway to further characterize guanine nucleotide regulation of [^{125}I]β$_h$-EP binding to SK-N-SH cell membranes. [USPHS grants DA05319 and DA03742.]

280.28

14 β-BROMOACETAMIDOMORPHINE SPECIFICALLY LABELS MU OPIOID RECEPTORS IN RAT BRAIN. D.K. Frey[1], J.M. Bidlack[1], A. Seyed-Mozaffari[2] and S. Archer[2]. [1]Dept. of Pharmacology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642 and [2]Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12181.

In the absence of a disulfide reducing agent, 14β-bromoacetamidomorphine (BAM) bound only reversibly to opioid binding sites in rat brain membranes and exhibited binding characteristics similar to those of morphine. However, when membranes were incubated with dithiothreitol (DTT), followed by the addition of BAM, and then extensive washing, BAM bound irreversibly to μ opioid binding sites. The binding of 0.25 nM [^{3}H][D-Ala^2,MePhe^4,Gly-ol^5] enkephalin (DAGO) and 0.8 nM [^{3}H]naloxone was inhibited to a maximum of 90% and 80%, respectively. BAM alkylation of membranes had no effect on the binding of δ and κ opioid ligands. The irreversible inhibition of μ opioid binding was dependent on the concentrations of DTT and BAM. With 20 mM DTT present, an IC_{50} value of 400 nM BAM was obtained for the irreversible inhibition of 0.25 nM [^{3}H]DAGO. The alkylation of the μ opioid binding site with BAM resulted in an apparent decrease in the number of binding sites without a change in affinity of the sites. These studies demonstrate that upon reduction of a specific disulfide bond in the vicinity of the μ binding site, BAM will alkylate this site, resulting in the specific affinity labeling of μ opioid receptors.
(Supported by USPHS grant DA 03742.)

280.29

PHOTOAFFINITY LABELING OF OPIOID RECEPTORS: REDUCTION OF NON-SPECIFIC BACKGROUND. J.C. SCHAEFFER, E.T. CONSORTI* AND T.M. SCHWAB*. Department of Chemistry, California State University, Northridge, CA 91330.

To investigate the molecular structure of opioid receptors, a potential photoaffinity label, 7α-[N-3-(4-azido-2-nitroanilino)propionyl]aminomethyl-6,14-endo-ethenotetrahydrooripavine, was prepared. Using a ^{3}H-naloxone/rat brain membrane binding assay, this compound exhibited an I_{50} of 0.47nM and totally inhibited specific ^{3}H-naloxone binding at 10nM. A tritiated analog of this label was synthesized and found to associate with rat brain membranes mainly in a non-specific manner; at 10nM only 10% of the total binding could be displaced by a 100-fold excess of unlabeled levallorphan. Octanol/buffer and membrane/buffer partition coefficients of 49 and 58, respectively, suggest that the label partitions into the hydrophobic core of the membranes. In order to decrease non-specific background before photolysis, a variety of mild extraction procedures were studied. Of these, gentle washing with 0.1% Triton X-100 of membranes after equilibration with label was the most effective. This method increased specific binding to 33% of total and increased specific binding/mg membrane protein by 8-fold. Also, several more polar candidate photoaffinity labels were synthesized and found to be potent inhibitors of ^{3}H-naloxone binding. Thus, a combination of Triton X-100 washing and a more polar label may offer the best solution to this problem. Supported by California State University Foundation, Northridge.

280.30

CHARACTERIZATION OF SIGMA RECEPTORS ON PC-12 CELLS: PHARMACOLOGICAL DIFFERENCES FROM RAT AND GUINEA PIG BRAIN INDICATE SIGMA RECEPTOR HETEROGENEITY. W.D. Bowen and S.B. Hellewell*. (SPON: R. Church) Div. of Biology and Medicine, Brown University, Providence RI 02912.

Sigma receptors have been shown to be present on the NCB-20 hybrid neurotumor cell line (Largent et al. Eur. J. Pharmacol., 124:183, 1986). We investigated the rat pheochromocytoma (PC-12) cell line for the presence of sigma receptors and compared the receptor characteristics to those of rat and guinea-pig brain. [^{3}H](+)-3-PPP bound to PC-12 cell membranes with K_d= 86 nM and B_{max}= 1539 fmol/mg protein. The corresponding values in rat brain membranes were 90 nM and 563 fmol/mg protein, while those of guinea pig brain membranes were 40 nM and 389 fmol/mg protein. [^{3}H]DTG bound to PC-12 membranes with K_d= 24 nM and B_{max}= 2024 fmol/mg protein. The corresponding values in rat brain were 30 nM and 651 fmol/mg protein. Thus, PC-12 cells contain high affinity binding sites for [^{3}H](+)-3-PPP and [^{3}H]DTG which exhibit parameters similar to those of rat and guinea pig brain. In competition binding studies using 3 nM [^{3}H](+)-3-PPP, unlabelled DTG exhibited nearly equal IC_{50} values in the three tissues (15-20 nM). However, the ability of (+)-benzomorphans to displace [^{3}H](+)-3-PPP differed markedly across tissues. (+)-Pentazocine exhibited IC_{50} values of 1.3, 37, and 1030 nM in guinea pig, rat, and PC-12 tissues, respectively. (+)-SKF 10,047 exhibited values of 70, 529, and >7,000 nM in guinea pig, rat, and PC-12 tissues, respectively. This is in contrast to the NCB-20 cell line where binding affinity of (+)-benzomorphans at sigma receptors was similar to guinea pig brain. These data suggest the existence of sigma receptor subtypes which bind (+)-3-PPP and DTG with similar affinity, but which differ in their affinities for (+)-benzomorphans. These subtypes may exist in varying ratios in different species. The PC-12 cell line possesses a novel sigma receptor with low affinity for (+)-benzomorphans. This cell line may prove useful in further investigations of the role of putative sigma receptor subtypes. (Supported by NIDA Grant #DA03776 and the Dystonia Medical Research Foundation.)

280.31

PHOTOLABELLING OF SIGMA RECEPTORS IN GUINEA PIG BRAIN, RAT BRAIN, AND PC-12 CELLS USING [^{3}H]AZIDO-DI-TOLYLGUANIDINE: EVIDENCE FOR RECEPTOR HETEROGENEITY. S.B. Hellewell* and W.D. Bowen. (SPON: R. Patrick) Div. of Biology and Medicine, Brown University, Providence RI 02912.

We have shown that [^{3}H](+)-3-PPP binding sites in guinea-pig brain, rat brain, and PC-12 cells have nearly equal affinity for DTG but differ markedly in affinity for the (+)-benzomorphans, (+)-pentazocine and (+)-SKF 10,047 (see abstract, this meeting). This suggests either species differences in sigma receptors or the existence of multiple sigma receptor types. Kavanaugh et al. (Proc. Natl. Acad. Sci. USA 85:2844, 1988) have demonstrated specific labelling of a 29 kDa sigma receptor polypeptide in guinea pig brain membranes using the photoaffinity probe [^{3}H]azido-di-tolylguanidine ([^{3}H]Az-DTG). Possible molecular heterogeneity in sigma receptors from these three sources was investigated using this probe.

Membranes from guinea pig brain, rat brain, and PC-12 cells were incubated with 10 nM [^{3}H]Az-DTG. After rapid centrifugal washing and resuspension in fresh buffer, the membranes were irradiated for 5 min with ultraviolet light of 254 nm. Membranes were then solubilized and subjected to SDS polyacrylamide gel electrophoresis. Radiolabelled proteins were visualized by fluorography. Labelling in presence of 1 μM haloperidol was used to determine specifically labelled bands. In guinea pig brain, a band of 28 kDa was strongly labelled, with a more weakly labelled band at 54 kDa. In rat brain, a band of 32 kDa was strongly labelled, with weakly labelled bands at 15 and 54 kDa. In PC-12 cell membranes, bands of 15 and 32 kDa were strongly labelled, with a more weakly labelled band at 58 kDa. These results indicate that sigma receptors occur in different molecular forms in different species and cell types and further supports the existence of multiple sigma receptor types. We wish to acknowledge Dr. Eckard Weber of Oregon Health Sciences Univ. for supplying [^{3}H]Az-DTG. (Supported by NIDA Grant #DA03776 and the Dystonia Medical Research Foundation.)

280.32

EVIDENCE FOR TWO POPULATIONS OF HALOPERIDOL-SENSITIVE SIGMA BINDING SITES IN GUINEA PIG BRAIN. A. A. REID, R. B. ROTHMAN, C. H. KIM*, A. E. JACOBSON* and K. C. RICE Lab of Clin Sci, NIMH and Section on Drug Design and Syn, Lab of Neurosci, NIDDK, Bethesda, MD 20892.

Benzomorphan opiates produce psychotomimetic effects in man. Binding sites thought to mediate these effects include the phencyclidine (PCP) receptor and the halperidol-sensitive sigma receptor. Ligand binding and autoradiographic studies support the hypothesis that sigma and PCP binding sites are distinct entities.

In order to further characterize the sigma receptor system in guinea pig brain, two sets of binding surfaces were generated, which involved displacement of ^{3}H-1,3,-di(2-tolyl)guanidine (^{3}H-DTG) by DTG, (+)-cyclazocine and (+)-pentazocine. The data from these experiments (134 data points) were pooled and fit to a one site binding model. The sum of squares (SS) was 0.98. When fit to a two site model, the SS decreased to 0.12, which was highly significant ($p<0.001$). The best fit parameter estimates (± SE) indicated the presence of two binding sites for ^{3}H-DTG, with Bmax values of 6780 ± 313 and 4500 ± 212 fmol/mg protein. The Kd's of DTG for the two sites were 191 ± 1.4 and 349 ± 4.8 nM, respectively; the Kd's for (+)-pentazocine were 20 ± 0.2 and 3462 ± 61 nM and for (+)-cyclazocine were 62 ± 0.4 and 6073 ± 92.5 nM, respectively. Pre-incubation of the membranes with di-o-tolyl-guanidine-isothiocyanate (DIGIT), a DTG site directed alkylating agent which does not bind to PCP sites, produced a 61 ± 5 percent decrease in ^{3}H-DTG binding (5 nM). Other studies indicated that drugs that bind to PCP receptors with high affinity such as PCP and TCP did not inhibit ^{3}H-DTG binding at concentrations of 1 uM. Taken collectively, these data demonstrate that ^{3}H-DTG labels two binding sites in a preparation of guinea pig membranes, which are distinguished by the benzomorphan opiates (+)-cyclazocine and (+)-pentazocine, and which are unrelated to the PCP receptor.

280.33

RECEPTOR BINDING PROPERTIES OF THREE WIDELY USED CLINICAL ANALGESICS (*d*-PROPOXYPHENE, MEPERIDINE AND *l*-METHADONE) IN THE GUINEA-PIG BRAIN. K.-Z. Wu* and T.-P. Su (SPON: D. Jasinski). Neuropharmacology Laboratory, NIDA, Addiction Res. Ctr., Baltimore, MD 21224.

d-Propoxyphene (PR; Darvon®), meperidine (MP; Demerol®) and *l*-methadone (MT; Dolophine®) are widely prescribed analgesics in most parts of the world. Nonetheless, a systematic examination of the interactions of these drugs with related receptors has been lacking although fragmented data may be obtained from the literature. This study examined the interactions of PR, MP and MT with *mu*, *delta*, and *kappa* opioid receptors and with *sigma* and phencyclidine (PCP) receptors in guinea-pig brain homogenates. All three drugs appeared to be most potent at *mu* receptors. Ki's for PR, MP and MT at the *mu* receptor were 82, 234 and 5 nM, respectively. The same orders of relative potencies were observed at both *delta* and *kappa* receptors. Ki's of PR at *delta*, *kappa*, *sigma* and PCP receptors were 4x, 17x, 524x and 150x higher, respectively, than its Ki at the *mu* receptor. The respective Ki's were 23x, 19x, 14x and 518x higher than the Ki at the *mu* receptor for MP and 24x, 174x, 328x and 276x higher for MT. Therefore, the analgesic effects of PR may derive from interactions at both *mu* and *delta* receptors and those of MP and MT from interactions mainly at *mu* receptors. MP may have psychotomimetic propensity, as its Ki at the *sigma* receptor is only 14 times its Ki at the *mu* receptor.

ALCOHOL III

281.1

CORTICOTROPIN-RELEASING FACTOR (CRF) IS ALTERED IN BRAINS OF RATS EXHIBITING HIGH PREFERENCE FOR ETHANOL. C.A. Naranjo, S.R. George, T. Fan* and L. Roldan*. Departments of Medicine and Pharmacology, University of Toronto, Toronto, ONT M5S 1A8 Canada.

Adult male rats were offered tap water and ethanol solution in a 2 bottle free choice paradigm. Ethanol concentrations during the preference acquisition period were increased sequentially to 6% at 4 day intervals. Rats were classified as having low preference if alcohol consumption was < 35% of fluid intake, moderate preference if consumption was between 35-50% and high preference if > 50%. A control group of animals were maintained on water alone. Animals were singly housed in an environmental room with a 12 hr light-dark cycle, were handled daily and had the position of the 2 drinking tubes reversed daily to prevent position bias. Rats were sacrificed by decapitation and brain regions assayed for CRF immunoreactivity with a RIA specific for rat CRF. Rats with high preference for ethanol had significantly lower CRF concentrations in medulla-pons compared to controls ($p=0.035$), and higher levels of CRF in hypothalamus outside of median eminence ($p=0.018$), whereas levels in median eminence did not differ. CRF concentrations in the neurointermediate pituitary of all rats given ethanol were higher than in controls ($p=0.035$) regardless of preference. No changes of CRF content were evident in frontal cortex, hippocampus, midbrain or cerebellum. These results suggest significant interactions of certain brain CRF systems in promoting ethanol consumption or mediating its consequences.

281.2

ETHANOL EFFECTS ON NMDA MODULATED PHOSPHOINOSITIDE HYDROLYSIS IN RAT CORTEX. R.A. Gonzales and L.D. Minor. Dept. of Pharmacology, LSU Med. Ctr., New Orleans, LA 70112.

The interaction of NMDA type glutamate receptors with muscarinic and α_1-adrenergic receptor mediated phosphoinositide (PI) hydrolysis was investigated. Rat cortical slices were labelled with [^{3}H]inositol, and the accumulation of [^{3}H]inositol phosphates in the presence of 8 mM LiCl was determined. Glutamate (GLUT) (1-10 mM) stimulated PI hydrolysis in a concentration-dependent manner, but did not alter the responses to 1 mM carbachol (CARB) or 10 µM norepinephrine (NE). NMDA inhibited the CARB-stimulated PI response with an IC50 of 10 µM and maximal (70%) inhibition at 100 µM. The inhibitory effect of 100 µM NMDA on CARB-stimulated PI hydrolysis was blocked by 1 mM 2-amino-5-phosphonovalerate (AP5). NMDA (0.3-3 mM) potentiated the NE-stimulated response in a concentration related manner, and this effect was not blocked by 1 mM AP5. Ethanol in vitro (100-500 mM) did not alter the PI response to CARB alone or NMDA + CARB. However, in vitro addition of ethanol (100-500 mM) inhibited PI hydrolysis stimulated by 10 µM NE alone or NE+NMDA (1 mM). The results suggest that NMDA type GLUT receptors can modulate receptor coupled PI hydrolysis positively or negatively depending on the receptor which is coupled. Ethanol's actions on NMDA modulated PI hydrolysis are also dependent on the type of receptor which mediates the PI response. (Supported by NIAAA grant 07297 and the ABMRF.)

281.3

EFFECT OF ETHANOL ON PHOSPHORYLATION OF SYNAPTOSOMAL (Na+K)-ATPase. PM Wixom and AY Sun (SPON: V St. Omer). Sinclair Comp. Med. Res. Farm and Biochem. Dept., Univ. of MO, Columbia, MO 65203

Ethanol is known to inhibit neural membrane-bound (Na+K)-ATPase, due to the hydrophobic interaction of alcohol with membrane components. When synaptic plasma membranes (SPM) were incubated with [^{32}P]-ATP, a 96 kDa protein was phosphorylated as revealed by SDS-gel electrophoresis and autoradiography. The phosphorylated protein was dephosphorylated upon the addition of 20 mM KCl and was sensitive to ouabain. In the absence of K^+, the phosphorylation of the 96 kDa protein was enhanced by ethanol, possibly by the exposure of additional active sites on the (Na+K)ATPase. Also, the dephosphorylation step upon the addition of K^+ was blocked by ethanol in a concentration-dependent manner. The transmembrane conformational changes accompanying dephosphorylation may be sensitive to the alteration of the microenvironment by ethanol. Thus, ethanol exhibits a biphasic mode of action upon the intermediate steps of synaptosomal (Na+K)-ATPase, exposing more active sites and simultaneously blocking the transmembrane process, resulting in activity changes as demonstrated earlier in our laboratory. (Supported in part by NIH grant AA02054.)

281.4

REGULATION OF NEURONAL GENE EXPRESSION BY ETHANOL. M. F. Miles. Dept. of Neurology and the Ernest Gallo Research Center, U.C.S.F., San Francisco, CA 94110.

The acute/chronic effects of ethanol on neuronal gene expression were investigated using the NG108-15 neuroblastoma-glioma hybrid cell line. In vitro translation coupled with 2-D gel electrophoresis identified prominent increases and decreases in the amount of specific mRNAs with ethanol treatment. Several mRNA species appeared only in the ethanol treated cells.

Northern blot analysis with certain marker genes showed that lactate dehydrogenase (LDH) M-type mRNA decreased in reponse to chronic ethanol while the heat shock cognate gene, Hsc-70 was a member of the induced class of ethanol responsive genes (ERGs). These changes were more prominent with isomolar amounts of increasing chain length aliphatic alcohols. This suggests that the membrane "destabilizing" effects of ethanol are causal in these alterations of gene expression.

These results indicate that ethanol produces prominent and complex alterations in neuronal gene expression that may play a role in the development of the tolerant/dependent phenotype. Further characterization of ERGs and mechanistic studies will be presented.

281.5

INCREASE IN NUMBER AND K_d OF MUSCARINIC CHOLINERGIC RECEPTORS IN HIPPOCAMPUS OF ADULT RATS EXPOSED TO ALCOHOL DURING THE BRAIN GROWTH SPURT. S.J. Kelly, A.C. Black, Jr., and J.R. West. Dept. of Anatomy, University of Iowa, Iowa City, IA 52242 and Dept. of Basic Sciences, Mercer University School of Medicine, Macon, GA 31207.

Since rats exposed to cyclic blood alcohol concentrations (BACs) with high peaks but not rats exposed to stable, moderate BACs during the brain growth spurt have been shown to be impaired on tasks that involve the septohippocampal cholinergic system, we examined the muscarinic cholinergic receptors in the hippocampus of these animals. An artificial rearing procedure was used to expose rats to the different patterns of BACs from postnatal day 4 to 10. Two control groups consisted of rats reared artificially but without alcohol and rats reared normally by dams. In adulthood, the rats were exposed to ether and decapitated. The hippocampi were assayed for muscarinic cholinergic receptors using QNB as the binding ligand. The dissociation constant and number of muscarinic cholinergic receptors in the hippocampus were increased by exposure to cyclic BACs with high peaks but not by exposure to stable, moderate BACs. Thus, changes in the septohippocampal system may underlie some behavioral changes seen in rats exposed to cyclic BACs with high peaks during the brain growth spurt. (Supported by NIAAA Grants AA05523 to J.R.W.and AA07000 to A.C.B.)

281.6

PATERNAL AND MATERNAL ALCOHOL CONSUMPTION INCREASE SUSCEPTIBILITY OF OFFSPRING TO INFECTION. E. Abel, L. Hazlett and R. Berk. Fetal Alcohol Ctr. and Depts. Anat. and Immunol., Wayne State U., Detroit, MI 48201.

Prenatal alcohol exposure is associated with a range of adverse effects ranging from spontaneous abortion and fetal alcohol syndrome at one extreme to subtle functional deficits in the absence of physical effects at the other. Nearly all studies of prenatal alcohol exposure have focussed on maternally-mediated effects. Recently, we have also found paternally-mediated effects and we report one such effect here.

Long-Evans male rats were fed liquid alcohol diets containing 35%, 17.5% or 0% ethanol derived calories (EDC) for a minimum of three weeks. Animals receiving the 17.5% and 0% EDC diets were pairfed to those given the 35% diet. These males were bred to females who were fed these same diets beginning on gestation day 8. The same pairfeeding procedure was used for females. At approximately 50 days of age, offspring were anesthetized with ether and a bacterial cell suspension containing a concentration of $1x10^8$ CFU of Pseudomonas aeruginosa was topically delivered to the surface of the incised corneas. The occular response to infection was macroscopically evaluated "blindly" at 18-24 hours after challenge, and then at every other day through the first week of infection.

After one week, almost all animals exhibited corneal perforation. There was a significant dose-related effect of both maternal ($p< .05$) and paternal ($p< .05$) alcohol exposure such that the higher the alcohol consumption, the earlier the time to perforation. There was no effect of sex of offspring or interaction between maternal and paternal factors (see Table 1).

Table 1

	Fathers			Mothers		
	35%	17.5%	0%	35%	17.5%	0%
Days	5.26	5.41	6.16	5.09	5.41	6.07

These results show that both maternal and paternal alcohol consumption increase susceptibility to infection in offspring. The fact that paternal alcohol consumption increases this risk is surprising and has not been previously reported. However, it supports other findings from our laboratory that such exposure can affect offspring, e.g., decreased locomoter activity.

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281.7

REGIONAL ANALYSIS OF BRAIN CHOLINERGIC ENZYME ACTIVITY IMMEDIATELY FOLLOWING POSTNATAL ALCOHOL TREATMENT OF RAT PUPS. K.E. Light and D.C. Serbus, Center for Addiction Studies, College of Pharmacy, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, AR 72205

Male and female rat pups were treated with ethanol (3g/kg/dose) twice daily by intragastric intubation from postnatal day (PN) 1 to 20. Control pups were treated identically by intubation of an equal volume of an isocaloric vehicle solution. On PN21 the rats were sacrificed and their brains were removed, dissected on ice, and frozen for subsequent regional analysis. The four regions involved in this study included: cortex (CX), striatum (ST), hippocampus (HP), and cerebellum (CB). Acetylcholinesterase (AChE) and choline acetyltransferase (CAT) activities were analyzed in aliquots of the S_1-supernatant using spectrophotometric and radioisotopic assays, respectively. AChE activity (micromoles hydrolyzed/ mg protein/hour) and CAT activity (nanomoles formed/mg protein/hour) showed regional differences in control animals with ST showing the greatest activity of both enzymes and CB the least. Pooled across gender, CAT activity was decreased within the CB by ethanol. Although no gender differences were noted between control pups for any of the endpoints measured, females had less AChE activity in the CB following ethanol exposure, when compared to males. Supported by AA06483.

281.8

PRENATAL ETHANOL EXPOSURE DELAYS ASTROCYTE DEVELOPMENT: A POSSIBLE MECHANISM FOR FETAL ALCOHOL SYNDROME S.F. Hoff and E. Anton*, Dept. Pharmacology, The Chicago Medical School, North Chicago, IL 60064.

Glial cells play a crucial role in CNS development by promoting neuronal migration, directional growth and survival. However, their contribution to the onset of Fetal Alcohol Syndrome has not been adequately studied. Pregnant Sprague-Dawley rats were fed an ethanol liquid diet throughout gestation. A control group was pairfed the liquid diet without ethanol and a third group was given free access to rat chow and water. At 30 and 60 days postnatal, sagittal sections from the brains of male offspring were stained for astrocytes by the Cajal's gold sublimate method. Astrocytes were counted in the hippocampal CA1 stratum radiatum, the dorsal and ventral dentate molecular layer, and the outer molecular layer of the cerebellar simple and paramedian lobules. The ethanol exposed group demonstrated a significant 17% reduction in the numbers of astrocytes in the hippocampal CA1 region at 30 days postnatal. All groups were at equivalent numbers at 60 days postnatal. Barnes and Walker (Dev. Br. Res. 1:333, 1981) previously reported a permanent loss of CA1 pyramidal neurons after prenatal ethanol exposure. Ethanol-induced deficits in astrocyte development and function may be responsible for the neuronal loss in the hippocampus and perhaps in other areas of the CNS. (Supported by NIAAA grant AA06156 to SFH)

281.9

HIGH AFFINITY [^{3}H]-OUABAIN BINDING SITES IN VARIOUS REGIONS OF RAT BRAIN AFTER ACUTE AND CHRONIC ETHANOL ADMINISTRATION. E. Voisin-Lestringant*, J. Karanian*, B. Giblin*, C. Gleiter*, M. Linnoila and S. Paul* SPON: P. Hanson. Clinical Neuroscience Branch, National Institute of Mental Health, Laboratory of Clinical Studies, National Institute of Alcohol Abuse and Alcoholism, Bethesda, MD 20892.

Recent evidence suggests that ethanol administration may affect Na^+ K^+ ATPase in rat brain, however the nature of this effect remains controversial. High affinity [^{3}H]-ouabain binding specifically labels the neuronal form of Na^+ K^+ ATPase. We examined the effects of acute and chronic ethanol administration on [^{3}H]-ouabain binding sites in membranes prepared from various brain regions of the rat. Acute ethanol administration (2g/kg i.p.) resulted in a significant increase in [^{3}H]-ouabain binding in brain stem (+56%) and hypothalamus (+45%) ($p<0.01$) but not in the cerebral cortex or cerebellum. Following chronic ethanol administration (rats were exposed to ethanol for 7 days in an inhalation chamber resulting in blood ethanol levels of 194±48 mg%), [^{3}H]-ouabain binding was significantly increased in cerebral cortex (44%), hippocampus (39%), hypothalamus (34%), cerebellum (33%) and brain stem (30%) ($p <0.01$). These data suggest that ethanol administration induces alterations in [^{3}H] ouabain binding to neuronal Na^+ K^+ ATPase which is apparent in some, but not all brain regions.

281.10

EXAMINATION OF HIPPOCAMPAL AREA CA3 IN LONG-SLEEP AND SHORT-SLEEP MICE FOLLOWING 3 MONTHS OF ETHANOL EXPOSURE Jeffrey A. Markham and Eva Fifková, University of Colorado, Boulder, CO 80309-0345.

The ultrastructural morphology of the connection between mossy fibers and dendritic spines in the CA3 region of the hippocampus was examined in long-sleep (LS) and short-sleep (SS) lines of mice following 3 months exposure to an ethanol-containing liquid diet (Bioserv). Ethanol-treated mice consumed an average of 24 gm ethanol/kg body weight/day with no line differences noted. Control mice received an isocaloric liquid diet. Morphological analysis revealed a slightly greater spine area and perimeter in both LS and SS ethanol-treated mice (NS). In addition, the length of synaptic appositions was found to be increased (+10%) in ethanol-treated LS mice compared to controls (NS). SS groups did not differ in this measure. In order to gain insight into the effects of ethanol on individual dendritic spines, we also calculated the percentage of spine membrane occupied by the synaptic density for each spine. Measurements were averaged for each mouse and group. Ethanol was found to have no effect on this measurement. However, the LS mice (both ethanol and control) were found to have a significantly greater percentage of spine membrane occupied by the postsynaptic density ($p<.01$) when compared to SS mice. The overall results show that in contrast to longer exposure times (e.g. Paula-Barbosa et al., *Soc Neurosci Abstr*, 13:75, 1987), 3 months of ethanol-exposure is insufficient to induce changes in the parameters measured in either the ethanol-insensitive SS or ethanol-sensitive LS lines of mice. However, differences in morphology between LS and SS mice may be involved in the differential physiological response of these mice to acute ethanol and other CNS depressants. *Supported by AA06196-04.*

281.11

PORTOCAVAL ANASTOMOSIS IN THE RAT: EFFECTS ON CEREBRAL AMMONIA METABOLISM: J.F.Giguère, G. Girard*, R.F. Butterworth. Lab. of Neurochem., André-Viallet Clin. Res. Ctr., Hôpital St-Luc, Montreal, Qué., Canada H3X 3J4.

Construction of a portocaval anastomosis (PCA) in both man and in experimental animals produces sustained hyperammonemia and associated neurological disturbances. In patients, an acute ammonia challenge resulting from gastrointestinal bleeding or protein ingestion results in neurological deterioration leading to coma. Four weeks after PCA, rats exhibit liver atrophy and region-selective increases of brain glutamine and ammonia (320% cerebral cortex, 200% brainstem). Following administration of a coma-inducing dose of ammonium acetate (5.2 mmol/kg i.p.) rats with PCA show a dramatic increase in brain ammonia (4.5 mM cerebral cortex, 3.4 mM brainstem) but further increases of glutamine are restricted to brainstem. The inability of cerebral cortex to remove blood-borne ammonia following PCA undoubtedly accounts for the disproportionately high concentrations of ammonia in this structure. These results suggest that the capacity for brain to remove ammonia is limited following PCA. Such a mechanism may play an important role in the pathogenesis of hepatic encephalopathy associated with portal-systemic shunting. [Supported by MRC (Canada)].

281.12

SELECTIVE CHANGES OF NEUROTRANSMITTER-PRECURSOR AMINO ACIDS IN AUTOPSIED BRAIN TISSUE FROM CIRRHOTIC PATIENTS WITH HEPATIC ENCEPHALOPATHY. J. Lavoie, M. Bergeron*, J.-F. Giguère, G. Pomier Layrargues* and R.F. Butterworth, Lab. of Neurochem., André-Viallet Clin. Res. Ctr., Hôpital St-Luc, Montreal, Que., Canada H2X 3J4.

A substantial body of evidence suggests that alterations of cerebral amino acids play an important role in the pathogenesis of hepatic encephalopathy (HE) associated with chronic liver disease. In order to evaluate this hypothesis, brain tissue was obtained at autopsy from 9 cirrhotic patients dying in hepatic coma and an equal number of age-matched controls. Amino acids were measured as their o-phthalaldehyde derivatives by HPLC with fluorescence detection. The following results were obtained: (i) A 3-fold increase of brain glutamine throughout brain; (ii) Glutamate decreased by 20-30% in cerebral cortex and caudate nuclei; (iii) No change of GABA content; (iv) 2-3 fold increases of phenylalanine and tyrosine in cerebral cortex and caudate nucleus.

Selective changes of neurotransmitter-precursor amino acids (glutamine, phenylalanine, tyrosine) may play an important role in the pathophysiology of HE associated with chronic liver disease. [Supported by MRC (Canada)]

281.13

EFFECT OF ACUTE ETHANOL ADMINISTRATION ON THE BRAIN AND PITUITARY B-ENDORPHIN SYSTEM IN MICE WITH VARIABLE SENSITIVITY TO ETHANOL. J.P. De Waele and C. Gianoulakis. Douglas Hospital Research Center, McGill University, Verdun, Quebec, Canada.

The objective of the present studies was to examine wether acute ethanol treatment has a differential effect on the pituitary and brain B-endorphin systems of three inbred strains of mice. These strains are selectively bred for their high (C57BL/6) or low (DBA/2) ethanol consumption. The content of immunoreactive B-endorphin (irB-EP) in the serum, anterior and neurointermediate lobes of the pituitary gland and 10 distinct regions of the brain as arcuate nucleus, nucleus accumbens, septum etc. was estimated at the resting state as well as 15 minutes after i.p. injection of either ethanol , 3.5g per Kg B.Wt., or saline. Results indicated that ethanol increased the release of B-endorphin from the pituitary gland. Ethanol treatment decreased the content of irB-EP in a number of brain areas as the nucleus accumbens and septum or had no effect as in the preoptic hypothalamic area. In addition, it was noticed that following acute ethanol treatment the content of irB-EP was decreased in the arcuate nucleus of the C57BL/6 mice but not of the DBA/2 or BALB/c mice. Thus the results indicate that there are strain specific differences in the response of the brain B-endorphin system to acute ethanol exposure in vivo.

281.14

ETHANOL-INDUCED HYDROLYSIS OF SIALOCONJUGATES IN BRAIN SLICES. J. Mathew* and W. R. Klemm (SPON: B. H. Rohde). Dept. Vet. Anat., Texas A&M U., College Sta., TX 77843

Acute ethanol reportedly decreases total sialic acid (SA) in brain. Here, we evaluated ethanol's effect on hydrolysis of brain and liver sialoconjugates. Mouse tissue slices were pulse labeled with ^{3}H-N-acetyl-D-mannosamine, the precursor of SA. Incorporation was linear for up to 4hr of incubation. When labelled slices were incubated with ethanol, liver SA was significantly affected only at 1M ethanol, while brain SA decreased in a concentration-dependent way. Sialidase activity decreased steadily with increasing concentrations of ethanol, indicating that the elevated hydrolysis was not attributable to enhanced sialidase activity. n-Propanol and t-butanol had the same degradative effect as ethanol; and 3mM pyrazole, an inhibitor of alcohol dehydrogenase (ADH), had no effect on ethanol-induced degradation of labeled SA. The data thus confirm the in vivo reports of ethanol-induced cleavage of SA and suggest that intoxication may be related to brain sialoconjugate hydrolysis as the cause.

ENDOCRINE CONTROL AND DEVELOPMENT III

282.1

ANDROGENS REGULATE DENDRITIC GROWTH AND RETRACTION DURING THE DEVELOPMENT OF A SEXUALLY DIMORPHIC RAT SPINAL MOTOR NUCLEUS. L.A. Goldstein, E.M. Kurz, and D.R. Sengelaub. Program in Neural Science, Department of Psychology, Indiana University, Bloomington, IN 47405.

Motoneuron number and size in the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) of rats are sensitive to androgens which determine their masculine pattern of development. This study demonstrates that SNB dendritic growth during postnatal development is also androgen dependent.

Male rats were castrated at postnatal day (P) 7 or P28 and treated with testosterone [either daily injections of .1mg testosterone propionate (TP) in oil or 45 mm silastic implants of testosterone] or an equal volume (.05ml) of vehicle alone or blank implants. An additional set of males was left intact and untreated. Motoneuron morphology was visualized with unilateral injections of cholera-toxin HRP (.3-.5 µl, .2%) into the bulbocavernosus, an SNB target muscle, at P7, P28, or P49 (n= 3-4 per group). After processing with TMB, dendritic length of all HRP-labeled SNB motoneurons was measured.

At P7, SNB dendritic length in normal males averaged 1463.2 µm per motoneuron. Dendritic length increased five-fold after P7 to an average of 7394.7 µm at P28, and this exuberant growth was then reduced by almost half to adult levels by P49 ($\bar{x}$=3868.6 µm). At P28, TP-treated castrates had a dendritic length similar to that of normal males ($\bar{x}$=7360.9 µm). Oil-treated castrates at P28 had a significantly shorter dendritic length ($\bar{x}$= 1449.2 µm) than that of normal males. At P49, dendritic length in oil-treated males ($\bar{x}$=2278.5 µm) continued to be significantly below that of normal males ($\bar{x}$=3868.6 µm); the early exuberant dendritic length was retained in androgen treated males ($\bar{x}$=6781.1 µm). These results suggest that in addition to regulating motoneuron number and size, androgens also regulate the dendritic growth and retraction of SNB motoneurons during development. Supported by NIH grant NS24877.

282.2

ANDROGEN TREATMENT DOES NOT PREVENT THE DEATH OF SEXUALLY DIMORPHIC RAT SPINAL MOTONEURONS FOLLOWING EARLY TARGET REMOVAL. A.R. Cover* and D.R. Sengelaub. Program in Neural Science, Department of Psychology, Indiana University, Bloomington, IN 47405.

In rats, the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) contains many more motoneurons in adult males than in females. Androgens produce this sex difference by regulating normally occurring motoneuron death during development, and this death is reduced to masculine levels in females treated with testosterone perinatally. Androgens may masculinize the SNB system by acting on the motoneurons or their target musculature, which are also dimorphic and androgen sensitive. We tested if androgens can act at sites other than the musculature by attempting to prevent or delay the death of SNB motoneurons with androgens consequent to muscle removal.

Female pups were masculinized with prenatal injections of testosterone proprionate (TP, 2 mg) from embryonic days (E)16-22. SNB target muscles (the bulbocavernosus and levator ani) were removed surgically on the day of birth [E23=postnatal (P) day 1], pups were cross-fostered, and either continued to receive TP (1 mg) on P1, 3, and 5 or were not treated further. Counts of SNB motoneurons were made prior to surgery at E22 and at P2, 4, and 10 (n=3-7 per group per day) and corrected for split nucleoli. At E22, TP-treated females had a fully masculine number of SNB motoneurons (282). Following muscle removal, SNB motoneuron number declined sharply, and postnatal androgen treatment produced no differences in the magnitude or timing of this loss. By P10, SNB motoneuron number in either group was not different from that of normal females (80-88). Thus, just as the lack of androgens results in muscle degeneration in normal females and a feminine SNB motoneuron death, muscle removal also produces a feminine SNB death. These results suggest that androgens act at, or in concert with, the perineal muscles to masculinize SNB. Supported by NIH grant NS24877.

282.3

EVIDENCE OF ANDROGEN RECEPTORS IN NEONATAL MALE PERINEAL MUSCLE. R. Fishman, L. Chism, & S.M. Breedlove, Dept. Psych., U. of Calif., Berkeley, CA 94720

The sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) innervates the perineal muscles bulbocavernosus (BC) and levator ani (LA) in adult male rats. Neonatal females possess both SNB motoneurons and BC/LA muscles at birth, but in the absence of androgen, these structures degenerate. Previous studies indicate that androgen directly interacts with the BC/LA to spare them, and thereby indirectly preserves SNB cells. To further examine the site of androgen action in masculinizing the SNB system, we assayed for the presence of androgen receptors in both the BC/LA and spinal cord of neonatal male rats.

BC/LA muscles and spinal cords of neonatal castrates were removed, separately incubated with tritiated methyltrienolone (+/- 100-fold cold ligand) for two hours, and assayed for the presence of androgen receptors using a glass fiber filter method. This procedure exploits the tendency of receptors to adhere to glass without compromising steroid binding.

Preliminary results suggest that specific binding occurs at both sites, but binding is approximately 5-fold greater per mg tissue in the muscles than in the spinal cord. It is not yet clear whether spinal levels of receptor binding are physiologically significant and therefore, comparison with Tfm spinal tissue is planned. These results demonstrate that receptors are present in newborn male BC/LA, confirming that androgen may act directly on these muscles to spare them. NIH# NS19790 & Muscular Dystrophy

282.4

2-D GEL ANALYSIS OF PROTEINS FROM BULBOCAVERNOSUS MUSCLE OF NEWBORN RATS. Nancy G Forger, G L Firestone, S M Breedlove, Depts. Psychology & Physiology, U. C. Berkeley, CA 94720.

The bulbocavernosus (BC) muscle is present in rats of both sexes at birth, but normally involutes in females during the first week of life. Exposure of females to androgen early in development permanently rescues BC muscles and their innervating motoneurons. We have utilized 2-dimensional gel electrophoresis to characterize and compare the proteins synthesized in the perinatal BC of males and females. BC muscles from 1-2 day old rats were incubated at 37 C_5 for 18-20h in serum-free medium containing 150 uCi ^{35}S-methionine. Equally radioactive aliquots of labeled proteins were separated using isoelectric focusing in the first dimension and SDS polyacrylamide gel electrophoresis in the second.

Autoradiography of the gels revealed that although the BC of newborn females has already undergone substantial involution, protein synthesis is essentially indistinguishable between the sexes. There were no consistent differences in the number or relative abundance of the > 200 proteins resolved. Despite gross morphological sex differences in the BC at birth, the fibers present in the female appear metabolically active and relatively normal. Since the female BC will completely degenerate unless rescued by androgen, it seems inescapable that androgen alters muscle gene expression, either directly or indirectly. We are currently testing whether BC proteins may be transiently induced or repressed by androgen.

282.5

MOTONEURONAL SEXUAL DIMORPHISM IN THE ABSENCE OF SEXUAL FUNCTION. Marianne Leslie, N.G. Forger, S.M. Breedlove, Psychology Dept, U.C. Berkeley, Berkeley CA 94720

The retrodorsolateral nucleus (RDLN) of the lumbo-sacral spinal cord innervates the intrinsic musculature of the foot. Motoneuron number in the RDLN is not sexually dimorphic (Jordan et al, '82), but our pilot study revealed a subtle sex difference in RDLN cell size. We now examine the relationship between RDLN neuron size and the mass of one of its target muscles, the flexor digitorum brevis (FDB). Tissues from gonadectomized (GdX) male, intact male and intact female Sprague-Dawley rats were compared. GdXs were performed at 90 days of age and all animals were sacrificed at 120 days. Spinal cords were frozen-sectioned at 50μ and thionin stained. Mean nuclear and soma areas were determined from camera lucida drawings of 20 RDLN cells from each animal. FDB muscles were removed and weighed.

There was a striking sex difference in FDB mass and RDLN soma and nuclear areas. RDLN size in GdX males was intermediate, while FDB mass was unaffected by castration:

		RDLN		FDB
	N	Soma(μ^2)	Nucleus(μ^2)	Mass(mg)
Intact males	12	1216 ± 31	340 ± 10	62 ± 3
GdX males	9	1140 ± 39	308 ± 10*	65 ± 3
Intact females	11	1083 ± 13**	302 ± 08*	43 ± 2**

(significantly different from intact males, *p<.05, **p<.001)

Such sexual dimorphisms may thus be widespread in rats and not limited to systems involved in sexual behavior.
Supported by NSF #BNS 8451367 & the McKnight Foundation.

282.6

DENERVATION ELIMINATES TESTOSTERONE'S ANABOLIC EFFECT ON BULBOCAVERNOSUS. M.N. Rand and S.M. Breedlove, Psych. Dept., U.C. Berkeley, Berkeley, CA 94720.

We previously showed that the anabolic effect of androgen on adult male bulbocavernosus and levator ani (BC/LA) muscles occurs at or near the muscle. The present study tested the idea that this effect may be due to direct action of androgen on BC/LA exclusive of motoneuronal innervation.

Adult male rats 60-120 days of age were castrated and implanted with two capsules, one containing testosterone (Sigma) and the other anti-androgen (SCH16423, Schering Corp.). These capsules had a single small diffusible Silastic surface and were sutured on each side of the BC so that the diffusible surface faced the muscle. The branch of the pudendal nerve innervating each BC/LA half was cut. After 30 days the animals were sacrificed and the BC/LA muscles dissected out. The muscles were trimmed, cut in half medially and then weighed without knowledge of their steroid treatment. Muscle half weights were compared using a repeated measures T-test.

There was no uniform effect of steroid treatment on muscle half weights. This is consistent with previous findings of no effect of systemic testosterone treatment on denervated LA -muscle weight in adult male rats (Buresova et al., J. Endocrinol. 54:3, 1972). The dystrophic effect of denervation may preclude testosterone's anabolic effect at or near the BC/LA muscle.

NSF #BNS 8451367 and the McKnight Foundation.

282.7

METABOLISM AND NUCLEAR UPTAKE OF [^{3}H]TESTOSTERONE IN THE BRAIN OF THE FETAL MACAQUE. R.W. Bonsall*, H.D. Rees and R.P. Michael. Dept. Psychiatry, Emory Univ. School of Medicine, Georgia Mental Health Inst., 1256 Briarcliff Road NE, Atlanta, GA 30306.

Testosterone is secreted by the fetal testis during gestation and is thought to have organizational effects on the brain. To study the way in which testosterone acts on brain cell nuclei, 3 female and 2 male fetuses (day 122-124 of gestation) were injected with 250 uCi [^{3}H]testosterone via the umbilical vein. After 60 min, a blood sample was taken from the heart, and brain and peripheral tissues were removed and homogenized in ice-cold buffer. Cell nuclei were purified by centrifugation through 2M sucrose and extracted with ether. Extracts were fractionated by reverse phase HPLC and radioctivity was identified by its coelution with internal standard steroids. Concentrations of radioactivity (dpm/mg DNA) were significantly higher (P<0.05) in hypothalamus than in seven other brain regions studied (amygdala, hippocampus, midbrain, cerebellar cortex and 3 samples of cerebral cortex), and a major proportion of this radioactivity (40% in males, 72% in females) coeluted with estradiol. Radioactivity in the form of estradiol was also detected in cell nuclei from the amygdala where it accounted for 33% of the radioactivity extracted. In contrast, radioactivity extracted from cell nuclei from the pituitary gland was largely (64%) in the form of testosterone and that from the male genital tract was largely (84%) in the form of dihydrotestosterone. Results demonstrated that aromatization of testosterone occurs in vivo in the hypothalamus and amygdala at this stage of gestation and indicate that the metabolite binds to receptors in cell nuclei. (Supported by USPHS Grant MH 40420 and by the Georgia Department of Human Resources).

282.8

ANDROGEN REGULATES GAP JUNCTIONS BETWEEN MOTONEURONS IN THE RAT SPINAL CORD. A. Matsumoto, A.P. Arnold, G.A. Zampighi*, and P.E. Micevych. Depts. Anat. and Psychol., Brain Res. Inst., UCLA Los Angeles, CA 90024.

Gap junctions are considered to play an important role in metabolic and electrical coupling between cells. We studied androgenic influence on the occurrence of gap junctions between androgen-sensitive motoneurons in the spinal nucleus of the bulbocavernosus (SNB). Adult male rat (Sprague-Dawley) were castrated and implanted with silastic tubes containing testosterone or nothing under Nembutal anesthesia. Four weeks later, animals were sacrificed and the somata and proximal dendrites of SNB motoneurons were examined by thin section and freeze fracture techniques. Of the gap junctions observed, 45% were somatodendritic, 35% were dendrodendritic, and 20% were somatosomatic. Castration decreased the number and length of gap junctions by 29% and 39% respectively. These values in castrates given testosterone were not significantly different from those in sham castrates. The evidence that the removal of testosterone by castration reduced the number and length of gap junctions, and that these changes were prevented by testosterone treatment suggests that androgen regulates the degree of coupling between SNB motoneurons which may allow for the synchronization of metabolic or electrical activity in the SNB. (supported by NIH grants NS23468 and HD15021).

282.9

THYROXINE STIMULATES NEURAL DEVELOPMENT OF THE PERIPHERAL OLFACTORY SYSTEM IN THE CLAWED FROG, XENOPUS LAEVIS. G.D. Burd. Depts. of Anatomy and Molecular & Cellular Biology, University of Arizona, Tucson AZ.

The olfactory nerve in tadpoles at stages 48-50 contains 10,000-15,000 axons, but it has 88,000-130,000 axons at stage 57. I hypothesized that thyroxine might be an important factor controlling the increase in the number of olfactory axons observed after the thyroxine surge at stage 54. To test this hypothesis, I blocked thyroxine synthesis with 1% propylthiouricil (PTU) in one group of tadpoles (stage 48) and implanted small pellets of thyroxine (10% in cholesterol) adjacent to the olfactory nerve in another group (stage 48). PTU-treated tadpoles, sacrificed when their age-matched controls reached stage 57, had 51% fewer olfactory axons than control tadpoles. Thyroxine-treated tadpoles had 10% more axons on the side of the implant. Other tadpoles with thyroxine implants for 7 days received a single I.P. injection of ^{3}H-thymidine (1 μCi) and were processed for autoradiography 3 days later. The olfactory epithelium of the hormone-treated animals contained 9 times more heavily labelled cells and several fold more lightly labelled cells than control tadpoles. These data suggest that thyroxine may increase the number of olfactory receptor cells, and thus axons, by stimulating neurogenesis. Supported by PHS NS25596, the Whitehall Fndn., and NIH-BRSG.

282.10

SPECIFIC ^{3}H-VINYLIDENE KAINIC ACID BINDING IS DECREASED IN HIPPOCAMPAL FORMATION OF 31-DAY-OLD HYPOTHYROID RAT. D.D. Savage, C.Y. Montano, S.Razani-Boroujerdi, M.A. Carrasco and L.L. Paxton. Dept. Pharmacol., Univ. New Mexico School of Medicine, Albuquerque, N.M., 87131.

Neonatal hypothyroidism (NH) has a profound effect on the development of the CNS. In rat, the hippocampal formation (HPF) is particularly sensitive to NH. We have previously observed a striking reduction in histochemically detectable HPF mossy fiber zinc, in both dorsal and ventral HPF, after perinatal treatment with propylthiouracil (PTU). ^{3}H-Vinylidene kainic acid (VKA) binds to the kainate-sensitive subtype of glutamate receptor binding site. In the HPF CA_3 region, VKA binding has a similar pattern of distribution as HPF mossy fiber zinc. Given this similarity and the effect of NH on HPF mossy fiber zinc, we examined ^{3}H-VKA binding sites in 31-day-old NH rats.

Timed pregnant Sprague-Dawley rat dams were given water containing either 0.02% PTU or untreated water from Day 18 of gestation until their litters were weaned at 31 days of age. Offspring were then sacrificed and their brains frozen. Serum thyroxine, triiodothyronine, total body weight and brain weight all indicated that the PTU-treated rats were severely hypothyroid. Eight μm sections of dorsal and ventral HPF were collected and processed for *in vitro* ^{3}H-VKA autoradiography.

Compared to the untreated controls, specific ^{3}H-VKA binding was reduced by 43% in the stratum lucidum of ventral HPF of 31-day-old PTU-treated rats. Specific ^{3}H-VKA binding was unchanged in the dorsal HPF. Experiments are in progress to determine whether the decrease in ^{3}H-VKA binding in the ventral HPF of PTU-treated rats is due to a change in the affinity or number of ^{3}H-VKA binding sites. The results indicate that NH affects ventral but not dorsal HPF ^{3}H-VKA binding, a pattern dissimilar to the effects of NH on HPF mossy fiber zinc.

(Supported by NIH-AA06548 and NIH-RR08139).

282.11

THYROID HORMONES AND GROWTH PLASTICITY OF THE RAT OLFACTORY MUCOSA. M. A. Paternostro* and E. Meisami. Dept. of Physiol., Univ. Illinois, Urbana, IL 61801.

The rat's olfactory mucosa (OM) grows markedly postnatally: between birth to day 25 (weaning) thickness increases by 40% and surface area (SA) by 8x, resulting in about 10x increase in the number of olfactory neurons (ON); another 2x increase in SA of OM occurs between day 25 to 50 and a 1.5x between 50 to 90 days. Thyroid deficiency (HT) retards brain development but its effects on olfactory system is poorly known, although HT patients are hyposmic. The reversible goitrogen propylthiouracil (PTU) was added to the litter's drinking water to inhibit thyroid secretion. Quantitative analysis of OM in HT rats at day 25 (weaning) revealed 40% reduction in SA and 30% in ON number. Basal and supporting cell nos. were proportionally reduced too. OM thickness was however unchanged. OM growth at later ages slowed down markedly in HT rats with no growth in the third month. When HT rats are allowed to recover by receiving PTU-free water, OM growth resumes remarkably so that by day 90, SA deficiency is fully compensated but OM is thinner and neuron number, while markedly increased, is not fully recovered. It is proposed that thyroid hormonal effects on OM growth may in part be mediated via submucosal connective tissue.

Supported by Univ. Of Illinois Research Board Award.

282.12

SEXUAL DIFFERENTIATION OF LARYNGEAL MUSCLE FIBER TYPE AND TENSION CHARACTERISTICS IN *XENOPUS LAEVIS*. Martha Tobias, Melanie Marin, and Darcy Kelley, Department of Biological Sciences, Columbia University, New York, N.Y. 10027

Male specific vocal behavior of *Xenopus laevis* frogs requires masculinization of the effector organ, the larynx. When the laryngeal nerve is stimulated at male mate call rates, male laryngeal muscle produces rapid tension transients in response to each stimulus pulse. Female laryngeal muscle produces a maintained tension at these rates (Tobias & Kelley, *J. Neurosci.* 1987). Sex differences in tension (transient vs. maintained) reflect the presence of exclusively fast twitch muscle fibers in males and predominantly slow twitch muscle fibers in females (Sassoon et al., *J. Neurosci.* 1987). At metamorphosis the fiber composition of laryngeal muscle is monomorphic and female-like.

In this study we determine when, during postmetamorphic development, fiber type and tension become masculine. The ability of male laryngeal muscle to produce tension transients is highly correlated (r=.87) with larynx weight. At metamorphosis the larynx weighs 0.008 gms; adult male larynx weighs 0.49 gms. Tension produced by male larynges weighing less than 0.05 gms is female-like, while tension produced by male larynges weighing more than 0.18 gms is male-like. Larynges weighing between 0.05 and 0.18 are partially masculinized and highly variable. These data are in good agreement with the fiber composition of laryngeal muscle at these stages. Laryngeal muscle composition is female-like (i.e. primarily slow twitch) at 0.06 gms larynx weight. Above 0.12 gms, laryngeal muscle composition is male-like (i.e. exclusively fast twitch). At larynx weights between 0.06 and 0.12 gms, fiber composition is transitional; there is a gradual decrease in the number of slow twitch fibers. These data suggest that tension characteristics, which are essential for sound production, are controlled by fiber type.

PAIN MODULATION: STRESS AND SENSORY STIMULATION

283.1

DISSOCIATION OF OPIOID AND NONOPIOID FORMS OF ANALGESIA BY ADULT MONOSODIUM GLUTAMATE TREATMENT IN RATS. N.S. Sathaye and R.J. Bodnar. Dept. of Psychol. and Neuropsychol. Doctoral sub-Program, Queens College, CUNY, Flushing, NY 11367.

Neonatal, but not adult administration of monosodium glutamate (MSG) selectively destroys circumventricular organs, especially the medial-basal hypothalamus (MBH). Neonatal MSG attenuates both nonopioid (continuous cold-water swim, CCWS, 2°C, 3.5 min) and morphine (5 mg/kg, SC) analgesia in adult rats. Since adult administration of MSG produces effects opposite to neonatal MSG for ingestive responses, the present study evaluated the effects of adult MSG administration (1-6 g/kg, SC) upon basal nociception on the tail-flick and jump tests as well as CCWS and morphine analgesia. While adult treatment with MSG failed to affect basal nociception, the 6 g/kg dose significantly potentiated CCWS analgesia and significantly decreased morphine analgesia. An osmolarity control (NaCl, 2.37 M) failed to exert effects. MSG (1 g/kg) generally failed to affect either form of analgesia. These data indicate a clear dissociation in opioid and nonopioid forms of analgesia by adult MSG, possibly through excitation of MBH neurons by MSG.

283.2

PRESENTATION OF A CAT PRODUCES INCREASED TAIL FLICK THRESHOLDS IN RATS. A. H. Lichtman and M. S. Fanselow*. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Using the formalin test, Lester and Fanselow (Behav. Neurosci.,99:756, 1985) have demonstrated that exposure to a cat produces a naltrexone reversible hypoalgesia in rats. Because much evidence indicates that different neural mechanisms underlie different types of pain, we examined whether exposure to cat would also produce analgesia in the tail flick test.

In Experiment 1, half of the rats were exposed to a cat and the other half of the subjects served as controls. Approximately 2 minutes after presentation of the cat or an equivalent period of time in control animals, 3 consecutive ascending series of tail shock were given until a tail flick was detected. Exposure to a cat significantly elevated the intensity of shock required to elicit a tail flick.

In Experiment 2, subjects were administered either naltrexone (7 mg/kg, ip) or saline and 15 minutes later half of the subject in each group were exposed to a cat, while the other half served as controls. As in Experiment 1, exposure to a cat increased tail flick thresholds, however, naltrexone failed to attenuate this decrease in pain sensitivity. These results suggest that opioid involvement in antinociception produced by the presence of a cat depends on the type of pain test used.

283.3

THE IMPACT OF CHOLINERGIC DRUGS ON MILD SHOCK-INDUCED ANALGESIA PARALLELS THEIR IMPACT ON MEMORY. J. W. Grau and M. W. Meagher. Dept. of Psychology, Texas A & M University, College Station, TX 77843.

We have previously shown that mild shock (3, 0.75 sec, 1.0 mA) elicits a strong analgesia in rats on the tail-flick test. We have suggested that the memory of the aversive event mediates the activation of the analgesic systems in this situation (Grau, J. W., Beh. Neurosci., 101: 272, 1987). If this is true, then cholinergic drugs which influence memory should have a parallel impact on the analgesia observed after mild shock.

To test this, we first assessed the impact of the cholinergic antagonist scopolamine (0, 0.1, 1.0, 10 mg/kg), a drug which others have shown can disrupt memory. We found that scopolamine also disrupts mild shock-induced analgesia. We then tested the impact of the anticholinesterase, physostigmine, a drug which others have reported can enhance memory. We found that a low dose of this drug (0.1 mg/kg) also enhances mild shock-induced analgesia.

Others have suggested that these analgesic effects can only be observed if subjects receive baseline testing prior to the administration of shock (Gower, A.J., & Tricklebank, M.D., Neuropharm., 25: 1161, 1986). We tested this hypothesis and found that mild shock induced a strong analgesia, and a low dose of physostigmine (0.1 mg/kg) potentiated this analgesia, irrespective of whether or not the subjects received baseline testing.

283.4

INTENSE, BUT NOT WEAK, SHOCKS INDUCE A TRANSIENT NONOPIOID, AND A LONG-LASTING OPIOID, ANALGESIA IN SPINAL RATS. M. W. Meagher and J. W. Grau. Dept. of Psychology, Texas A & M University, College Station, TX, 77843.

Elsewhere we have shown that 3 brief (0.75 sec) or 3 long (25 sec) tail-shocks (1 mA) elicit analgesia on the tail-flick test. Here we examined whether these shock schedules directly activate intraspinal analgesic systems by examining the impact of T2 cord transection. We found that spinal transection entirely eliminated both examples of analgesia, which suggests that these analgesias are mediated by supraspinal systems. Since others (Watkins et al., Brain Res., 245: 97, 1982) have suggested that more severe shock schedules elicit a spinally mediated non-opioid analgesia, we then examined whether increasing shock intensity or duration would induce analgesia in spinal rats. We found that increasing the intensity of the three 25 sec shocks from 1 mA to 3 mA induced a strong analgesia, but that increasing the duration of the three 1 mA shocks from 25 sec to 75 sec did not. Since both of these manipulations yielded the same coulometric product, this result suggests that the coulometric hypothesis cannot be used to predict the conditions under which intraspinal analgesic systems are directly activated. Finally, we tested whether the analgesia induced by the three 25 sec 3 mA shocks was opioid mediated. We found that naltrexone (14 mg/kg) had no impact on the analgesia observed 2 and 4 min after shock, but that it totally blocked the analgesia observed at 6, 8 and 10 min post-shock.

283.5

VAGINOCERVICAL STIMULATION SUPPRESSES NOXIOUS SENSORY INPUT AT THE SPINAL CORD; 2-DG AUTORADIOGRAPHIC EVIDENCE. Byron M. Johnson*, Claudia Pott, Allan Siegel, Norman T. Adler, and Barry R. Komisaruk. Inst. Animal Behavior, Rutgers Univ. and Dept. Neurosci., Univ. Med. Dent. N.J., Newark, N.J. 07102, and Dept. Psych., Univ. Penn., Phila. 19104.

Based on 2-DG autoradiography and computerized densitometry, we present evidence that a significant component of vaginocervical stimulation (VS)-produced analgesia occurs at spinal cord level L5 in rats. Four groups were used (n=4/gp): unilateral foot pinch (FP), VS, FP+VS, no stimulation. FP activated ipsilateral laminae 1-2 significantly more (16.7%) than did FP+VS (Mann-Whitney U-test, $p<0.03$, 2-tailed). FP activated ipsilateral laminae 1-2 significantly more than the contralateral laminae 1-2 ($p<0.03$, Wilcoxon test, 1-tailed). The difference between right and left laminae 1-2 in the FP group was significantly greater than either the right-left difference in the control group ($p<0.03$) or in the FP+VS group ($p<0.03$; Mann-Whitney U-tests, 2-tailed). These findings support our hypothesis that VS suppresses responses to noxious stimulation at the spinal afferent level. Support: NIH:1RO1NS22948-02 and GRS5- S06RR08223-04; Rutgers Univ. Provost's Fund & Busch Foundation

283.6

POST-SURGERY ELECTROCORTICOGRAM (ECoG) ZERO-CROSSING INDEX INCREASE REVEALED FOLLOWING NALOXONE IN THE RAT. Vahn A. Lewis, Univ. of Texas, Dental Branch, Houston, Tx. 77459

Wall (1) has proposed that an behavioral arousal follows injury. The ECoG Zero Crossing Index (ZI) is increased during arousal(2). In another study a trend toward an increased Z index was observed following a surgical procedure(SP). In this study, the effect of a SP on the ZI was assessed in the presence of 10 mg/kg naloxone (NX). 13 Sprague Dawley rats were used. Bipolar ECoG, neck muscle EMG electrodes and intrajugular catheter were implanted. This SP was the experimental treatment. Four hours following surgery the rats were administered NX and 30 minutes of ECoG activity was recorded. Control data was recorded one week following surgery when the rats were administered surgical anesthetics followed 4 hours later by NX. ZI was measured by computer. The surgical rat's ZI was 28% greater than that for control $P=<0.05$. The current report would support the hypothesis that a NX reversible inhibitory factor is present 4 hours post surgery and can act to mask Wall's post injury arousal.

1. Wall, P.D.,Pain, 5(1979)253-264. 2. Chouvet, et. al, Waking and Sleeping (1980),9-31,1980.

283.7

ROLE OF SUBSTANCE P (SP) METABOLISM IN SP-INDUCED DESENSITIZATION IN MICE. O.J. Igwe*, X. Sun* and A.A. Larson. Dept of Veterinary Biology, University of Minnesota, St. Paul, Minnesota 55108.

Intrathecal (i.t.) injection of SP in mice results in a behavioral syndrome characterized by caudally-directed biting and scratching (CBS). This behavior has been postulated to reflect nociception and is produced by injections of as small as 7.5 pmol of SP. Repeated injection of SP at 90 sec intervals has been shown to result in desensitization to the SP-induced CBS (Larson, Pain 33: 367, 1988). Both the SP-P (NK-I) receptor subtype and activation of the endogenous opioid system have been implicated in the induction of SP desensitization. The effects of various peptidase inhibitors [phosphoramidon, SQ20881 and bacitracin] on SP-mediated CBS in mice were examined in order to assess the roles of "enkephalinase", angiotensin converting enzyme and "bacitracin sensitive enzymes" in the behavioral syndrome. The protease inhibitors themselves did not induce CBS at the doses used but significantly increased both the magnitude and the duration of the SP response when coadministered i.t. Protease inhibitors also prevented the development of SP-induced desensitization over the same dosage interval. Coadministration of N-terminal fragments of SP (SP_{1-4}, SP_{1-7} and SP_{1-9}) at doses that did not elicit the CBS response when administered alone, decreased the magnitude of SP-induced CBS. The inhibition was dose-related for SP_{1-7}. Administration of the C-terminal fragment SP_{3-11}, which elicits CBS, did not result in desensitization to CBS after multiple i.t. injections at 1 min intervals. The present results strongly suggest that N-terminal metabolic fragments of SP are involved in the modulation of SP-induced desensitization at the spinal cord level. Supported by USPHS Grants DA04090, DA04190 & DA00124.

283.8

MODULATION OF SPINAL NOCICEPTIVE TRANSMISSION BY VAGAL AFFERENT STIMULATION (VAS) IN THE RAT. K. Ren, A. Randich and G.F. Gebhart. Departments of Pharmacology and Psychology, University of Iowa, Iowa City, IA 52242.

VAS has been shown to modulate nociceptive behavior. To distinguish between modulation of the sensory event from the motor response to the noxious stimulus, the effect of VAS on responses of dorsal horn neurons to noxious heating of the skin was studied. Unit activity was recorded extracellularly in anesthetized, paralyzed rats. VAS was delivered to the central end of the cut cervical vagus nerve. All units responded to mechanical stimuli and 50°C heating of the skin. Responses of 55% of neurons to noxious heating were facilitated, followed by inhibition, as intensities of VAS increased; 34% of the units were only inhibited and 9% only facilitated. VAS decreased the slope of the stimulus response function (SRF) at intensities inhibiting unit responses to noxious heat, but had no effect on the slope of the SRF at lower, facilitatory intensities of VAS. The apparent latencies for VAS effects were 91 ± 11ms for inhibition and 278 ± 59ms for facilitation. The effects of VAS on spinal unit activity was dissociable from hemodynamic changes produced by VAS. The results establish that vagal input to the CNS has modulatory effects on spinal nociceptive transmission. Differences between VAS-produced facilitatory and inhibitory effects suggest a selective and complex influence of vagal input on central processing of somatosensory information.

283.9

COUNTERIRRITATION: INHIBITION OF VISCERAL AND CUTANEOUS NOCICEPTION. T.J.Ness and G.F.Gebhart. Dept. Pharmacol., Univ. of Iowa, Iowa City, IA 52242.

Counterirritation is the phenomenon whereby one noxious stimulus reduces the sensation of pain arising from another noxious stimulus. The present study examined the interactions between visceral and cutaneous nociceptive mechanisms in the rat using colorectal distension (DIST, 40-100 mmHg, 10-40 s), heat (50-60°C) and pinch as noxious stimuli. Pseudaffective reflexes and spinal dorsal horn neuronal responses were evoked by these stimuli. The magnitude of these reflex and neuronal responses was decreased in an intensity-dependent fashion by conditioning noxious stimuli presented elsewhere in the body. Specifically, the following was observed:

(1) Dorsal horn neurons excited by radiant heating of the hindpaw were inhibited by conditioning noxious DIST.

(2) Dorsal horn neurons excited by noxious DIST were inhibited by conditioning noxious heat and pinch.

(3) The tail flick reflex evoked by heating of the tail was inhibited by conditioning noxious DIST.

(4) The visceromotor reflex (contraction of abdominal muscles) evoked by DIST was inhibited by conditioning noxious heat and pinch.

Supported by NS19912, DA02879 and a Life and Health Insurance Medical Research Fellowship.

283.10

CONVENTIONAL AND ACUPUNCTURE-LIKE TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION (TENS) EXCITES SIMILAR TYPES OF PERIPHERAL AFFERENT FIBERS. M.F.Levin and C.W.Y.Chan. School of Physical and Occupational Therapy, McGill University, Montreal, Canada H3G 1Y5.

Two types of TENS, commonly used clinically for pain relief, are: 1) Conventional (conv-) TENS, applied at low intensity and high frequency; and 2) Acupuncture-like (acu-) TENS, applied at high intensity and low frequency. They are alternately reported to excite only large diameter, or large and small diameter, afferent fibers. The purpose of our study was to resolve this controversy by determining which peripheral afferent fiber(s) is(are) activated by the two types of therapeutic TENS.

Seventeen healthy volunteers, aged 19 to 30 years, participated in the study. Two main types of TENS-like stimulation were delivered to the median nerve at the wrist: 1) conv-TENS: single pulses at an intensity of 3 x T = sensory threshold; and 2) two kinds of acu-TENS: single pulses at 0.1 Hz, and trains of 100 Hz pulses at 4 Hz, both delivered at an intensity greater than 3 x T. For averaging purposes, 30 compound action potentials per type of stimulation were recorded with surface electrodes placed over the median nerve in the cubital fossa. Nerve conduction velocities were computed as the distance divided by the time to the peaks of the negative and of the positive waves of the compound action potentials.

Our results showed that the mean conduction velocities of the afferent fibers excited by conv-TENS, single pulse and short train acu-TENS ranged from: 65.4±SD 9.5 to 50.3±5.0, 61.4±5.5 to 46.4±7.4, and 54.8±6.2 to 41.3±5.4 m/sec respectively. In man, large diameter afferent fibers conduct between 80 and 55 m/sec. Thus, conv- and acu-TENS activated similar fiber types, predominantly in the A-beta range. This finding suggested that the pain-relieving effects of these two types of TENS may be mediated by the activation of similar peripheral afferent fibers.

283.11

VASOPRESSIN-INDUCED SCRATCHING AND ANTINOCICEPTION. C.L. Thurston, J.G. Campbell*, E. Carstens, and L.R. Watkins. Animal Physiol., U.C. Davis, Davis, CA 95616

Lumbosacral intrathecal (IT) injection of vasopressin (VP) produces antinociception and scratching bouts. These experiments characterize the antinociception and scratching with respect to receptor specificity, interactions with opiates, and possible relationships to convulsant activity. IT injection of a V1-specific VP antagonist (dEt2AVP, 50 ng, gift from Dr. M. Manning) blocked IT VP-induced antinociception and scratching. The opiate antagonists naloxone (1 mg/Kg IP) and naltrexone (10 mg/Kg IP) both attenuated the antinociceptive effects of VP. Naltrexone, but not naloxone, potentiated the scratching bouts. IT injection of morphine (20 ug) tended to potentiate the VP-induced scratching, but the effect was not significant. IT injection of the anticonvulsant valproic acid (5 and 7.5 ul) had no effect on either IT VP-induced antinociception or scratching. To further clarify possible convulsant activity, some rats were injected IT at the high thoracic level with either VP (25 ng), morphine (200 ug), or substance P (40 ug). Convulsant activity induced by these substances would be indicated by motor activity initiated at the dermatome level of drug injection, whereas a sensory effect of the drug would be indicated by scratching directed at the dermatome level of drug injection. Thoracic injection of all 3 drugs induced hindlimb scratching directed more rostrally (face, neck, shoulders, forelimbs, sternum and upper ribs) than lumbosacral injection (flanks and abdomen). These data suggest that IT VP-induced scratching involves activation of V1-like VP receptors, interaction with opiate systems, and probably activation of sensory fibers as opposed to motor convulsions. These data also show that IT VP-induced antinociception is mediated by V1-like VP receptors, at least partially opioid in nature, and does not appear to result from convulsant activity.

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283.12

BASES OF PHENYLEPHRINE (PHE) ANALGESIA. LR Watkins & CL Thurston. Dept. Animal Physiol., Univ. CA at Davis, Davis, CA 95616.

Acute hypertensive states can produce analgesia, via unknown mechanisms. The purpose of the present study was to define neural & potential hormonal bases of analgesia induced by i.v. infusion of the hypertensive agent PHE. Since PHE can (1) excite baroreceptor afferents to the n. tractus solitarius (NTS), (2) release pituitary ACTH & B-endorphin, & (3) mimic the action of stress-induced (nor)epinephrine release, neural & hormonal pathways are both possible. All rats were implanted with right jugular & left carotid cannulae for PHE infusion & blood pressure/heart rate monitoring, respectively, & were tested ≥ 1 day later in the unanesthetized state using tail flick (TF) as the measure of pain responsivity. TF latencies were recorded prior to PHE, each 5 min of the 26 min PHE infusion & through 50 min post-PHE. Pretreatment with dexamethasone (DEX, 3 mg/kg 16 hr pre-PHE) abolished PHE-analgesia. This DEX dose blocks PHE-induced ACTH (but not B-endorphin) release, suggesting involvement of the pituitary-adrenal axis. However, acute block of all adrenal hormones (via closure at time of testing of pre-implanted adrenal ligatures) did not alter baseline TF latencies & did not block PHE analgesia. Thus PHE analgesia is not due to adrenal hormones. Since PHE induced copious urination only in DEX rats, this suggests that PHE normally releases vasopressin (VP) from the paraventricular hypothalamus (PVH), possibly via the known NTS-PVH link. Since (1) pilot studies suggest that spinal cord dorsolateral funiculus (DLF) lesions block PHE analgesia, (2) PVH sends VP projections to the spinal cord via the DLF, & (3) intrathecal (IT) VP produces analgesia via V1-like VP receptors & partly via opiate receptors, the effects of IT V1 antagonist & naloxone were tested. The V1 VP antagonist, but not naloxone, blocked PHE analgesia. To date, then, these studies suggest that PHE analgesia may be mediated by a baroreceptor-NTS-PVH-DLF pathway leading to VP release at spinal levels. NIH NS20037; V1 antagonist a gift from Dr. M. Manning.

283.13

SPATIAL SUMMATION IN HUMAN PAIN PERCEPTION. D.K. Douglass*, E. Carstens and L.R. Watkins (SPON: P.A. Pappone) Dept. Animal Physiology, University of California, Davis, CA 95616.

While spatial summation has been shown electrophysiologically to occur in reflexes involving unmyelinated "pain" fibers, previous psychophysical research has suggested that there is no spatial summation in human pain perception. However, the majority of these psychophysical studies have looked only at pain threshold, or at a single stimulus intensity. The intent of this study is to explore summation over a range of suprathreshold thermal stimuli. Visual analog scales (VAS) were used to rate the sensory and affective components of pain elicited by 45-51°C contact heat applied to the volar forearm with Peltier thermodes (area=0.42cm^2 each). To compare possible summation effects within and between dermatomes, two separate dermatomes were used, one innervated primarily by the musculocutaneous nerve (D1) and one by the medial cutaneous nerve (D2). These nerves enter the spinal column at the sixth cervical and first thoracic vertebral level, respectively. Five trained subjects attended two sessions each. In session 1, one thermode was placed an equal number of times in D1 and D2. In session 2, two thermodes were placed, 5 cm apart, an equal number of times on the same dermatome (D1+D1 or D2+D2) and across dermatomes (D1+D2). Comparisons were made between VAS ratings of pain produced by one vs. two thermodes. Both sensory and affective responses were significantly greater with two thermodes than with one (ANOVA, $p<.005$), with this effect being greater at the higher temperatures. We interpret this as spatial summation of pain, and preliminary data suggest that this summation is greater across dermatomes than within one dermatome. Supported by NIH Grant NS20037 (NINCDS) and UCD Grant D-1554.

283.14

EFFECTS OF INTRATHECAL ADENOSINE RECEPTOR AGONISTS ON NOCICEPTIVE, MOTOR AND BLADDER FUNCTION IN THE RAT. M. Sosnowski*, C.W. Stevens and T.L. Yaksh (SPON: P.R. Wilson). Mayo Clinic, Rochester, MN 55905.

We analyzed the effects of intrathecal (IT) adenosine analogues given through chronically implanted IT catheters and antagonists on nociceptive (tail flick: TF; hot plate: HP), motor dysfunction (MD) and bladder function in awake rats. Following IT administration A_1 (CHA and L-PIA) and A_2 (NECA) agonists resulted in dose-dependent increases in HP and TF response latencies, significant MD and blockade of the volume evoked micturition response.

	IT ED_{50} Values (nmol)			
	HP[1]	TF[1]	MD[2]	Bladder Block[2]
NECA	1.0	0.5	5.0	1.5
L-PIA	0.3	0.2	3.0	-
CHA	0.5	1.3	5.0	1.5

[1]mean, n=9-16; [2]lowest dose at which 50% or more of animals affected.

The BP and HR were continuously monitored during injection of 1.5 NM of NECA and 3 nmol of L-PIA. In spite of significant hindlimb paralysis, no significant changes were recorded. Pretreatment with IT caffeine (2 µmol) antagonized the antinociceptive and the motor dysfunction produced by L-PIA (1 nmol and 3 nmol). These data support a possible role of spinal A_1 and A_2 receptors in modulation of thermal nociceptive processing and bladder function, as well as motor outflow. (Supported by grant DA-02110.)

283.15

PHARMACOLOGY OF INTRATHECAL STRYCHNINE EVOKED ALLODYNIA: A PROBABLE ROLE OF EXCITATORY AMINO ACIDS IN A MODEL OF CENTRALLY MEDIATED HYPERESTHESIA. T.L. Yaksh and G.J. Harty*. Mayo Clinic, Rochester, MN 55905.

The intrathecal (IT) administration of strychnine (STRY; doses <30 µg/3 µl) results in minimal behavioral changes in the unanesthetized rat. Tactile stimuli applied in the dermatome acted upon by the IT STRY, however, results in prominent vocalization and aggressive behavior (touch evoked agitation; TEA). In the anesthetized rat following IT STRY such tactile stimulation yields marked (>50 mm Hg) increases in MABP. IT administration of doses of µ (morphine; 45 µg) δ (DADL; 30 µg) or α_2 (ST-91; 45 µg) which readily block the nocisponsive behavior evoked by a variety of somatic or visceral stimuli, produced only a mild (<25-35%) reduction in the TEA score evoked by IT STRY. In contrast, agents known to act as excitatory amino acid antagonists resulted in a clear dose dependent reduction in TEA at doses which did not produce significant motor dysfunction. The ordering of activity was: MK-801 > AP5 > kynurenic acid > SKF10047 > ketamine. The hypertensive effects of IT STRY were similarly diminished by IT MK-801. These data suggest that 1) light tactile stimuli can evoke pain-like behavior in the presence of subconvulsive doses of IT STRY; 2) that these effects are refractory to opioid and adrenergic modulatory effects; and 3) that the tactile evoked spinally mediated dysesthesia is mediated by an NMDA class of glutamate receptors. (Supported by grant DA-02110 (TLY).)

283.16

EVIDENCE FOR THE INVOLVEMENT OF BOTH THE MU AND DELTA OPIOID RECEPTOR SUBTYPES IN THE ANTINOCICEPTIVE EFFECTS OF OPIATES IN THE VENTROMEDIAL MEDULLA (VMM). N.R.F. Al-Rodhan and T.L. Yaksh. Dept. of Neurologic Surgery, Mayo Clinic, Rochester, MN 55905.

We have examined the effects of several selective opioid ligands following their administration (0.5 µl), through chronically implanted guides placed stereotaxically into the VMM. Antinociception was measured using 1) the hot plate (HP; 52.5°C; baseline latency = 9.7 ± 2.9 sec; 60 sec cutoff) and the thermally evoked tail flick test (TF; baseline latency = 2.8 ± 0.4 sec; 6 sec cutoff). The ordering of agonist potency on the HP and TF was: sufentanil > β-endorphin > PL017 = DPDPE = DSTLE >> meptazinol = U50488H ≈ 0. The antinociceptive effects of all agonists were naloxone-reversible. The irreversible mu-selective non-competitive antagonist β-funaltrexamine (β-FNA; 5 nmol) given at the site 24 hrs in advance produced a significant antagonism of SUF, β-End, PL017, DSTLE but not DPDPE (the most selective delta agonist). These results suggest that in the VMM, both the mu and delta receptors are involved in the antinociceptive effects of opioids to thermal noxious stimuli as measured by the HP and TF tests. These results are in contrast to our previous observations in the PAG where similar experiments suggested the sole involvement of the mu receptor (Al-Rodhan and Yaksh, Soc. Neurosci. Abst. 13:638, 1987). (Supported by the Mayo Fdn.; the King Faisal Specialist Hosp., Saudi Arabia (NRFA); NIH grant NS-16541 (TLY).)

283.17

CHOLINERGIC INHIBITION OF A SPINAL NOCICEPTIVE REFLEX IN THE RAT: COMPARISON OF SENSORY AND MOTOR EFFECTS. A.M. Lohof, M.M. Morgan, B. Beilin*, and J.C. Liebeskind. Department of Psychology, UCLA, Los Angeles, CA 90024-1563.

Intrathecally-administered cholinomimetic drugs can produce antinociception. Cholinergic binding sites exist in both the dorsal and ventral horns of the spinal cord, suggesting roles in both sensory and motor functions. Prior studies have not addressed in detail the possible contribution of a motor deficit in the production of nociceptive reflex inhibition. Three experiments were designed to quantify motor function as well as nociceptive reflex inhibition after intrathecal administration of a cholinergic agonist. Rats were anesthetized with pentobarbital and implanted with an intrathecal catheter terminating at the level of the lumbar enlargement. Pain sensitivity was assessed with the tail-flick test. 1) Anesthetized animals received carbachol HCl (5 µg) or artificial CSF in a volume of 10 µl followed by a 5 µl flush; treatment with carbachol produced a pronounced increase in tail-flick latency. This effect was eliminated in animals pretreated with scopolamine (0.5 mg/kg). 2) A group of unanesthetized animals receiving intrathecal carbachol or CSF one day after catheter implantation also showed inhibition of the tail-flick with no apparent motor deficit produced by carbachol. 3) To quantify motor function, a third group was observed in an open field after intrathecal administration of carbachol or CSF. A small decrease in spontaneous motor activity was evident after carbachol treatment. These results indicate that stimulation of spinal cholinergic receptors may alter function in both the dorsal and ventral horns, but it seems unlikely that the inhibition of nociceptive responses is entirely attributable to a motor deficit. (NIH grant NS07628)

283.18

THE TAIL-FLICK REFLEX: INTERACTIONS BETWEEN TESTING SITE, PENTOBARBITAL ANESTHESIA, TRIAL, AND INTER-FLICK-INTERVAL. J.T. Cannon, E.W. Walsh*, D.B. Roat* and R.J. Liskowicz*. Dept. of Psychology, University of Scranton, Scranton, PA 18510-2192.

The tail-flick response to heating is one of the most widely used indices of nociception in rodents. Ness, Jones and Gebhart (Brain Research, 426:169-172, 1987) recently reported that the site of heating caused systematic changes in flick latencies in either pentobarbital anesthetized or spinalized rats.

Here we examined interactions between tail-flick latencies and a number of variables, including site of testing. Forty male, albino rats (350-450g) were studied during the dark phase of a 12-12 hr light-dark cycle. Animals were divided into 4 groups of 10 that received 30 tail-flick trials at 1 or 2 min intervals while awake or anesthetized (pentobarbital, 55 mg/kg, IP, 40-70 min prior to testing). Five tail spots, separated by 2 cm beginning 2 cm from the tail tip, were tested in a random fashion by blocks of 5 with no spot being retested in sequence. The .05 level of significance was used throughout.

A 2 (interval) x 2 (anesthesia) x 5 (testing site) x 6 (trial blocks) ANOVA revealed significant main effects for trial blocks and testing site. There were no significant 2-way interactions. The only significant 3-way interaction was between flick intervals, anesthesia and trial blocks.

Overall, tail-flick latencies decreased across the first 3 trial blocks. The decrease in latencies across trials was greatest in anesthetized animals tested at 2 min intervals. Unexpectedly, our longest flick latencies were obtained at both the most proximal and the most distal tail sites. In contrast, Ness et al. (1987) found that latencies increased across testing sites from distal to proximal.

We agree with the main conclusions of Ness et al.; controls should be incorporated in tail-flick research to eliminate the possibility of confounds due to latency differences that are testing site-dependent and, when possible, testing site should be included as a variable in statistical analyses of tail-flick data.

283.19

THE EFFECT OF PENTOBARBITAL ON GABAERGIC MODULATION OF NOCICEPTIVE THRESHOLD. Edward J. Drower and Donna L. Hammond. CNS Diseases Research, G.D. Searle & Co., Skokie, Illinois 60077

Microinjection of GABA receptor agonists and antagonists in the n. raphe magnus (NRM) and n. reticularis gigantocellularis parsα (NGCpα) produces hyperalgesia and hypoalgesia, respectively, suggesting that these neurons are subject to GABAergic regulation. This study examined the potency of pentobarbital (P) pretreatment on the potency of GABA receptor agonists or antagonists microinjected in the NRM/NGCpα. Rats were injected with P prior to microinjection of either a $GABA_A$ receptor antagonist (bicuculline; BIC, 40 ng) or agonist (THIP; 300 ng) in the NRM/NGCpα. Alterations in nociceptive threshold were determined using the tail flick test. In rats pretreated with 7.5 mg/kg s.c. P, the effects of THIP or BIC on nociceptive threshold were comparable to their effects in untreated rats. Following administration of 50 mg/kg s.c. P, the hypoalgesia produced by microinjection of BIC was augmented as compared to its effect in the unanesthetized rat. This observation is consistent with previous biochemical studies indicating that barbiturates modulate the GABA receptor. It additionally suggests that the tonic inhibition of NRM/NGCpα neurons is enhanced in anesthetized rats.

284.1

INTRATHECAL MORPHINE CAUSES NALOXONE-REVESIBLE SUPPRESSION OF REFLEX RESPONSES TO NOXIOUS AND INNOCUOUS STIMULI IN PATIENTS WITH CHRONIC SPINAL CORD LESIONS. M.C. Wainberg*, R.M. Herman. (SPONS: M.J. Perlow) Catholic Med. Ctr., Manchester, NH 03102; Good Sam. Med. Ctr., Phoenix, AZ 85006.

In normal animal and man, intraspinal morphine (MOR) inhibits reflex motor responses to noxious cutaneous stimulation, but not to innocuous stimulation. Such specificity of action was examined in 8 patients with "spasticity" secondary to complete (3) and incomplete (5) suprasacral spinal cord lesions, each exposed to one or more injections of intrathecal (i.t.) MOR (50-400 μg). Reflex EMG discharges from bilateral proximal and distal lower limb, and striated ano-urethral muscles were elicited by noxious (electrical) and innocuous (brush) cutaneous stimulation of the foot and penis (or clitoris). All stimuli induced widespread discharges in the sphincter, and proximal and distal lower limb muscles, often producing flexor, "mass", motor contractions. At all doses, i.t. MOR suppressed nociceptive and non-nociceptive reflexes; in patients with sensory discrimination of pain and touch, i.t. MOR reduced pain perception while tactile perception was unaltered. Naloxone, i.v. (≥10 μg/kg), readily reversed the actions of i.t. MOR. These results suggest that i.t. MOR acts on opiate receptor sites located in the dorsal horn (sensory: nociceptive reflexes), and in the ventral horn (motor: non-nociceptive reflexes); apparently, the ventral site is released from suprasacral inhibition by spinalization. (Partial Support: Cath. Med. Ctr., Spinal Cord Soc.).

284.2

ROLE OF ADENYLATE CYCLASE IN SPINAL ANTINOCICEPTION PRODUCED BY MORPHINE, NORADRENALINE AND BACLOFEN. D. Nicholson* and J. Sawynok. Dept. Pharmacology, Dalhousie Univ., Halifax, Canada.

Pertussis toxin and agents which increase cyclic AMP levels inhibit opioid effects in cultured spinal cord ganglion explants (Brain Res. 370, 1986, 61; 400, 1987, 185). In vivo pretreatment with pertussis toxin inhibits spinal antinociception by morphine, noradrenaline and baclofen (Eur. J. Pharmacol. 146, 1988, 65). In this study, we examined the effects of forskolin (adenylate cyclase activator) and Ro 201724 (phosphodiesterase inhibitor) on antinociception (tail flick and hot plate tests) in rats implanted with chronic indwelling intrathecal cannulas. Pretreatment with forskolin (10 μg) did not affect or slightly reduced antinociception produced by morphine (5-10 μg), inhibited noradrenaline (5-15 μg) and increased the effect of baclofen (0.8 μg). Pretreatment with Ro 201724 (30 μg) inhibited the action of morphine but did not affect noradrenaline or baclofen. These results provide some in vivo support for inhibition of adenylate cyclase in spinal antinociception produced by morphine and noradrenaline (although their actions do not appear identical) but not by baclofen. (Supported by MRC Canada)

284.3

SUFENTANIL, K-AGONIST U-50,488H, AND D-ala[2]-met[5]-ENKEPHALINAMIDE (DAME) INHIBIT SUBSTANCE P RELEASE FROM PRIMARY SENSORY NEURONS. **H. M. Chang,*[1] R.M. Kream,*[2] and G.G. Holz[2]. Dept. Anesthesia, Harvard Medical School, Children's Hospital, Boston, MA[1] and Dept. Anesthesia Research, Tufts University-New England Medical Center, Boston, MA[2].**

Presynaptic inhibition of transmission in nociceptive sensory pathways may result from activation of opioid receptors located on spinal terminations of primary sensory neurons. Specifically, opioids may act to suppress excitation-secretion coupling in sensory nerve terminals, thereby blocking transmitter release at first order synapses in the spinal dorsal horn. The mechanism underlying this phenomenon may be studied *in vitro* using primary cultures of embyonic dorsal root ganglion neurons. In this model system, effects of opioid receptor-specific ligands may be assessed by measuring their affect on the electrically-evoked release of substance P (Holz et al., *J.Neurosci.* 8(2): 463-471). Here, we report that opioid agonists with preferential affinity for μ (sufentanil), κ (U-50,488H), and δ (DAME) receptors inhibit the release of substance P in a high affinity, dose-dependent manner, exhibiting saturability, stereoselectivity, and blockade by specific opioid receptor antagonists.

TREATMENT	% INHIBITION	TREATMENT	% INHIBITION
Sufentanil		**DAME, 5 μM**	**54 ± 2**
20 nM	**13 ± 2**		
100 nM	**35 ± 6**	**Dextrorphan, 50 μM**	**n.s.**
500 nM	**54 ± 13**		
U-50,488H		**Sufentanil, 500 nM**	**n.s.**
5 μM	**22 ± 15**	**+ Naloxone, 25 μM**	
10 μM	**32 ± 7**		
25 μM	**43 ± 8**	**U-50,488H, 10 μM**	**n.s.**
		+ Naltrexone, 25 μM	

284.4

EFFECTS OF μ OPIOID AND EXCITATORY AMINO ACID (EAA) AGONISTS ON PROJECTION NEURONS IN RAT SPINAL CORD. S. Lei and G. L. Wilcox, Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Electrophysiological studies of EAAs in the spinal cord have suggested their involvement in spinal synaptic excitation. Previous studies in this laboratory have shown that hyperalgesia, biting and scratching induced by intrathecal (i.t.) NMDA was inhibited by extremely low doses of the highly selective μ opioid agonist, DAMGO, and that this action was naloxone-reversible. By contrast, the selective δ opioid agonist DPDPE had no effect. DAMGO, at much higher doses near the toxic threshold, had little effect on Quisqualate (Quis) or Kainate action. Electrophysiological studies in this laboratory have shown selective excitation of nociceptive neurons by NMDA and Quis or the more selective agonist, AMPA. To examine the generality of the DAMGO-NMDA interaction, we tested DAMGO for inhibition of NMDA- and AMPA-induced excitation in further electrophysiological studies.

Extracellular recordings were conducted in rat spinal cord. Rats were anaesthetized with urethane (1.2g/kg, i.p.), paralyzed with gallamine (27mg/kg, i.v.) and artificially ventilated. Laminectomies were performed to expose both the lumbar and cervical enlargements of the spinal cord. The cervical laminectomy allowed antidromic activation of axons ascending in the lateral funiculi of the spinal cord. Eight barreled compound microelectrodes were used to apply drugs iontophoretically and to record from single spinal neurons.

Of a small initial sample, one third of AMPA-excited cells and one third of NMDA-excited cells were inhibited by DAMGO. In one cell, EAA-induced excitation was enhanced by morphine, and this enhancement was not blocked by i.v. injection of naloxone (0.3mg). Preliminary results agree with behavioral studies showing DAMGO to be more selectively inhibitory than morphine. However, they do not support a one-to-one association between NMDA excitation and DAMGO inhibition.

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284.5

MULTIPLICATIVE INTERACTION BETWEN INTRATHECALLY APPLIED OPIOIDS AND COCAINE INVOLVES δ RECEPTORS. S. C. Roerig*, K. F. Kitto*, S. Lei, and G. L. Wilcox, Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455

Previous studies have suggested that cocaine (i.t.) potentiates spinal morphine antinociception possibly through α_2-adrenergic receptors (Lei and Wilcox, Neurosci Abst. 1987). The present studies were designed to evaluate which opioid receptor types were involved since morphine interacts with both μ and δ receptors. Caudally directed biting and scratching behavior elicited during the first min after i.t. injection of substance P (SP) (10ng) was counted in male mice (ICR, 17-23 g). When co-injected with SP, morphine sulfate (MS), cocaine HCl (Co), the μ agonist DAMGO (Tyr-D-Ala-Gly-NMe-Phe-Gly(ol)) and the δ agonist DPDPE (D-Pen2-D-Pen5-enkephalin) inhibited the response. Opioids were then co-administered with Co in constant dose ratios and interactions were evaluated by isobolographic analysis. ED50 (95% CI) values were:

Drug Alone	Drug Combination
Co-10 (7.9-12) nmol	
MS-5.5 (4,7-6.6) nmol	Co-1.0 (0.9-1.2)nmol+MS-0.9 (0.8-1.1) nmol
DAMGO-0.17 (0.12-0.24) pmol	Co-6.7(6.0-7.6)nmol+DAMGO-0.08(0.07-0.10)pmol
DPDPE-0.62 (0.47-0.85) nmol	Co-0.8(0.6-0.9)nmol+DPDPE-0.04(0.03-0.05)nmol

Interactions between Co and MS and between Co and DPDPE were multiplicative, while the interaction between Co and DAMGO was additive. Thus, it appeared that opioids interact at spinal δ receptors to produce a multiplicative interaction with cocaine (i.t.). These results agree with those previously found for the same opioids co-administered with clonidine (i.t., α_2-adrenergic agonist) using the tail flick antinociceptive test (Roerig and Fujimoto, Fed. Proc. 1988), further supporting the role of spinal δ receptors in the opioid-adrenergic multiplicative interaction.

(Supported by USPHS grants DA-01933 and DA-04274)

284.6

MORPHINE SUPPRESSES NOXIOUS STIMULATION-EVOKED EXPRESSION OF THE *C-FOS* ONCOGENE IN SPINAL CORD NEURONS. R.W. Presley*, D. Menétrey*, J.D. Levine, and A.I. Basbaum (SPON: H.L. Fields). Depts. of Anesthesia, Anatomy, and Internal Medicine, UCSF, CA 94143.

The *c-fos* oncogene encodes a nuclear protein (Fos) that is associated with the regulation of differentiation in a variety of cell types. In spinal cord neurons, *c-fos* expression is evoked by peripheral noxious stimulation (Hunt et al, *Nature* 328:1987). Here we report on the effect of subcutaneous morphine on *c-fos* expression evoked in a tonic pain model, formalin injection into the hindpaw of rats.

After perfusion with 4% paraformaldehyde, 50 μm frozen sections of lumbar spinal cord were cut and immunostained with a rabbit antiserum directed against the *c-fos* protein. There were no *c-fos*-immunoreactive neurons in the spinal cords of unstimulated rats, either with or without morphine pretreatment. Formalin injection produced maximal *c-fos* activity at the L4-5 levels, and was confined to the side of the cord ipsilateral to the stimulus. After one hour, there was dense labelling of *c-fos* positive neurons in laminae I and II; many fewer cells were found in laminae III, IV, and V. Peak expression of *c-fos* occurred at two hours post-injection with similar dense labelling in Laminae I and II, but also with many cells labelled in laminae V and VII, and some in laminae III, IV, VIII, and X. After subcutaneous morphine (1.0 mg/kg) 30 minutes prior to formalin injection, *c-fos* expression was not significantly different from control. At a dose of 2.5 mg/kg, suppression was only noted in laminae V, VII, VIII, and X; however, morphine (5 or 10 mg/kg) profoundly reduced *c-fos* activity in all laminae. This was most apparent in the deeper laminae, V, VII, VIII, and X, but there was also a substantial reduction in *c-fos* expression in laminae I and II. This effect was naloxone reversible.

These data indicate that, in addition to the expected contribution of superficial dorsal horn neurons, there is a delayed activation and greater morphine susceptibility of deeper dorsal horn and more ventral neurons, which may be important features of "tonic" pain states.

284.7

OPIOID RECEPTOR SUBTYPES MEDIATING ANTINOCICEPTION IN THE FORMALIN AND TAIL-IMMERSION TESTS. L.Chen*, F.V.Abbott, Depts of Psychiatry and Pharmacology, McGill Univ. Montreal, PQ, Canada H3A 1A1

The receptor interactions underlying antinociceptive effects of morphine are complex and vary with to the nociceptive test and during the time course due to the presence of an active metabolite (Abbott et al 1986; Abbott & Palmour in press). The present studies used - funaltrexmine (FNA) and naloxonazine (NXINE) to explore antinociceptive mechanisms in the formalin (FN) and tail-immersion (TI) tests. FNA (4 g icv 4h before) blocked morphine in both tests indicating kappa receptor involvement is unlikely. NXINE (1 g icv 4h before) split the morphine time-effect curve into 2 components in the TI test: the late but not the early effects were attenuated. Sufentanyl, like the early component, was unaltered. Morphine-6-glucuronide (M6G) effects were attenuated by NXINE in the TI test. In the FN test NXINE had little effect on either the early or late components of morphine. The data imply 2 distinct mechanisms in the TI test. In the FN test morphine effects are mediated by receptors sensitive to FNA and insensitive to NXINE.

284.8

EFFECTS OF THE CHOLECYSTOKININ (CCK) ANTAGONIST, LORGLUMIDE (LGM) ON SPINAL MORPHINE-INDUCED ANTINOCICEPTION AND TOLERANCE. D.E. Kellstein and D.J. Mayer, Dept. of Physiology, Medical College of Virginia, Richmond, VA 23298.

Previous studies in this laboratory demonstrated that acute intrathecal (IT) injection of proglumide, a CCK antagonist, enhanced IT morphine analgesia whereas chronic (22 day) treatment attenuated opioid antinociception (S.N. Abs. 1987, 13, 1592). The present study investigated the effects of IT LGM (Rotta Research Lab.), a more potent analog of proglumide, on IT morphine analgesia and tolerance. Rats were implanted with lumbar IT catheters and allowed one week to recover. Morphine (1 µg, IT) analgesia was assessed weekly with a tail-flick assay during and after 22 days of LGM (7 ng, IT) treatment. On day one, LGM pretreatment reliably ($P<0.01$) enhanced morphine antinociception, but this enhancement was absent on days 8 and 15 and replaced by inhibition ($P<0.05$) of morphine on day 22. After daily LGM injections were terminated, opioid analgesia was unaltered by LGM pretreatment on days 29 and 36. Weekly co-administration of LGM with morphine had similar effects except that attenuation of morphine was not observed. In a separate study, daily IT co-injection of morphine (1 µg) with LGM (70 ng; does not alter morphine acutely) for 6 days prevented induction of opioid tolerance; i.e., analgesia was equivalent to that in non-tolerant rats and reliably ($P<0.01$) greater than in tolerant animals. These results suggest a complex interaction between spinal CCK and opioid systems, since 1) chronic IT injection of LGM (or proglumide), at a dose which enhances IT morphine acutely, inhibits analgesia, whereas 2) IT co-administration of morphine with a dose of LGM which has no acute effect upon morphine analgesia prevents development of tolerance. (Supported by HHS awards DA05316 and DA00576).

284.9

A ROLE FOR HISTAMINE H_2-RECEPTORS IN MORPHINE ANTINOCICEPTION. K.R. Gogas[1], L.B. Hough[1], M.B. Eberle*[1], R.A. Lyon[1], S.D. Glick[1], S.J. Ward*[2], R.C.Young*[3] and M.E. Parsons*[3]. [1]Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208; [2]Dept. of Pharmacology, Sterling Winthrop Research Institute, Rensselaer, NY 12144; [3]Smith, Kline and French Res. Ltd., The Frythe, Welwyn, Hertfordshire, England AL6 9AR.

To test the hypothesis that brain H_2-receptors are involved in the mediation of opiate antinociception, the effect of the first brain-penetrating H_2-antagonist, zolantidine, was determined on several opiate actions in male Sprague-Dawley rats. Zolantidine (s.c.) caused a dose-related inhibition of morphine antinociception in both the tail-flick (ID50=.135 umol/kg, Emax = 87%) and hot plate tests, with no effect on baseline responding. Morphine-induced increases in locomotor activity were also reduced by zolantidine, but no effect was seen on morphine-induced hyperthermia, catalepsy or lethality. Zolantidine (10^{-5}M) did not antagonize mu, delta or kappa agonists in vitro and showed at least 35-fold higher affinity at the H_2-receptor than at serotonergic, adrenergic or dopaminergic binding sites in brain. Furthermore, zolantidine had no effect on morphine brain levels measured by GC-MS methods, indicating the absence of a drug-drug interaction. To further clarify the role of H_2-receptors in zolantidine's anti-opiate activity in vivo, the potencies of 7 structural congeners of zolantidine on the H_2-receptor and on morphine antinociception were determined. Over 3 orders of magnitude, the rank order of potency of the compounds for inhibiting morphine antinociception was highly correlated ($p < .05$) with their potency as H_2-antagonists, suggesting an H_2-receptor involvement in opiate antinociception. (Supported by DA-03816 and Sterling Winthrop Research Institute).

284.10

DOPAMINERGIC MEDIATION OF D-AMPHETAMINE- AND MORPHINE-INDUCED ANALGESIA IN THE FORMALIN TEST. M.J. MORGAN and K.J.B. FRANKLIN, Dept. of Psychology, McGill University, P.Q., Canada, H3A 1B1.

D-amphetamine induced dose-dependent analgesia in the formalin test. Furthermore, sub-effective doses synergised with sub-effective doses of morphine to produce strong analgesia. Selective depletion of striatal dopamine by intra-VTA administration of 6-OHDA abolished analgesia produced by D-amphetamine or morphine. D-amphetamine analgesia was dose-dependently attenuated by the selective D1 and D2 antagonists SCH 23390 and pimozide, and was eliminated by the mixed antagonist alpha-flupentixol (0.5 mg/kg). Flupentixol also blocked morphine analgesia. Pimozide (0.5 mg/kg) and the high dose of SCH 23390 (0.1 mg/kg) increased the analgesia produced by low doses of morphine (0.75 to 3 mg/kg) and antagonized the effect of higher morphine doses. A low dose of SCH 23390 (0.01 mg/kg) was found to increase the analgesic activity of morphine and morphine-amphetamine mixtures. These data suggest that dopamine, acting at both D1 and D2 receptors, is involved in D-amphetamine- and morphine-induced analgesia in the formalin test.

284.11

DIFFERENTIAL EFFECTS OF POST-NATAL MORPHINE TREATMENT UPON OPIATE, OPIOID-MEDIATED AND NON-OPIOID-MEDIATED ANALGESIA IN ADULT RATS. D. Arjune and R.J. Bodnar. Dept. of Psychol. and Neuropsych. Doctoral Sub-Program , Queens College , CUNY , Flushing , NY 11367.

Post-natal exposure to opiates decreases hot-plate and tail-flick latencies and produces short-term reductions in opiate receptor number. The present study evaluated the effects of post-natal morphine (0 , 1 and 20 ug , SC , Days 1-7) upon subsequent analgesia induced by either morphine (2.5 and 5 mg/kg , SC) or continuous or intermittent cold-water swims (CCWS , ICWS , 2°C , 3.5 min) in adult rats. As established previously , male rats displayed significantly greater analgesic responses than female rats. Post-natal morphine significantly potentiated morphine analgesia in adult females , but slightly decreased these responses in adult male rats. In contrast , post-natal morphine failed to affect CCWS or ICWS analgesia in either gender. These data indicate an important interaction between gonadal steroids and post-natal exposure to opiates in the mediation of opiate , but presumably not non-opioid analgesic responses.

284.12

DIFFERENTIAL DEVELOPMENT OF THE ANALGESIC EFFECTS OF FOCAL MORPHINE AND GLUTAMATE ADMINISTRATION TO THE PERIAQUEDUCTAL GREY AREA OF THE RAT. L. A. Tive* and G. A. Barr. Biopsychology Dept., Hunter College of the City University of New York, New York, N.Y., 10021 and Dept. of Psychiatry, Albert Einstein College of Medicine, Bronx, N.Y.

The periaqueductal grey area (PAG) of the midbrain supports both opiate induced analgesia (OA) and stimulation produced analgesia (SPA) in many species including man. The ventral aspect of the PAG mediates SPA and OA that show cross-tolerance to each other and to systemic morphine and are naloxone reversible. The dorsal region of the PAG supports SPA that is neither cross-tolerant with morphine nor naloxone reversible. In this study, SPA and OA supported by the dorsal and ventral PAG were compared in developing rats. Ten day old pups were implanted with cannula aimed at either the dorsal or ventral PAG area. Injections of glutamate were administered to either the dorsal or ventral region in doses of 0, 60, or 180 mM/0.2 µl and subjects were tested for analgesia against noxious thermal and mechanical stimuli applied to the forepaw, hindpaw and tail. Morphine was administered to the ventral PAG in doses of 0, 2, or 6 µg/0.2 µl and analgesia was measured in the same manner. Glutamate injections to the dorsal PAG produced dose dependent analgesia against mechanical but not thermal stimuli. Glutamate administration to the ventral PAG did not produce analgesia against either stimulus type. Morphine administration to the ventral PAG induced robust, dose dependent analgesia against the thermal, but not the mechanical stimulus, that was stronger in the forepaw and hindpaw than in the tail. These results indicate that the SPA and OA supported by the ventral PAG area can be distingushed developmentally despite their pharmacological similarities in the adult. These two types of analgesia may be mediated by concurrent, rather than identical, spinopetal neuronal systems. The SPA supported by the dorsal and ventral aspects of the PAG can also be distinguished by their ontogeny indicating that they may be subserved by distinct neuroanatomical substrates.

285.1

TEMPORAL CHARACTERISTICS OF EXPLORATORY FINGER MOVEMENTS AND ACTIVATION OF MECHANORECEPTORS. E.Kunesch*, F.Binkofski*, H.-J.Freund. Dept. of Neurology, University of Düsseldorf, FRG

Little is known about the discharge patterns of skin mechanoreceptors (MRs) during exploratory finger movements. We recorded from single afferent nerve fibers innervating low threshold MRs of the finger pads during natural exploratory finger movements which were consistently performed at frequencies between 0.5–2 Hz. The velocity profiles of these movements along one single scan path were bell-shaped. When the object passed the receptive field of a slow (SA I, SA II) or fast (FA I, FA II) adapting unit, a discharge burst was elicited. Discharge rate within the burst increased with movement velocity.

Passive touch experiments in monkeys have shown that the spatial characteristics of grated surfaces are encoded in the population response rather than in the firing characteristics of single MRs (Darian-Smith I. et al., J.Physiol. 309:117,1980). We calculated the sequential activation of FA I MRs on the basis of their density distribution and movement velocity during natural exploratory movements. The maximal number of sequential receptor hits approximated 700/s. The limitation of exploratory finger movements during active touch to serial scan frequencies below 2 Hz may indicate that the central demodulator neurones cannot resolve higher sequential sampling rates.

285.2

SLOWLY ADAPTING TYPE I MECHANORECEPTOR DISCHARGE AS A FUNCTION OF DYNAMIC FORCE VERSUS DYNAMIC DISPLACEMENT STIMULATION OF GLABROUS SKIN OF RACCOON AND SQUIRREL MONKEY HAND. Benjamin H. Pubols Jr. The Robert S. Dow Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209.

The discharge rate of slowly adapting Type I (SAI) mechanoreceptive afferent fibers has been shown to be adequately described as a power function of displacement velocity (dynamic displacement) over a wide range of values (Pubols and Pubols, 1983). Apparently, the effect of rate of change in force (dynamic force) on dynamic discharge of glabrous skin mechanoreceptors has not been investigated in any mammalian species.

In 10 raccoon SAI units and 8 squirrel monkey SAI units, the effects of dynamic force and dynamic displacement on discharge rate during ramp stimulation were compared. In all 18 cases, power function exponents, b, were higher for the effect of displacement ramp velocity (range = .39-.70) than for the effect of dynamic force (range = .09-.59) on ramp discharge frequency. Thus, these SAI mechanoreceptors are more sensitive to variations in displacement velocity than to variations in dynamic force.

A possible explanation lies in the fact that the relationship between dynamic force and dynamic displacement is, itself, nonlinear, dynamic displacement being a power function of dynamic force, with values of $b < 1.0$. (Support: NS-19487, USPHS.)

285.3

STIMULUS INTEGRATION IN CUTANEOUS RECEPTIVE FIELDS. E.P. Gardner and C.I. Palmer. Dept. of Physiology, NYU Sch. Med., NY, NY 10016.

RA afferents innervate clusters of Meissner's corpuscles, showing 12-17 points of high sensitivity within their receptive fields (RFs). Pacinian corpuscles (PCs) also integrate information from a wide skin area. In order to determine whether RAs and PCs summate inputs from multiple sites within their RFs, we used the OPTACON (144 miniature probes arranged in a 6 column x 24 row matrix) to vary the density of tactile stimuli to the glabrous skin of the macaque monkey's hand. A four ms pulse was applied simultaneously to the central four columns of single rows, or to groups of 2-5 rows spanning 4.4 mm on the skin. Active rows were shifted in 1.1 mm steps across the OPTACON at 10, 20 or 40 ms interpulse intervals.

75% of RAs fired one spike/pulse to single rows, yielding uniform sensitivity in the RF, while PC responses peak at the center. Neither RAs nor PCs summate inputs from multiple stimulation sites. Simultaneous activation of 2-5 rows evokes the same or fewer spikes per pulse than the most effective individual row tested alone. Primate RAs and PCs thus follow activity in the dominant branch or terminus, suppressing additional inputs. Moving patterns evoke responses as long as at least one row stimulates the RF; broader patterns evoke longer spike trains. Suppression of activity was observed only at 100 Hz pulse rates using broad bars. Simultaneous activation of 5 adjacent rows at 100 Hz suppressed responses to the last 1 or 2 rows in 8 of 16 RAs, and 10 of 13 PCs. All RAs and 11 of 13 PCs responded to the entire set of 5 rows at 50 Hz, corresponding to a lateral speed of 55 mm/s. Recovery from stimulation of adjacent receptors is rapid, and nearly complete at intervals as short as 10 ms, allowing RAs and PCs to encode stimulus width by their duration of firing.

Multiple spike responses showed similar recovery cycles. Eight of 24 RAs responded with two spikes to OPTACON pulses to the RF center when tested with widely spaced single row patterns, or the leading edge of a multiple row array. Double spikes were reduced to one when 5 active rows in the RF were spaced 1 mm apart, at all Hz, suggesting that skin mechanics rather than receptor adaptation attenuate activity. Uniform sensitivity, short recovery cycles, and selection of a dominant branch, allow RAs to transmit information concerning maximum rate of curvature change from a wide skin area. As a stimulus shifts across the RF, this most salient stimulus feature is transmitted repetitively by each RA at the expense of fine grain localization. *(Supported by USPHS Research Grant NS11862.)*

285.4

TEMPORAL SUMMATION IN VIBROTACTION IS NOT PERIPHERALLY MEDIATED. C.M. Checkosky* and S.J. Bolanowski, Jr. (SPON: R Verrillo). Dept. Physiol., Univ. Rochester Med. School, Rochester, N.Y. 14642.

Temporal summation refers to the psychophysical phenomenon in which increases in stimulus duration produce decreases in the stimulus amplitude required for threshold detection. For bursts of sinusoidal stimuli having durations from 10–300 msec, the effect in both audition and vibrotaction produces a 5.3-fold increase in sensitivity, requiring a -3dB change in sensitivity per doubling of duration. For audition, the effect is centrally located, as shown for binaural-stimulus conditions, but no experiments have assessed the locus of the effect for vibrotaction. Vibrotactile thresholds mediated by the Pacinian-corpuscle (PC) fiber population in skin exhibit temporal summation. If the phenomenon is peripherally located, then activity recorded from individual PCs should show the -3dB/doubling effect. PCs isolated from cat mesentery were stimulated with sinusoidal displacement bursts of different durations and the intensity of the stimulus adjusted for a fixed criterion response of 1 impulse/burst. The average decrease for increases in stimulus duration was only -1dB/doubling of duration. The result indicates that temporal summation is not a property of PC fibers but that, as for audition, the process probably occurs centrally. The slight effect that did occur may be statistical in nature, in that longer durations may provide for greater probability that an impulse will occur. Support by NS23933

285.5

EFFECTS OF STATIC INDENTATION ON THE PACINIAN-CORPUSCLE RECEPTOR POTENTIAL. S.J.Bolanowski, Jr. and C.M.Checkosky* Dept. Physiol., Univ. Rochester Med. Sch., Roch., NY 14642

The receptor-potential (RP) of cat Pacinian corpuscles (PC) in response to sinusoidal displacements exhibit asymmetric, full-wave rectification. Furthermore, there are apparently two populations of PC types, one more sensitive to stimulus compression, the other to stimulus withdrawal. Based on these findings, we have proposed the presence of two groups of neural elements within each PC, the neural elements producing half-wave rectification with the two groups in polarity opposition. The RP is thus dependent upon the relative contribution to it from the two groups. If true, then changes in the RP should occur with selective deactivation of one group of elements. We have assessed this by varying the static indentation of the vibrating probe, noting that small static indentations should permit only the most sensitive group of elements to be excited, revealing a half-wave rectified RP. Increasing indentations should then produce concomitant changes in the RP shape as the other group becomes more active, eventually resulting in a full-wave rectified RP. The experiments show that changes in static indentation do indeed produce changes in the RP shape. The effect, however, is not half-wave to full-wave, but considerably more complicated. The results indicate that while the two-group hypothesis may still be correct, the transductive mechanism in each element does not half-wave rectify but more likely produces some degree of full-wave rectification. Supported by NS 23933.

285.6

CENTRAL PROJECTIONS OF DEEP AND SUPERFICIAL VIBRISSAL NERVES IN RAT MYSTACIAL PAD. E.L.Rice, J.Arvidsson, K. Johansson*, and H.Aldskogius. (SPON: R. Oesterreich) Anatomy Dept., Albany Medical College, Albany, NY 12208; Anatomy Dept., Karolinska Institute, Stockholm, Sweden

Each vibrissal follicle-sinus complex (F-SC) in the rat mystacial pad is innervated by a single, large deep vibrissal nerve (DVN) and several, small superficial vibrissal nerves (SVN's). DVN's and SVN's supply a similar variety of sensory endings, but differ in their distribution within each F-SC (Rice et al., JCN 252:154: 1986). Thus, DVN's and SVN's may have different functions and central projections. In 52 adult rats, HRP was applied to various combinations of isolated, transected DVN's (Arvidsson, JCN 211:84, 1982) or SVN's, or WGA-HRP was selectively applied to intact sites of SVN innervation. The distribution of TMB-revealed label differed in all the trigeminal nuclei, especially in the laminated portion of nucleus caudalis (L-NC). Label from a DVN formed a continuous column that angles from deep lamina III in rostral L-NC to lamina V in caudal L-NC. The somatotopic organization of the DVN's is in the transverse plane. Label of SVN's from each F-SC formed discreet loci in lamina I and in deep lamina II and superficial lamina III at a mid-level in L-NC. SVN somatotopic organization is in a longitudinal plane with only the dorso-ventral axis in register with the DVN organization. (Support: Swedish Medical Research Council)

285.7

TRIGEMINAL AFFERENT PROJECTIONS IN A DIVER, THE MUSKRAT

S. DeLisa Jarrell* and S.O.E. Ebbesson (SPON: D. Feist) Insts. Arctic Biol. and Mar. Sci., Univ. Alaska Fairbanks, Fairbanks, AK 99775

The diving response (DR) comprises reflex apnea, bradycardia and peripheral and visceral vasoconstriction, conserving oxygen for the brain and heart while maintaining central blood pressure. It is exhibited by aquatic representatives of all the vertebrate classes, and appears related to protective responses elicited under a variety of other conditions. It has been described as an "external narial reflex" in muskrats, triggered by wetting the nares and blocked by coating them with vaseline. Similar but less intense responses can be elicited in anesthetized muskrats by stimulation of the soft palate (SP) or anterior larynx (AL). In a preliminary search for connections that may be involved in mediating the DR, we have traced neural projections from the nares, SP and AL in muskrats. This report concerns projections to the sensory trigeminal complex.

Muskrats (*Ondatra zibethicus*) were injected with horseradish peroxidase (HRP) or wheatgerm agglutinin-HRP under halothane anesthesia, and transcardially perfused with fixative 24-48 h later under deep pentobarbital sodium anesthesia. Serial sections of frozen brains were thawed onto slides, and sets at intervals of 100 µm were reacted for HRP using tetramethylbenzidine; adjacent sets were Nissl stained. Projections from the nares included: dense, extensive terminals in marginal and gelatinous layers and sparse terminals in magnocellular layer of caudal spinal trigeminal subnucleus (Sp5C); paratrigeminal nucleus (Pa5); interpolar (Sp5I) and oral (Sp5O) subnuclei (with processes of labeled facial nerve cells extending into Sp5O); and principal trigeminal nucleus (Pr5). Projections from SP to Sp5C were sparse and restricted to the dorsal margin at lower levels; Sp5I, Sp5O and Pr5 received moderate, diffuse projections, whereas Pa5 was heavily labeled. AL injections produced labeling of Pa5, Sp5I, Sp5O and Pr5. In addition, projections from the nares were traced in feral Norway rats with results similar to those for muskrats except that Pa5 was not labeled.

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SOMATOSENSORY CORTEX II

286.1

NON-INVASIVE APPROACH TO 3 - DIMENSIONAL MAPPING OF THE HUMAN SOMATOSENSORY CORTEX. L. Narici°, L. Modena°*, V. Pizzella*, G.L. Romani&, P.M. Rossini#*, G. Torrioli*, Istituto di Elettronica dello Stato Solido, CNR Roma, Italy.

We show the results of a series of totally non-invasive neuromagnetic measurements on healthy human subjects aimed at resolving different cortical sensory areas activated by somatosensory stimulations of different body parts (wrist, elbow, ankle, etc.). The localizations of these areas agree well with the known somatosensory homunculus, which has been mapped in the past by a series of very invasive measurements. A few neuromagnetic localizations of sensory areas activated by periferal nerve stimulations have been reported previously, here we show that the entire homunculus can be reconstruct via non-invasive measurements.

Whenever the measured field presents a dipolar structure, the same technique permits to follow, non invasively, in three dimensions, the successive activation of different cortical areas following the presentation of the sensory stimulation. The time resolution is limited by the acquisition bandwith, and in the cases presented here, is of the order of 1 ms. The spatial resolution, limited by the external noise, is about 5 mm. The fitting routine gives the position, direction and strength of the equivalent dipole generating the measured field.

The presented results enphasize the usefulness of the neuromagnetic technique as a powerful tool aimed at studying non-invasively, the brain functional behaviour.

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286.2

DIFFERENT CONTRIBUTION OF SI AND SII CORTICES TO SCALP-RECORDED SEPs IN MAN. H.Hämäläinen, J.Kekoni*, K.Reinikainen*, R.Näätänen*, M.Sams*, and M.Hämäläinen*. Dept. Psychol., Univ. Helsinki, and Low Temp. Lab., Helsinki Univ. Technol., Finland.

Somatosensory evoked potentials were measured to tactile pulses and vibration applied to the fingertip in order to determine the scalp topography of different SEP components.

The first distinct contralateral response was an anteriorly negative and posteriorly positive peak at about 50-ms latency. On the ipsilateral side, no polarity reversal was seen. The isopotential field analysis revealed that at this latency a clearly dipolar electrical field pattern was located on the contralateral hemisphere. Similar pattern could be produced by fitting a dipole at the contralateral SI somatosensory area. Therefore this early response is probably generated by activation of the contralateral SI cortex, and the responses seen ipsilaterally are due to volume conduction.

The early response was followed by a negative (N70) deflection and by a bilateral positive (P100) peak. The isopotential field analysis revealed that N70 cannot be explained by a single contralateral generator, but rather by several which might be bilateral. Furthermore, the isopotential field pattern at the latency of P100 was best accounted for by generators placed bilaterally at the approximated sites of SII cortices.

286.3

MANY-NEURON RECORDINGS DEFINE SYNCHRONOUS ACTIVITY AND BEHAVIORAL GATING IN THALAMIC AND CORTICAL NETWORKS. J.K. Chapin, H.-C. Shin, and I.M. Patel, Dept. Physiology, Hahnemann U., Philadelphia, PA 19102

As part of an effort to define neural circuit bases for higher order somatic sensory processing we have developed a technique for simultaneous recording and discrimination of up to 12 single neurons in awake, behaving rats. For this, arrays of 25µ microwires were chronically implanted in the forepaw subregions of the somatosensory (SI) cortex and/or VPL thalamus. Sensory responses of these neurons could be tested by stimulating through electrodes chronically implanted in the forepaw. Previously we had shown that sensory transmission to SI and VPL neurons is gated according to phase of the forelimb step cycle. Here we found that several different temporal patterns of gating exist among the simultaneously recorded neurons, demonstrating that sensory inputs are dynamically routed along different paths through thalamic and cortical networks according to the phase of limb movement. Another use of this technique was to construct spike-triggered histograms to cross-correlate the activity of pairs of neurons. Most often these histograms exhibited relatively broad peaks around the trigger point, indicating that the two neurons were driven by some sort of common input. When these were observed during sensory stimulation it was possible to measure precise response latency relationships between the two neurons. Such histograms were also used to define phase relationships between neurons during episodes of synchronous firing, which often occurred during resting behavior. Finally, some spike-triggered histograms exhibited discrete short latency post-spike responses, indicating functional connections between the neurons. Thalmocortical functional projections were observed, which were phasically suppressed during limb movements, as would be predicted from the earlier gating studies. Cortico-thalamic functional connections were also observed, indicating a cortical inhibitory effect on thalamic neurons. Finally,cortico-cortical functional connections were observed, whose strength varied according to the phase of limb movement. These studies thus provided evidence at the synaptic level that information transmission within thalamocortical networks is dynamically modulated during behavior.

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286.4

VIBROTACTILE INTENSITY DIFFERENCE THRESHOLDS MEASURED BY TWO METHODS. G.A.Gescheider, S.J.Bolanowski, Jr. and R.T. Verrillo. Dept. Psychol., Hamilton College, Clinton, NY 13323 and Institute for Sensory Research, Syracuse Univ., Syracuse, NY 13244.

The difference thresholds for the detection of changes in vibration amplitude was measured as a function of the intensity and frequency of stimuli delivered through a 2.9 cm^2 contactor to the thenar eminence. Stimuli were either 25- or 250- Hz sinusoids or narrow-band noise centered at 250 Hz. Thresholds were measured by two-interval, forced-choice tracking under two methods of stimulus presentation. In the two-burst method, subjects had to judge which of two 700-ms bursts of vibration separated by 1000 ms was more intense. In the increment-detection method with continuous pedestal, subjects had to detect an increment in the amplitude of vibration. In the increment-detection method with 1500 ms pedestal bursts, the subject had to detect which of two successively presented pedestals contained an amplitude increment. Thresholds were consistently lower for detecting increments in the amplitude of continuous vibration than for detecting amplitude differences between successive bursts or amplitude increments in successive bursts. A "near miss" to Weber's Law was found for both sinusoidal and noise stimuli under both methods of stimulus presentation. The difference threshold was not affected by stimulus frequency. Supported by NS 24255 and NS 09940.

286.5

VIBRISSAL TACTILE DISCRIMINATION IN THE RAT. G.E. Carvell* and D.J. Simons (SPON: T.-K. Hung), Depts. of Physical Therapy and Physiology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

A testing procedure was developed to investigate discriminative tactile behavior in the rodent whisker system. Blindfolded adult rats were trained to jump to one of two platforms for a food reward. The edge of each platform had a rough or smooth surface. The distance between the start platform and the discrimination platforms was adjusted so that rats could contact the discriminanda with their whiskers by stretching across the gap. Discriminanda consisted of metal surfaces that were periodically cleaned to eliminate possible odor cues. Most animals learned to correctly discriminate between a smooth and a rough surface in 10-14 days. Frame-by-frame video analyses confirmed that the rats used only their whiskers to contact the discriminanda. The search phase was characterized by large head and whisker movements. Upon contact, the head remained relatively stationary and whisking amplitude was markedly reduced. Rats maintained whisker contact for many 10's of msecs during which time the hairs were continually bent. Thus whiskers do not engage objects as independent, rigid levers. Results demonstrate that rats can use their vibrissae to obtain detailed tactile information from the environment. Supported by NIH NS 23047.

286.6

RESPONSES TO TENDON VIBRATION IN MONKEY SOMATOSENSORY CORTEX. N. Bite* and W.A. MacKay. Dept. of Physiology, Univ. of Toronto, Toronto, Ont. M5S 1A8

Muscle tendon vibration has been shown to distort position sense. For example, vibration of the biceps muscle of humans produces the illusion of extension about the elbow joint. A possible explanation for this illusion is that the activation of muscle receptors in the biceps informs the cortex that the muscle has been stretched.

The purpose of the present study was to determine whether cells in the somatosensory cortex which are sensitive to joint position, could also be activated by tendon vibration.

Single unit chronic recording was performed in an awake, unanesthetized cynomolgous monkey. All of the somatosensory areas from 3a to 5 were examined. Once a sensitive cell was found, vibration was applied to the appropriate tendon at ~80 Hz, using a small solenoid driven by a function generator.

Cells which fired tonically for joint positioning gave a sustained response when vibration was applied, while phasic cells produced only an initial burst of spikes. The proportion of tonic cells responsive to vibration varied as follows: Area 3a, 12/18; Area 1, 5/22; Area 2 and 5, 15/40. The results suggest that the most likely locus for the kinesthetic illusion produced by tendon vibration is Area 3a. Supported by MRC Canada.

286.7

SI CORTICAL NEURONS PARTICIPATE IN THE ENCODING PROCESS BY WHICH MONKEYS PERCEIVE THE INTENSITY OF NOXIOUS THERMAL STIMULATION D.R. Kenshalo, Jr., E.H. Chudler, F. Anton* and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892

In the present series of studies, we have obtained additional evidence that the activity of SI cortical nociceptive neurons correlates with the monkey's ability to detect small increases in noxious temperatures. Monkeys initiated a trial by pressing an illuminated button. A contact thermode, positioned on the upper lip, subsequently increased in temperature from 38° to 46°C (T1). After a random foreperiod of 4 to 10 sec, the thermode increased in temperature an additional 0.2° to 0.8°C (T2). The monkey received a fruit juice reward for releasing the button within 2 sec of the onset of T2. The activity of 18 wide-dynamic-range neurons was recorded while the animal performed the thermal detection task. In addition, mean detection speed (MDS) to the onset of T2 was determined.

The monkeys' ability to detect near threshold T2 (0.2°C at 46°C) temperature increases was correlated with the discharge of SI neurons. A correct detection of T2 by the monkey resulted in a statistically significant increase ($p<.01$) in discharge frequency of SI neurons as compared to occasions when the monkey did not detect T2. In addition, increases in the foreperiod resulted in an increase in both the MDS and peak neuronal discharge.

These data support the hypothesis that SI cortical nociceptive neurons are involved in the encoding process by which monkeys perceive the intensity of noxious thermal stimulation.

286.8

QUANTITATIVE DIFFERENCES IN PREMOVEMENT ACTIVITY OF PRIMARY SOMATOSENSORY CORTICAL NEURONS DURING VISUAL VERSUS VIBRATORY CUED HAND MOVEMENTS. R.J. Nelson and V.D. Douglas*. Department of Anatomy & Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

We measured the timing and magnitude of premovement activity changes of SI neurons associated with movements made after vibratory and visual go-cues. Rhesus monkeys were rewarded for making reaction time flexion (against a 0.07 Nm load) or extension (with load) wrist movements in response to palmar vibration of 27, 57 or 127 Hz, or to a visual cue. Cues remained on until the animal had moved. Single SI neuronal activity and wrist position were digitized by conventional means. This study met NIH animal utilization guidelines. Non-stimulus related premovement activity changes for vibratory and visually triggered movements were compared for each neuron. Changes deviating from the mean background by ≥ 50% for 30 consecutive msec were deemed significant, and determined the response onset. Response magnitudes were calculated as the absolute value of the mean response activity minus the mean background activity.

The responses of 72 neurons have been analyzed. Fifteen showed no significant premovement responses. Of the remaining 57, 43 and 47 had earlier premovement activity for at least one vibratory frequency or no premovement response during visually-cued trials during flexion and extension movements, respectively. Activity changes occurred earlier (mean= 22.4msec, prob<.001) and response magnitudes were less (mean=-4.3 spikes per sec, prob<.03; paired t-test), for extension movements during vibratory as compared to paired visually-cued trials. Means for flexion trials were not significantly different (prob>.05). Linear regressions were fit to the magnitude data using the formula *vib-response= constant+ Coeff * visual-response*. This resulted in these relationships:

Variable	Value	S.E	T	P 2 tail	Variable	Value	S.E.	T	P 2 tail
Flex const	12.45	5.25	2.38	0.02	Flex Coeff	0.69	0.09	7.72	0.00
Ext const	5.18	3.51	1.46	0.15	Ext Coeff	0.81	0.06	13.33	0.00

These results suggest that premovement activity onset times and magnitudes differ quantitatively, depending upon the type of stimulus used to elicit movement and may suggest that different mechanisms are at work during the premovement interval under the two stimulus conditions. Supported by USAF Grant AFOSR 85-0217 to RJN.

286.9

SI CELL ACTIVITY DURING MOVEMENT: CYTOARCHITECTONIC CORRELATES. Michel Prud'homme, Dan A.D. Cohen and John F. Kalaska, Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, H3C 3J7.

We studied SI cutaneous cells in a two-dimensional reaching task requiring movements of the arm in 8 directions away from a central starting position. We demonstrated previously that the probability and strength of movement-induced activity correlated with certain receptive field properties (D.A.D. Cohen et al. (1986), Neurosci.Abstr. 12:1430). Many of these penetrations have been reconstructed. Slowly-adapting cells are more common in area 3b than area 1 and are concentrated in intermediate layers. Rapidly-adapting cells are found in all laminae. Cutaneous cell activity during arm movement is more intense in area 3b than area 1. Movement-induced activity is weakest in lamina 2 and strongest in deep laminae, especially lamina 5. Cells with certain distinct patterns of movement-related activity are selectively located in certain laminae. For instance, cells which are excited in all 8 movement directions are found in lamina 4, and cells which are inhibited in all directions are concentrated in deep laminae. Proprioceptive cells are concentrated in areas 3a and 2. These areas are much more active overall during movement than are areas 3b and 1. Supported by MRC Group Grant in neurological sciences.

286.10

MODIFICATION OF SOMATOSENSORY CORTICAL (SI) RESPONSE PROPERTIES BY GABA SYSTEMS. T. Kaneko, T.P. Hicks and C.A. Stark. Dept Med Physiol, Fac Med, Univ Calgary, Calg, Alta, Canada.

From somatosensory experiments comparing effects of GABA with baclofen (B) it seems that not only bicuculline (BMI)-sensitive processes but also those activated by B are involved in controlling response properties of SI neurones (Kaneko & Hicks, Brain Res 443: 360-366, 1988). To help understand the roles of diverse types of SI inhibition, we tested compounds related to different classes of GABA receptor on SI receptive field (RF) properties. B-induced suppressions of excitations were reversable & stereospecific, the (+)isomer being inactive. Sometimes B increased airpuffer responses on certain RF subregions while suppressing others, an effect not mimicked by M. Glutamate-induced and VPL-evoked firing was differentially sensitive to suppression by B and M (M>B & B>M, resp.) THIP acted similarly to GABA & M but with reduced potency. THIP-induced suppressions of puffer firing, but not those by B, were blocked by BMI. Phaclofen (<200 nA) invariably failed to affect B's actions yet occassionally reduced puffer or glutamate excitations. δ-amino-n-valerate did not block effects of B even while showing potent effects of it's own. The spectra of suppressant effects produced by GABA, M & THIP suggest that actions observed by ejected GABA are due almost solely to $GABA_A$ processes, those mediated by $GABA_B$ being masked. Diffusional constraints & uptake processes also intervene here. $GABA_B$ processes are extant and are likely to be active presynaptically on distal sites from the somata and additionally may be involved in presynaptic inhibitory events

286.11

QUANTITATIVE MEASUREMENTS OF RECEPTIVE FIELD EXPANSION BY BICUCULLINE ON CAT SI NEURONS. K. Alloway, P. Rosenthal*, and H. Burton. Department of Anatomy and Neurobiology, Washington University School of Medicine, St Louis, MO 63110

We recorded responses of 50 SI neurons to punctate stimuli before and during iontophoresis of bicuculline methiodide (BMI) in barbiturate anesthetized cats. Constant amplitude indentations were applied to depilatated skin along linear proximal-distal and radial-ulnar axes that intersected the most sensitive site in the receptive field (RF). Responses were largest at the center of the RF and gradually decreased at more distant points. During BMI, responses increased at all sites previously tested, including sites outside the original RF. The extent of a cell's RF was measured from the center to the boundary along four directions. RFs were usually oblong, having a mean radius of 4.0 mm in the ulnar or radial direction and 8.6 mm in the proximal or distal direction. BMI increased the length of these dimensions by an average of 132% and 160% in the ulnar/radial and proximal/distal directions. RFs located on the distal paw tended to expand more proportionally than RFs found on the wrist or arm. This suggests that GABAergic inhibition might be more important for limiting RF size in distal areas that contribute more towards making a tactile discrimination.

The spatial distribution of inhibition was determined by subtracting the mean normalized responses during control conditions from the corresponding responses during BMI and plotting the difference along the axes of the RF. This created a symmetrical, tent-like curve with a dip located at the center of the RF. These results suggest that inhibitory processes have a center-surround organization that serves to restrict the effect of excitatory inputs. NIH NS22012

286.12

VASOACTIVE INTESTINAL POLYPEPTIDE-IMMUNOREACTIVE (VIP-IR) NEURONS IN THE RAT SOMATOSENSORY CORTEX. E. Muly, S.M. Lu, and C.-S. Lin. Dept. Neurobiology, Duke University Medical Center, Durham NC 27710.

The laminar distribution of VIP-IR neurons in the granular and dysgranular regions and posteromedial barrel subfield (PMBSF) of SI and in SII were examined and compared. Further, the relation of VIP-IR neurons to the barrels of the PMBSF was studied.

Pigmented rats were perfused with PLP fixative. VIP immunocytochemistry was done on coronal and flattened tangential sections of cortex by the PAP method.

Cells, fibers and terminals in the somatosensory cortex were well stained and most neurons were of the bipolar class. The laminar distribution of VIP-IR neurons was determined in coronal sections. Labeled neurons were present in every layer, with the highest density in layer II/III. Labeled neurons appeared to be more prevalant at the borders between laminae. No significant difference was found between the laminar distribution in the granular and dysgranular regions and the PMBSF of SI and in SII. The relationship between VIP-IR neurons in layer IV and the barrels of the PMBSF was studied in tangential sections. Labeled neurons sometimes appeared to be preferentially located in the barrel walls or septa. Serial sections are being examined to assess this possibility.

Supported by NS-06233.

286.13

SOME PYRAMIDAL NEURONS IN THE SOMATIC SENSORY CORTEX OF CATS CONTAIN TACHYKININ IMMUNOREACTIVITY. F. Conti, M. Fabri, L. Abdullah*, T. Manzoni. Inst. of Physiol., Univ. of Ancona, Ancona, (Italy) and *Dept. of Anatomy, Univ. North Carolina, Chapel Hill (Usa).

Neuropeptides have never been convincingly demonstrated in pyramidal neurons. We have reinvestigated the issue of peptide-containing pyramidal neurons by using policlonal antisera raised in rabbits against substance P (SP) or substance K (SK) conjugated to keyhole limpet hemocyanin with glutaraldehyde. Adult cats were perfused with paraformaldehyde and 25μm vibratome sections were incubated with anti SP (1:1.250) or anti SK (1:500) sera. Neuronal cell body visualization required colchicine pre-treatment. In this case, SP- and SK- positive neurons were present in SI and SII. The majority of these neurons were non-pyramidal but a fraction (about 10%) of tachykinin-containing cells was composed of clear pyramidal neurons, with triangular perikarya, basal dendrites, axons emerging from the base and, most importantly, a long vertical apical dendrite. Experiments are currently in progress to ascertain whether SP and SK immunoreactivities co-exist in the same neurons and whether they are co-localized with glutamate.

286.14

NEURONS IN THE SOMATOSENSORY CORTEX OF THE AWAKE RESTRAINED RAT: SINGLE UNIT RECORDINGS AND MICROIONTOPHORESIS. M.H. Bassant*, J.M. Baleyte* and Y. Lamour, INSERM, U 161, 75014 PARIS, FRANCE.

Experiments were performed on unanaesthetized rats using a chronic restraint system. The animal's head was held in a stereotaxic apparatus by means of two metallic tubes fixed on the skull with dental cement. After a period of habituation, the rat tolerated easily this painless holding system for several hours. Electrode consisted of a recording micropipette (filled with 1M NaCl and 2% pontamine blue) attached to a multibarreled micropipette for iontophoresis. At the end of each track, pontamine blue was ejected. Electrodes penetrations were reconstructed on camera lucida drawings of frontal brain sections. The mean spontaneous activity of the cortical somatosensory neurons was 6.8 ± 8 impulses per second (n = 111, with 68% of spontaneously active cells), compared to about 2 impulses per second under urethane anesthesia. Virtually all cells were activated by glutamate and inhibited by GABA. 64% of the neurons were excited by acetylcholine and 53% by carbachol. The effects of cholinergic agonists-observed for the first time in awake rats - are significantly different from those previously obtained under urethane anaesthesia (respectively 32 and 34.5% of neurons excited). Most of the cholinoceptive neurons were located in infragranular layers.

286.15

LAMINAR DISTRIBUTION OF GLUTAMATE DECARBOXYLASE IMMUNOREACTIVITY IN THE RAT SmI BARREL CORTEX. N. D. Akhtar and P. W. Land. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Immunocytochemical methods were used to examine the distribution of glutamate decarboxylase (GAD) in the vibrissa region of the rat somatic sensory (SmI) barrel cortex. GAD positive cell bodies are present in all cortical laminae and in the white matter beneath SmI. GAD stained neurons are nearly uniformly distributed among the cortical laminae with slightly higher densities occurring within laminae II and VI. In lamina IV immunoreactive cells are clustered in the sides of the smaller, anterior barrels, but in general the distribution of stained cells bears little relationship to the location of the barrel centers.

GAD positive puncta appear on the surface of stained and unstained cell bodies as well as in the neuropil. Puncta are largest and most darkly stained in lower lamina III and lamina IV where they are organized into patches, each of which is centered upon a cortical barrel. The intervening septal regions are much less GAD immunoreactive. Lamina Vb is densely stained, laminae II/III, Vc and VI have moderate levels of GAD staining and laminae I and Va are least reactive.

The morphological details of GAD positive neurons are enhanced in sections from rats receiving intraventricular colchicine injections. Here it is seen that the stained cells are non-pyramidal in shape. Interestingly, colchicine pretreatment reveals a sublaminar organization of GAD stained puncta in SmI. While puncta staining is markedly attenuated throughout cortical laminae in treated animals, two prominent bands persist: one in lower lamina IV and one in lamina Vb. A similar pattern of puncta staining is present in untreated animals that have been deprived of vibrissa input for prolonged periods of time. GAD containing neurons in SmI thus are a heterogeneous population of cells that exhibit laminar and perhaps sublaminar differences in their functional characteristics. (Supported by NIH grant NS 23047)

287.1

DEMONSTRATION OF REGIONALLY SPECIFIC N-ACETYL-ASPARTYLGLUTAMATE IMMUNOREACTIVITY IN THE CORPUS STRIATUM OF THE RAT. Moffett, J.R. Cassidy, M. Neale, J.H. and Namboodiri, M.A.A. Biology Dept. Georgetown University, Washington, DC 20057.

The acetylated dipeptide, N-acetylaspartylglutamate (NAAG) was found by immunohistochemistry to be selectively localized in the ventral striatum of the rat. Exhaustive carbodiimide coupling prior to aldehyde post-fixation greatly enhanced NAAG-like immunoreactivity (NAAG-IR) as compared with mixed carbodiimide/formaldehyde fixations. The cellular NAAG-IR in the corpus striatum was almost exclusively localized in the pallidum, confluent ventrally with heavily labeled neurons of the hypothalamus. Multipolar neurons surrounding fascicles of the internal capsule were labeled throughout the globus pallidus. The neuropil between pallidal neurons exhibited punctate NAAG-IR associated with neuronal processes, presumably representing NAAG containing synaptic terminals. In contrast, the caudate and putamen were almost devoid of NAAG positive neurons. These results suggest that NAAG may be functionally involved in the extrapyamidal motor system, in the striatal output projections from the pallidum to the thalamus or midbrain. (Supported by Grant DK 37021)

287.2

ANISOMYCIN CAUSES EXACERBATION OF THE IMINODIPROPIONITRILE (IDPN)-INDUCED DYSKINETIC SYNDROME IN RATS. J.L. Cadet, R. Stennet† M. Katz† V. Jackson-Lewis and S. Fahn. Columbia University, Department of Neurology, New York, NY 10032

Chronic administration of IDPN causes a complex motor syndrome consisting of lateral and vertical neck movements, circling, and hyperactivity. The syndrome develops after 7 daily injections of the drug. The syndrome is accompanied by increases in serotonin levels in the caudate and nucleus accumbens of rats (Cadet, J.L. et al. Brain Res. (in press)). The present study evaluates the role of protein synthesis in the development of these abnormalities.

Rats were treated with IDPN alone, anisomycin alone, the combination of IDPN and anisomycin, or saline. Administration of IDPN alone causes the persistent abnormalities as expected. Anisomycin alone caused no behavioral changes. The combination of the two drugs caused significant exacerbations of all aspects of the syndrome.

The present data and previous ones on striatal protein phosphorylation in similarly treated animals (Cadet, J.L. et al. Soc. Neurosci. Abst 13:462, 1987) raise the possibilitiy that both translational and post-translational processes might be involved in the development of the IDPN-induced persistent spasmodic dyskinetic syndrome. Biochemical data on the serotonin system will be presented.

287.3

BEHAVIORAL EFFECTS OF MUSCIMOL-INDUCED PREVENTION OF TRANSSYNAPTIC DEGENERATION FOLLOWING UNILATERAL IBOTENIC ACID LESIONS OF THE CAUDATE NUCLEUS M.D. Lindner, T. Schallert. Dept. of Psychology and Institute for Neurological Sciences University of Texas, Austin, Texas 78712

Saji and Reis (Science,235:66-69,1987) recently developed a procedure to prevent the transsynaptic degeneration of cells in the ipsilateral substantia nigra following unilateral ibotenic acid infusions into the caudate nucleus. They demonstrated that GABA-ergic cells in the caudate were destroyed by the neurotoxin and that chronic intraventricular infusions of muscimol prevented the subsequent degeneration of target cells in the substantia nigra. With more cells surviving, the assumption has been that treatment with a GABA-agonist facilitates restoration of function following the loss of GABA-ergic projections.

However, no behavioral observations were obtained. Thus, it remained possible that muscimol might facilitate, disrupt, or have no effect on functional outcome after striatal lesions. GABA agonists have been found to retard recovery of function following cortical lesions (Schallert et al., 1986; Brailowsky et al., 1986; Hernandez et al., 1988), but the neurochemical circuitry was very different.

Saji & Reis's procedure was replicated and the results of a battery of behavioral tasks which are sensitive to the effects of this lesion, including motor, postural, and somatosensory tasks, will be reported. Supported by NS-23964.

287.4

THE FUNCTION OF DOPAMINE IN THE STRIATUM: A SHARPENER OF SENSORY INFORMATION? L.L.Brown, Albert Einstein College of Medicine, Bronx, NY 10461.

14C Deoxyglucose autoradiographic mapping studies were carried out in awake, restrained rats receiving a tactile stimulus, and either with or without a unilateral 6-OHDA lesion of the substantia nigra. The tactile stimulus was a stroke of a nylon bristle (0.5 g or 2.5 g pressure) across 1 cm of the forelimb above the wrist at the rate of 3-4/sec. EMG was recorded. Lesion effectiveness for significant dopamine depletion of striatum was verified with an apomorphine rotation trial and by tyrosine hydroxylase staining of histological sections. Glucose utilization (GU) was estimated using the technique of Sokoloff et al. (1977). In the cortex of non-lesion animals, the tactile stimulus caused focal increases in GU in SI, and in the striatum a region of no difference from the other side or an increase (-2 ± 2 to $+7\pm2$ %) surrounded by a significant decrease (-8 ± 1 to -13 ± 2 % for the two pressure groups). Thus there was a "spared" or increased focal area in dorsolateral striatum with a decreased surround. In the lesion rats, the focal area was larger, showed a higher GU rate than non-lesion rats, and there was no significant decreased surround region. The results suggest that the striatum plays a role in the localization of tactile stimuli, and that dopamine plays a role in increasing the signal-to-noise ratio for this information by decreasing surrounding activity.

287.5

ETHANOL ALTERS LOCOMOTOR AND TONE EVOKED UNIT ACTIVITY IN NEOSTRIATUM OF RAT. R.-S. Lee, N. Shimizu*, and D.J. Woodward. *Dept. of Cell Biol. & Anat., The Univ. of Tx. Southwestern Med. Ctr. at Dallas, TX 75235.*

The objective of the present study was to determine whether ethanol modulates (1) the increases in neostriatal neuronal activity during treadmill (TM) locomotion; and (2) the neostriatal response to a tone stimulus used as a cue to the onset of each TM on/off cycle. Adult Long-Evans hooded rats were used for chronic recording of single neostriatal neurons. Animals were trained to walk on a treadmill (30 sec on/30 sec off) and a tone (800 Hz, 0.2 sec, 60 dB) served as a cue (0.5 sec before TM-onset) for the onset of TM locomotion. Ethanol (0.6-1.2 g/kg, i.p.) suppressed the TM-induced increased firing by a maximum of 35% (no change in TM response in 3 of 15). This effect was observed within 10 min and peaked at 10 to 30 min. The spontaneous firing rate during resting state remained unchanged in 8 of 15 neurons or decreased by 40% in 4 of 15 cases. The effect of ethanol on neostriatal unit activity lasted for 0.7-1.5 hours, well beyond the acute effect of ethanol on observable locomotor behavior (such as ataxia). Ethanol also suppressed the learned tone response, which recovered earlier than TM response. At a dose of 1.2 g/kg ethanol there was a decreased resting firing rate and an increased delay (1-1.5 sec) for the peaks (in 2 of 4 cases) in unit responses which correlated with the first steps during each cycle of TM exercise. These observations suggest that ethanol at moderate doses exerts a substantial effect on neural processing in neostriatum which may degrade the linkage between sensory and motor units. *(Supported by AA-3901, DA-02338 and the Biological Humanics Foundation.)*

287.6

SOMATOMOTOR ACTIVITY OF GLOBUS PALLIDUS UNITS C.Manetto*and T.I. Lidsky. CUNY Med.Sch., N.Y., N.Y. 10031

Units were recorded in the globus pallidus (GP) of awake cats. Animals were painlessly restrained during recording sessions in a device that permitted a variety of activities including buccolingual movements. Buccolingual movements were displayed by the cats spontaneously and also were elicited by delivering a small volume of milk.

A majority (75%) of the sampled cells changed firing rate during buccolingual movements. For 57% of the responsive cells, activity changes were related to the sensory concomitants of ingestion rather than the movements per se. For example, the majority had perioral somatosensory receptive fields that were stimulated by the delivery of milk. It should be noted that, in this respect, the activity of GP cells was quite similar to that of striatal neurons described in earlier work by this laboratory.

The remaining responsive neurons (43%) became active in relation to movement. Typically, such cells fired during all buccolingual movements regardless of the initiating conditions. However, a subgroup (27%) responded only during movements that were triggered by milk delivery. Although movement related responses similar to those seen in the GP have also been observed in the striatum, there were two noteworthy differences. 1) A higher proportion of GP cells showed movement related activity. 2) The responses of GP cells were time-locked to individual phasic contractions of jaw muscles. Similar specificity was never observed in striatal neurons. (NINCDS:NS 21418)

287.7

THE HUMAN SUBCOMMISSURAL BASAL FOREBRAIN. N. Sakamoto*, J. Pearson and B. Reisberg. New York University Medical Center, New York, NY 10016

The human subcommissural pallidum (often called the ventral pallidum) extends ventrally in finger-like processes interdigitating with "pseudostriatal" cell groups structurally and chemically similar to the nucleus accumbens. Islands of Calleja and small islands of putaminal gray matter are scattered in the "pallido-pseudostriatal interdigitation complex" (PPSIC). Large neurons of the nucleus basalis interpolate with the lateral side of the PPSIC. The PPSIC abuts onto the olfactory bulb and nucleus of the diagonal band. Enkephalin-immunoreactive (EIR) woolly fibers are densely packed in the external and subcommissural components of the pallidum but are scarce in the interdigitating process which have a dense population of SPIR woolly fibers. Thus the subcommissural pallidum is chemo-architectonically "duplex", consisting of a enkephalinergic dorsal component resembling the external segment of the globus pallidus and a substance Pergic ventral component resembling the internal segment. The duplex structure is seen in monkey subcommissural pallidum but ventral interdigitations are not present.

287.8

LESIONS OF THE PRIMATE SUBTHALAMIC NUCLEUS (STN) REDUCE TONIC AND PHASIC NEURAL ACTIVITY IN GLOBUS PALLIDUS.

I. Hamada* and M.R. DeLong (SPON: R.T. Richardson). Depts. of Neurology and Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

In the primate, lesions of the STN result in severe dyskinesias of the contralateral limbs. These have previously been postulated to result from removal of inhibitory STN input to the internal segment of globus pallidus (GPi), which gives rise to the major output from the basal ganglia. Recent evidence, however, suggests that STN input to GPi is excitatory (Kita, H. and Kitai, S.T. J. Comp. Neurol. 260:435-452, 1987). To examine these issues, we recorded neural activity in both GPi and GPe (external segment of pallidum) of a rhesus monkey before and after lesions of the STN. The monkey held a manipulandum in a small zone and received torque perturbations of the elbow. After collecting control data, small amounts of ibotenic acid or kainic acid were injected into several STN locations, producing acute (5-10 minutes latency) contralateral dyskinesias of the arm and leg. Recordings were begun after recovery from acute dyskinesia (5 days). Tonic discharge rates were significantly lower than control and phasic responses to torque perturbation, particularly increases in discharge, were significantly reduced in number both in GPi and GPe. These findings provide support for the view that the STN provides a major excitatory input to both GPe and GPi and suggests that decreased GPi output may play a role in the pathogenesis of dyskinesias.

287.9

SINGLE UNIT ACTIVITY OF PRIMATE CAUDATE NUCLEUS IN A PRECUE TASK. D. Jaeger, S. Gilman and J.W. Aldridge. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48104

We have examined caudate nucleus unit activity responses to the presentation of visual stimuli that precue the target position of learned reaching movements. The behavioral task used requires a monkey to touch one of a set of 8 target knobs that have stimulus lights mounted on them. A subset of these lights was flashed for 2 s before a go-cue triggered the reaching movement. The locations of these lights precued partial to complete information about the required target position of the following movement. For example, partial information could indicate movement direction or movement distance. One to 4 single units were recorded simultaneously with a multiwire electrode from caudate nucleus during task execution. Of 72 recorded caudate units, 68 (94%) had responses to the task. Responses related to precue presentation were found in 43 units (60%). Precue responses were excitatory and lasted 200 to 800 ms. These responses could occur during precue presentation or following precue offset, lasting through the hold phase that preceded the go-cue. Each unit with a precue dependent response was activated only for a subset of all precue types presented. Some responses during the reaching movement occurred only when a certain precue preceded the movement. Units located within 1 mm from each other often showed similar response types during task execution. A cross-correlation analysis showed that there was no close coupling in the activity of such units. Units with precue specific responses often showed additional responses during other phases of the task. Precue responses were not predictive of such other response types. This independence of responses in different task phases suggests that units are dynamically associated with different neural assemblies. The functional properties of unit responses in caudate nucleus indicate involvement of this structure in the recognition of task requirements and preparation for further task execution. NIH grants NS19613, NS07222

287.10

A COMPARISON OF AUDITORY AND VISUAL GO-CUES ON SINGLE UNIT ACTIVITY IN THALAMUS R.N.S. Sachdev *, C. Hekmatpanah *, D. Jaeger, S. Gilman, and J.W. Aldridge. Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104

The aim of this investigation is to compare the properties of single units that respond during a movement in cat thalamus. Unit responses associated with movements initiated by visual and / or auditory go-cues were compared to determine whether responses depend on stimulus type. Unrestrained cats were trained to make discrete paw movements with the right limb. The task began after an initial hold period in the rest position. Following this hold period a go-cue - flashing light, sound, or both - was presented to trigger the movement. The randomly presented go cues remained on throughout the movement, and were turned off 250-500 ms after the movement was completed. Thalamic unit activity was assessed by constructing peri-event time histograms and cumulative sum diagrams for each discriminated unit collected during the task. Sixty-two units from the thalamus were analyzed. Sixty-one percent of the units had responses starting 80-550 ms before the movement. These responses typically lasted for the duration of the movement. Eight percent of the units altered their discharge only during the movement and 31% had no response. The responses of 55% of the movement related units did not depend on the type of cue. The remaining 45% exhibited responses dependent on the cue. The cue-dependent responses were distinguished on the basis of excitation, inhibition and latency. For example, 12% of the movement related units had a shorter latency with the sound cue than with the visual cue. Also, other units were excited during movements with one cue and inhibited with the other cue. These results suggest that the sensory features of a stimulus may have a role in determining the movement-related response characteristics of the thalamic neurons. NIH grants N519613, NS07222 and Hereditary Disease Foundation.

287.11

ORGANIZATION OF EFFERENT CONNECTIONS OF THE TWO PALLIDAL SEGMENTS IN PRIMATE AS REVEALED BY PHA-L ANTEROGRADE TRACING METHOD. Y. Smith, A. Parent and J. Dumas*. Lab. of Neurobiology, Fac. of Med., Laval Univ., Québec, Canada.

The lectin Phaseolus vulgaris-leucoagglutinin (PHA-L) was used as an anterograde tracer to study the pattern of termination of pallidofugal fibers arising from the external (GPe) and the internal (GPi) pallidal segments in squirrel monkey (*Saimiri sciureus*). Small iontophoretic PHA-L injection in the dorsal half of GPe led to profuse anterograde labeling in the central core of the subthalamic nucleus. In addition a small to moderate number of fibers detached from the pallidosubthalamic axons to descend toward the substantia nigra (SN) where they formed small, diffuse plexuses in the lateral two-thirds of the pars reticulata (SNr). Other anterogradely-labeled fibers were visualized in smaller number in the dorsal part of the GPi, the putamen (PUT) and the caudate nucleus. At thalamic level, a very small number of fibers were seen in caudal intralaminar nuclei. Finally, at brainstem level few labeled fibers were scattered in the mesencephalic reticular formation (MRF) and periaqueductal gray (PAG). Following PHA-L injection in the dorsal two-thirds of GPi, a rich contingent of anterogradely-labeled fibers traversed the internal capsule and coursed through the lenticular fasciculus to invade massively the ventral anterior/ventral lateral (VA/VL) thalamic nuclei where they formed several small plexuses that were mainly confined to the VA and the oral part of VL. In addition a significant number of fibers extended more caudally to arborize profusely in the centre médian (CM) and centromedial (CeM) intralaminar nuclei, the dorsal part of zona incerta and the ventral two-thirds of the lateral habenular nucleus. It is worth noting that numerous pallidofugal fibers crossed the midline at the CeM level. At striatal level a small number of labeled fibers oriented dorsoventrally occurred in the dorsomedial part of PUT. Other fibers arising from the injection site invaded the dorsomedial third of GPe where they arborized in all directions. Finally, few labeled fibers were scattered in the SNr, MRF and the area of the pedunculopontine nucleus. (Supported by MRC, FRSQ and FCAR).

287.12

DISTRIBUTION OF SEROTONIN IMMUNOREACTIVITY IN PRIMATE BASAL GANGLIA. B. Lavoie*, Y. Smith and A. Parent (SPON: M. Filion). Lab. of Neurobiology, Fac. of Med., Laval Univ., Québec, Canada.

A 5-hydroxytryptamine (5-HT) antibody was used to study the serotoninergic innervation of the basal ganglia in the squirrel monkey (*Saimiri sciureus*). At midbrain level, numerous 5-HT-immunoreactive axons of the dorsal raphe neurons coursed laterally and rostrally toward the substantia nigra (SN) where they arborized into a multitude of heterogeneously distributed 5-HT terminals. The latter were particularly abundant in the pars reticulata and pars lateralis, but much less so in the pars compacta of SN. Other 5-HT fibers arched dorsally and reached the subthalamic nucleus where they formed a dense network of varicosed fibers. Numerous fibers also traversed the internal capsule and arborized profusely in the globus pallidus (GP). The external pallidum was much less densely innervated than the internal pallidum which contained numerous 5-HT varicosed fibers and terminals arranged in a typical band-like pattern. At striatal levels, the 5-HT terminals were particularly abundant in the ventral striatum comprising nucleus accumbens, in the ventrolateral region of the putamen (PU) and the ventromedial aspect of the caudate nucleus (CD). In both CD and PU the number of 5-HT terminals decreased progressively along the rostrocaudal axis and several large and elongated zones rather devoid of 5-HT immunoreactivity were visualized, particularly in CD and dorsal PU. These zones of poor 5-HT immunoreactivity were in register with similar areas devoid of tyrosine hydroxylase immunoreactivity, but the correspondence with other neurochemical markers remains to be established. (Supported by MRC, FRSQ and FCAR).

287.13

THE EXACT DISTRIBUTION OF STRIATALLY-PROJECTING THALAMIC NEURONS IN THE MONKEY. C.A. Hunt and E.G. Jones, Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Wheatgerm agglutinin-horseradish peroxidase (WGA-HRP, Sigma, 2%) was injected into the caudate nucleus and putamen of adult monkeys, using approaches that avoided contamination of the cerebral cortex. After large injections, retrogradely labeled cells were present throughout the intralaminar complex: Large numbers in the rhomboid, dorsal central lateral, centre médian, and parafascicular nuclei, with fewer in the central medial and paracentral nuclei and in the remainder of the central lateral nucleus. Small injections into the putamen resulted in a small number of labeled neurons in the centre médian nucleus. Labeling of cells in other nuclei could usually be attributed to HRP spread to non-striatal structures. However, a consistent finding was the labeling of a few neurons in the ventral anterior nucleus (VA), including its magnocellular part, even when spread of HRP to the cortex was minimal. The intralaminar nuclei are thus the major sources of the thalamostriate projection in the monkey, with a likely contribution from VA.

Supported by NIH Grant NS22317 and NIMH Grant MH09424.

287.14

CHANGES IN PUTAMEN ACTIVITY IN BEHAVING PARKINSONIAN MONKEYS. E.B. Montgomery, Jr. and S.R. Buchholz*. Dept. of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110

At the last meeting we reported single unit recordings in the putamen of a normal monkey trained to perform wrist extension and flexion movements. The major finding was the high incidence of neurons (6 of 14 related to the tasks) best related to and active in advance of the achievement of final target. In the same monkey made parkinsonian by intravenous injection of 1.2 mg/kg of MPTP, only 1 out of 8 units in the putamen showed this pattern. These neurons may represent the major output function of the putamen since they were active before reaching final target and were lost following MPTP induced disruption of putamenal physiology.

Following MPTP most putamenal neurons were related to and active after the "go" signal or at or after movement onset. These patterns, also seen in normal monkeys, may represent input responses from areas not affected by MPTP.

287.15

CHOLECYSTOKININ BINDING IN MACAQUE NEOSTRIATUM. M.F. Kritzer, R.B. Innis, and P.S. Goldman-Rakic, Yale Univ. Sch. Med., New Haven, CT. 06510.

Cholecystokinin (CCK) binding sites were labeled with [3H]- and [125I]-CCK-8 in macaque neostriatum by in vitro receptor autoradiography. Non-specific binding was assessed using 1 uM CCK-8; analysis included comparison of Nissl and AChE stained sections with autoradiograms.

CCK binding in macaque striatum was highly selective. Patches of reduced binding were evident in much of the striatum; comparison of binding and AChE staining showed poor correlation with striosomal organization. A striking feature of binding, however, was the sharp demarcation of heavily labeled areas located ventromedially throughout the striatum and weakly labeled areas located dorsolaterally. Thus, heavy label in the ventromedial striatum contrasted with sparse label in the dorsolateral one-third of the body of the caudate and nearly all of the putamen lateral to the pallidum. The dense binding in the ventromedial striatum overlies regions innervated by association cortex (Selemon & Goldman-Rakic, J. Neurosci. 5: 1985) whereas dorso- lateral regions of light binding appear to correspond to areas innervated mainly by motor cortex (Kunzle, Brain Res. 88: 1975). The present results have a parallel in our previous finding that CCK binding in macaque cortex is dense in association regions of cortex and diminished in motor and premotor regions (Kritzer et al., J. Comp. Neurol. 263: 1987). Supported by MH38546, NS22807.

287.16

DOPAMINERGIC, SEROTONERGIC, CHOLINERGIC AND B-ADRENERGIC SYSTEMS IN NORMAL AND SCHIZOPHRENIC STRIATUM WITH REFERENCE TO STRIOSOME/MATRIX COMPARTMENTS J.N. Joyce, N. Lexow*, B. Neal and A. Winokur, Departments of Psychiatry and Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA

Quantitative autoradiography was used to examine the pre- and postsynaptic elements of several transmitter systems in the striatum of post-mortem brains derived from non-disease controls and of long-term hospitalized schizophrenics. As previously described, the matrix could be visualized with AChE histochemistry, [^{3}H]hemicholinium-3 labeling of choline uptake sites on cholinergic terminals or [^{3}H]mazindol labeling of DA uptake sites on DA terminals. In all cases the matrix was enriched as compared to less dense labeling of the striosomes. Serotonin uptake sites labeled with [^{3}H]CNMI were densest in the ventral striatum and tended to be enriched in the striosomes. DA D2 receptors were denser in the matrix, whereas D1 receptor patches could be seen to be congruent with or avoid the D2-rich zones. The density of M1 receptors was greater dorsally in the striatum, with distinct patches of higher density apparent in all regions of striatum. M2 receptors were more homogeneously distributed and less numerous. None of the serotonin receptors examined (5-HT$_{1a}$, 5-HT$_{1c}$, 5-HT$_2$) bore any apparent relationsip to the patterning of serotonin terminals, as visualized with [^{3}H]CNMI. In contrast, B$_1$ (but not B$_2$ receptors) receptors were densest where serotonin terminal labeling was highest. In the striatum of the schizophrenic cases, D2 receptor density was increased 1.8-fold without any change in D1 receptor density or DA terminal labeling. **Funded by NIH NS19597 to AW and a grant from the Scottish Rite Foundation to JNJ**

287.17

ORGANIZATION OF PRIMATE BASAL GANGLIA "MOTOR CIRCUIT": 1. MOTOR CORTEX (MC) AND SUPPLEMENTARY MOTOR AREA (SMA) PROJECT TO COMPLEMENTARY REGIONS WITHIN MATRIX COMPARTMENT OF PUTAMEN G.E. Alexander, V.E. Koliatsos, L.J. Martin*, J. Hedreen, J. Hamada* and M.R. DeLong, Depts. of Neurology, Pathology and Neuroscience, The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

We have proposed recently that in primates there are several parallel circuits that link distinct, structurally and functionally segregated, portions of cortex, basal ganglia and thalamus (Alexander, et al., Ann. Rev. Neurosci. 9:357-381, 1986). To investigate the organization of the basal ganglia-thalamocortical "motor circuit", we have carried out a series of combined anatomical and physiological studies, the results of which are outlined in the present series of abstracts. In this first study, the organization of cortical inputs to the electrophysiologically-defined arm area of the putamen was examined in the rhesus monkey. Tritiated aminoacids and WGA-HRP were injected into the respective arm areas of MC and SMA, both of which were identified by microstimulation. SMA terminals were distributed over a large part of the putamen arm area. MC terminals were distributed ventrolateral to the SMA terminal field. Both sets of terminals were confined to the matrix zone, as defined by AChE histochemistry. The densely labeled central portions of the two terminal fields were clearly separated, whereas the peripheral zones of sparse labeling showed some overlap. These results suggest that within the putamen arm area segregation of inputs from anatomically distinct precentral motor fields is largely maintained.

287.18

ORGANIZATION OF PRIMATE BASAL GANGLIA "MOTOR CIRCUIT": 2. PUTAMINAL PROJECTIONS TO INTERNAL (GPi) AND EXTERNAL (GPe) GLOBUS PALLIDUS ORIGINATE IN DISTINCT NEURONAL POPULATIONS WITHIN THE MATRIX COMPARTMENT. V.E. Koliatsos, L.J. J. Martin*, J. Hedreen, G.E. Alexander, J. Hamada*, D.L. Price, and M.R. DeLong, Depts. of Neurology, Pathology and Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Double retrograde tracing utilizing the fluorescent dyes Fast Blue (FB) and Diamidino Yellow (DY) was employed to show the anatomical relations of striatal neuronal populations projecting to GPi and GPe in the rhesus monkey. GPi and GPe were mapped electrophysiologically and their arm areas identified; each pallidal segment was injected using a compound recording-injecting system. AChE histochemistry was used to visualize patch-matrix striatal compartments. Retrogradely labeled populations of putamen neurons were confined primarily to the matrix zone. Retrogradely labeled neurons were clustered in groups, which were comprised almost exclusively of neurons projecting to either GPi or GPe. Target-specific groups were peripherally intermixed and a few double-labeled neurons (not exceeding 10% of the total of overlapping cells) were noted in the areas of intermixing. These results further support the concept of segregation of striatal efferent systems. We are currently analyzing the precise relations between GPe- and GPi-projecting neurons and cortical afferents to the putamen.

287.19

ORGANIZATION OF PRIMATE BASAL GANGLIA "MOTOR CIRCUIT": 3. RELATIONS OF STRIATAL MICROEXCITABLE ZONES TO AFFERENT AND EFFERENT PROJECTIONS. M.R. DeLong, I. Hamada*, G.E. Alexander, V. Koliatsos, L.J. Martin*, and J. Hedreen. Depts. of Neurology, Pathology and Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

In previous electrophysiological studies we have shown that the primate putamen is functionally heterogeneous (Alexander & DeLong, J. Neurophysiol. 53:1401-1416, 1417-1429, 1985, Crutcher & DeLong, Exp. Br. Res. 53:244-258, 1984). Microstimulation along individual electrode tracks through the putamen has revealed discrete regions from which uniform motor responses are evoked. These regions have been termed striatal microexcitable zones (SMZ). In order to characterize the 3-dimensional structure of SMZs and their relations to the anatomical organization of the striatum, the microexcitable putamen was stimulated at 100 um intervals with closely spaced (1mm) microelectrode penetrations. We found that SMZs are irregularly shaped 3-dimensional structures whose maximal dimensions may reach several mm along the rostro-caudal axis. When these findings were examined in relation to the results of anatomical studies carried out in the same animals (see preceding abstracts), we found that: 1) SMZs were located in the central portion of the putamen, which appeared to consist only of matrix, as defined by acetylcholinesterase staining, 2) arm SMZs appeared to overlap with regions receiving dense inputs from the arm area of the motor cortex but not from the supplementary motor area, and 3) SMZs overlapped with clusters of neurons projecting to GPe and GPi.

287.20

ORGANIZATION OF PRIMATE BASAL GANGLIA "MOTOR CIRCUIT": 4. VENTROLATERAL THALAMUS LINKS INTERNAL PALLIDUM (GPi) AND SUPPLEMENTARY MOTOR AREA (SMA). J.C. Hedreen, L.J. Martin*, V.E. Koliatsos, I. Hamada*, G.E. Alexander and M.R. DeLong. Depts. of Neurology and Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The concept of a closed basal ganglia-thalamocortical "motor circuit" linking motor areas of cortex, basal ganglia and thalamus has been proposed on the basis of diverse physiological and anatomical studies in different animals (see Alexander, et al., Ann. Rev. Neurosci. 9:357-381, 1986). In order to demonstrate the continuity of this circuit in individual animals, injections of two different anterograde/retrograde tracers were made into two non-contiguous stations of this circuit. Wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was injected into the arm region of the SMA and Fast Blue (FB) into the GPi. Retrograde and anterograde transport of WGA-HRP to thalamus from SMA was largely confined to pars oralis of the ventrolateral thalamic nucleus (VLo). FB-labeled terminals from GPi were scattered within VLo, and often overlapped the distribution of VLo cells projecting to SMA. As described in companion abstracts (Alexander, et al. and Koliatsos, et al.), the arm area of putamen was anterogradely labeled from SMA, and these labeled terminals overlapped with cells projecting to GPi. These studies provide further evidence for the maintenance of structural and functional segregation between the "motor" and other basal ganglia-thalamocortical circuits.

287.21

ORGANIZATION OF PRIMATE BASAL GANGLIA "MOTOR CIRCUIT": 5. CORTICAL AND PALLIDAL CONNECTIONS ARE SEGREGATED WITHIN THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS (PPN). F.H. Baker, L.J. Martin*, V.E. Koliatsos, J. Hedreen, D.L. Price, I. Hamada*, G.E. Alexander, and M.R. DeLong. Depts. of Neurology and Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Despite reports that the PPN projects to globus pallidus (GP) and receives input from internal GP (GPi) and the supplementary motor area (SMA) in primates, it is not known whether GP and cortical circuitries converge or remain segregated within PPN. It is also not known whether PPN projections to GP arise from cholinergic or non-cholinergic subpopulations of PPN neurons. We, therefore, injected the retrograde marker Fast Blue (FB) into GPi and the anterograde tracer wheat germ agglutinin-horseradish peroxidase (WGA-HRP) into SMA. Retrogradely labeled FB neurons were found throughout PPN (pars compacta and dissipata), with a predominant dorsomedial distribution. Combined retrograde tracing and ChAT immunofluorescence revealed that <2% of these neurons were cholinergic and that pallidopetal neurons were only minimally admixed with cholinergic PPN neurons. Pallidal neurons were predominately found dorsal and ventral to cholinergic neurons. GPi terminals were distributed medially in the same general area as GPi-projecting neurons. SMA terminals were primarily distributed in lateral PPN and showed minimal overlap with GPi-projecting neurons and GPi terminals. The results indicate that cortical and pallidal circuitries in PPN are largely segregated, with a lateral-medial topography, and that pallidopetal PPN neurons are noncholinergic.

LEARNING AND MEMORY: PHARMACOLOGY III

288.1

DIRECT COMPARISON OF THA AND 3,4 DAP, METHANESULFONYL FLUORIDE, PHYSOSTIGMINE AND CLONIDINE: DIFFERENTIAL EFFECTS ON IMPROVING MEMORY IN AGED MONEYS. Bartus, R.T. and Dean, R.L. Lederle Laboratories, Pearl River, NY 10965

The effects of several different drugs recently suggested to have therapeutic value for treating the cognitive symptoms of aging and Alzheimer's were evaluated for their ability to reduce memory impairments in aged, test-sophisticated Cebus monkeys (20 to 30 years old). Included were tetrahydroaminoacridine (THA), 3,4 diamino pyridine, methanesulfonyl fluoride, physostigmine and clonidine. Several doses of each drug were tested (P.O.) in each of ten different monkeys, allowing for direct and extensive comparison of each drug's efficacy in this model.

The results of this comparative test revealed several potentially interesting findings: (1) all drugs, except clonidine, produced improvement in a portion of the monkeys tested; (2) as in many past tests with aged monkeys and humans, wide variations in most effective dose, per subject, were observed; (3) different monkeys responded more effectively to one drug than another; and (4) under these tightly controlled conditions, physostigmine and methanesulfonyl fluoride produced the most reliable and robust effects ($p<.005$), in more monkeys, than did either THA ($p<.05$) or 3,4 DAP ($p<.10$). In contrast, neither acute, nor subchronic doses of clonidine improved performance of any monkey, while some exhibited clear impairments at higher doses.

288.2

DIFFERENTIAL EFFECTS OF CHOLINERGIC, DOPAMINERGIC, ADRENERGIC AND HISTAMINERGIC ANTAGONISTS INJECTED INTO THE HIPPOCAMPUS ON THE PERFORMANCE OF A T-MAZE WORKING-MEMORY TASK IN THE RAT. G.N.O.Brito, L.S.O.Brito, S.P.Silva and F.A.R.Prado. Lab.Neuropsicol.Exp., Setor de Neurociencias, Univ.Fed.Fluminense, Niteroi, RJ 24210, Brasil.

Previous research in our laboratory demonstrated that injections of scopolamine (a muscarinic antagonist) into the dorsal hippocampus impaired the performance of a working-memory task (discrete alternation), but did not alter the performance of a reference-memory task (visual discrimination) in a T-maze (Brito, G. et al., Psychopharm., 81: 315, 1983). In the present study, three groups of rats were tested on discrete alternation after bilateral intrahippocampal injections (1.0 ul), repectively, of scopolamine (39 mM) and sulpiride (19 mM, D2 blocker); propranolol (34 mM, beta blocker), prazosin (34 mM, alpha 1 antagonist) and yohimbine (34 mM, alpha 2 blocker); and antazoline (3 mM, histamine H1 antagonist), cimetidine (6 mM, H2 blocker) and betahistine (1 mM, H3 antagonist). The results demonstrated that only the injections of scopolamine disrupted the performance of the discrete alternation task. We conclude that the effects of intrahippocampal injections of scopolamine on working memory in rats is related to the specific blockade of cholinergic mechanisms, and not to the induction of imbalance among different neurotransmitter systems in the hippocampus.

Research supported by grants from CNPq and FINEP.

288.3

PHARMACOLOGICAL DISSOCIATION OF THE HEART RATE AND SOMATOMOTOR COMPONENTS OF THE ORIENTING RESPONSE IN PREWEANLING RATS. Jane A. Saiers*, Rick Richardson* and Byron A. Campbell. Dept. Psychology, Princeton Univ., Princeton, NJ 08544

In response to a discreet auditory stimulus, rats exhibit a quick, lateral head jerk and a 40-50 bpm deceleration in heart rate. Both the head jerk and bradycardia are components of the orienting response (OR), which is presumed to reflect stimulus-directed attention. This research examined the effects of cholinergic and dopaminergic blockade on the OR to an 80 dB tone. In Experiment 1, scopolamine hydrobromide was found to inhibit the heart rate OR in a dose-dependent manner ranging from little effect at .125 mg/kg to complete inhibition of the cardiac OR at .5 mg/kg. No attenuation of the heart rate OR was observed in the saline control animals. The head jerk response, unlike the cardiac response, was undiminished by scopolamine. In the second experiment, haloperidol, administered at dosages ranging from 0 to 10 mg/kg attenuated the behavioral response (head jerk) in a dose dependent fashion but had no effect on the cardiac component of the OR. These results demonstrate that the autonomic and behavioral components of the orienting response are independent representations of a common central process. These results specifically contradict the view that the heart rate OR is simply a matter of cardiac-somatic coupling, in which cardiac deceleration is attributed to a change in somatic activity.

288.4

MEMORY FACILITATION PRODUCED BY DOPAMINERGIC AND CHOLINERGIC AGENTS: EFFECTS ON WIN-SHIFT AND WIN-STAY RADIAL MAZE TASKS. M.G. Packard, W. Regenold, R. Quirion, N.M. White. Department of Psychology and Douglas Hospital Research Centre, McGill University, Montreal, Quebec, Canada.

The role of DA and ACh receptor subtypes in the acquisition of two memory tasks was examined. The receptors were manipulated with S.C. injections of an ACh agonist (M2 receptor antagonist) AFDX-116 (1mg/kg), d-amphetamine (2mg/kg), a DA-D1 receptor agonist SKF38393 (2mg/kg), a DA-D2 receptor agonist LY171555 (2mg/kg), or vehicle controls. On a win-stay task (sensitive to caudate lesions) a light cue signalled the location of food in 4 randomly selected arms on each trial. Rats were given one trial per day and injected following trial 5. D-amphetamine, AFDX-116 and LY171555 (but not SKF38393) significantly improved performance compared to controls. On the win-shift task (sensitive to hippocampus/fornix lesions) food was available in all arms. Rats were trained to perform accurately with a 15 min delay between choices 4 and 5. They were then injected after choice 4 and tested 18 hours later. LY171555, but not AFDX-116 improved performance on this task. This is one of only a few reported cases of a direct behavioral effect of "pure" D2 receptor activation. These results suggest that post-training D2 receptor activation may influence both win-stay and win-shift memory. In contrast, post-training cholinergic activation influenced only win-stay memory; although a cholinergic substrate may be involved in win-shift memory it was not affected by immediate post-training activation.

288.5

EFFECTS OF NORADRENERGIC DEPLETION AND CHOLINERGIC ANTAGONISM ON COMPLEX MAZE PERFORMANCE IN YOUNG F-344 RATS. M. Chachich, E. Spangler*, K. Smith*, G. Wenk*, and D. Ingram*. Gerontology Research Ctr, Baltimore, MD 21224 and Dept. Psychology, Johns Hopkins U., Baltimore, MD 21218.

The muscarinic cholinergic (ACh) antagonist, scopolamine hydrochloride (SC), and the central norepinephrine (NE) neurotoxin, N-(2-chloroethyl)-N-ethyl-2-bromo benzylamine hydrochloride (DSP4), were given in combination to evaluate their effects on performance in a complex 14-unit T-maze. Male F-344 rats 3-mo old were given i.p. injections of either 0.9% saline (SAL) or DSP4 50 mg/kg bodyweight 2 wk prior to maze training. Rats were pretrained to criterion in 1-way active avoidance (0.8 mA) in a straight runway for 3 days with 10 trials/ day. On day 4 rats received 15 trials in a 14-unit T-maze. The rat had 10-sec to locomote past each of 5 gates to avoid shock (0.8 mA) enroute to the goal box. Each rat received either SAL or SC 0.25 mg/kg or 0.5 mg/kg (i.p.) 30-min prior to maze training. Drug groups were: SAL/DSP4, SAL/SC=0.25, SAL/SC=0.50, DSP4/SAL, DSP4/SC=0.25, DSP4/SC=0.50. Following maze training all rats were decapitated and the post-decapitation reflex (PDR) latency (sec), a behavioral assay for NE depletion, was measured. Dependent measures of maze performance included errors, alternation errors, runtime, shock frequency and duration. Analysis of variance was conducted for each dependent measure of maze performance. While a significant main effect of SC was observed on all measures of maze performance (p's<0.05) except shock duration, no main effect of DSP4 was observed in any of these measures (p's > 0.05). No interaction of SC and DSP4 was observed except in the alternation error measure where the DSP/SAL group exhibited superior performance during initial trials. PDR latencies for the DSP4-treated rats were significantly higher than for the SAL rats, $t(59)=8.61, p<0.01$, indicating that NE levels were depleted in DSP4-treated rats. These results further implicate ACh systems in learning this task and in age-related impairments previously observed, but suggest that central NE systems may not be critically involved.

288.6

STRIA TERMINALIS LESIONS ATTENUATE THE EFFECTS OF POSTTRAINING OXOTREMORINE AND ATROPINE ON MEMORY. I.B. Introini-Collison, Y. Arai*, and J.L. McGaugh (SPON: Gary D. Novack). Center for the Neurobiology of Learning and Memory and Department of Psychobiology. University of California, Irvine. CA 92717.

Previous evidence indicates that stria terminalis lesions prevent the memory-modulating effects of posttraining amygdala stimulation, as well as the facilitatory effects of epinephrine or naloxone and the impairing effects of beta-endorphin. The present experiments examined the effects of ST lesions on retention in rats given the muscarinic agonist oxotremorine (50 ug/kg; ip) or the muscarinic antagonist atropine (3.0 mg/kg; ip) immediately posttraining. Sprague Dawley rats (250-300 g) were submitted to sham operation or to bilateral lesions of the stria terminalis. One week later, they were trained in an inhibitory avoidance task and immediately after training they received saline, oxotremorine or atropine. Two weeks later they were trained on a Y-maze discrimination response and received saline or oxotremorine immediately postraining. Retention of each task was tested one week after training. Stria terminalis lesions did not affect retention of either response, but significantly attenuated both the enhancing effects of oxotremorine and the impairing action of atropine on memory. These findings support the view that the amygdala is involved in the memory-modulating effects of the cholinergic system.

Supported by NIMH grant MH12526 and ONR grant N00014-87-K0518.

288.7

EFFECTS OF CONCURRENT MANIPULATIONS OF CHOLINERGIC AND NORADRENERGIC FUNCTION ON INHIBITORY (PASSIVE) AVOIDANCE AND PLACE LEARNING. M.W. Decker and J.L. McGaugh. Dept. of Psychobiol. and Ctr. for Neurobiol. of Learn. and Mem., U. C., Irvine, CA 92717.

Cholinergic/noradrenergic interactions in learning and memory and in developmental plasticity have been identified, but whether such interactions generally characterize neural plasticity is not yet clear. To test the generality of this phenomenon, we evaluated the memory-impairing effects of scopolamine, a muscarinic antagonist, in normal and NE-depleted mice.

Administration of scopolamine i.p. 20 min before training impaired 24 h retention of inhibitory avoidance training (at doses of 0.1, 0.3 and 1.0 mg/kg) and the acquisition of place-training in a water maze (at a dose of 1.0 mg/kg). NE-depletion (to about 44% of control levels in hippocampus), which was produced by 50mg/kg DSP-4 i.p., did not affect performance on either task and did not alter the effects of scopolamine. NE-depletion did, however, impair the retention of place-learning when mice were retested on this task 16 d after initial learning. Since NE-depletion did not affect acquisition, this retention deficit is likely due to more rapid forgetting by NE-depleted mice. Interestingly, normal acquisition but rapid forgetting has also been observed in aged rodents displaying deterioration of the noradrenergic system. Thus, our results support the notion that noradrenergic dysfunction plays a role in age-related memory decline. Our failure to observe an enhancement of the disruptive effects of scopolamine by NE-depletion similar to that previously reported with rats in the radial arm maze, is likely due to species and task differences. Further, our NE-depletions were less extensive than in the previous report. Still, our findings suggest that cholinergic/noradrenergic interactions in learning and memory may be limited to certain forms of memory.

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288.8

EFFECTS OF NORADRENERGIC DEPLETION ON NALOXONE-INDUCED MEMORY FACILITATION. A.H. Nagahara*, I.B. Introini-Collison, K.C. Liang, M. Kim* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology. University of California, Irvine, CA 92717.

Recent findings have indicated that beta-noradrenergic mechanisms in the amygdala participate in naloxone-induced memory facilitation. To further analyze this possibility Sprague Dawley rats (250-300 g) were bilaterally implanted with amygdala cannulae. One week later they were injected in the amygdala with water or the noradrenergic neurotoxin DSP 4 (30 ug/2.5 ul). They were then trained on an inhibitory avoidance task (IAT) and two weeks later on a Y-maze discrimination response (YMD). Immediately following training they were given intra-amygdala injections of buffer or naloxone (0.1 - 10.0 ug/1.0 ul). Pretreatment with DSP 4 significantly attenuated naloxone-induced memory facilitation (IAT: 0.1 ug; YMD: 0.3 ug). Further, in those rats pretreated with DSP 4, naloxone only facilitated retention when injected at the highest dose (IA: 10 ug). The blocking effects of DSP 4 on naloxone-induced memory facilitation appear to be related to the depletion of NE, since such a blockade was prevented by pretreatment with desipramine (DMI)(25 mg/kg; 4 and 1 hr before DSP 4). As expected, DSP 4 depleted NE in the amygdala (-24 %), and this depletion was prevented by DMI. The levels of NE in the amygdala correlated with the performance of the rats during the retention test. Unexpectedly, DSP 4 also decreased 5-HT levels in the amygdala (-27 %). No other changes in amine levels were found in any of the structures studied (amygdala, hypothalamus, hippocampus or striatum). We interpret these findings as providing further support for the view that naloxone-induced memory facilitation depends on the levels of NE in the amygdala.

Supported by NIMH grant MH12526 and ONR grant N00014-87-K0518.

288.9

LESIONS OF THE STRIA TERMINALIS ATTENUATE THE EFFECTS OF INTRA-AMYGDALA INJECTIONS OF NOREPINEPHRINE ON MEMORY. K.C. Liang & H.-C. Yao*. Dept. of Psychology, Natl' Taiwan Univ., Taipei, TAIWAN 10764, R.O.C.

Previous evidence indicates that post-trial injections of norepinephrine (NE) into the amygdala (Amyg.) affect retention of learned responses. The present study was designed to examine whether the memory modulatory effect of intra-Amyg. NE injections is attenuated by transecting a major Amyg. connection pathway--the stria terminalis (ST).

Male Sprague-Dawley rats received bilateral implantation of cannulae into the center of the Amyg.. Half of the rats also received radio frequency lesions (2.5 mA/30 sec) of the ST (ST-), while the rest received sham ST operations (ST+). Two weeks after surgery, all rats were trained on a one-trial step-through inhibitory avoidance task (1 mA/1 sec footshock). Immediately after training, rats received intra-Amyg. injections of vehicle or 0.2, 1.0 or 5.0 ug of NE (1 ul/Amyg.). Retention performance tested 24 hrs later indicated that in the ST+ rats, post-trial intra-Amyg. injections of 0.2 ug NE enhanced retention ($U=17$, $p<0.05$), while post-trial intra-Amyg. injections of 5.0 ug NE tended to impair retention ($U=26.5$, $0.05< p <0.10$). In contrast, various doses of NE injected into the Amyg. after training failed to affect retention in the ST- rats. These findings suggest that the integrity of the ST is critical for the memory modulatory effects of NE injected into the Amyg. Supported by a grant NSC 76-0301-H002-02 from NSC of R.O.C.

288.10

EFFECTS OF SCOPOLAMINE WITH DSP4 OR PCPA, ON WORKING AND REFERENCE MEMORY OF RATS. P. Prior and R.E. Hinson. Dept. of Psychology, Univ. of Western Ontario, London, Ont., N6A 5C2

This study investigated the effects of depletion of norepinephrine (NE) or serotonin (5HT) on scopolamine-induced deficits in 16-arm radial maze performance. After training, animals were placed into 4 groups, to receive different treatments prior to 2 maze tests, 3 days apart. One group received an i.p. injection of 400 mg/kg PCPA (5HT synthesis inhibitor) on each of the 3 days before the first maze test. A second had one i.p. injection of 50 mg/kg DSP4 (NE neurotoxin) 10 days before the first test day. Two other groups received saline in the pre-test period. The PCPA, DSP4 and one of the saline groups received 1.0 mg/kg scopolamine hydrobromide i.p. 30 min before one maze test, and 1.0 mg/kg scopolamine methylbromide before the other. The fourth group received only saline before tests. Assay after the second test showed 5HT depletion to 11% of control in the PCPA group, and to 60% of control in the DSP4 group. Working (re-entries into already visited baited arms) and reference (entries into the 8 never-baited arms) memory errors were elevated in the scopolamine and scopolamine+DSP4 compared to the analogous methylscopolamine conditions. Interestingly, working errors were not increased with PCPA+scopolamine; in fact they were decreased compared to all conditions including control. Serotonin synthesis blockade by PCPA may ameliorate some memory deficits produced by scopolamine in this task.

288.11

NORADRENALINE DEPLETION BY DSP4, LIKE CLONIDINE, IMPROVES SPATIAL LEARNING IN FORNIX SECTIONED RATS. S.J. Sara, C. Dyon-Laurent and C. Maho, Lab. of Psychophysiology, CNRS, LPN2, 91198 Gif/Yvette, France. (SPON: R.B. Messing)

The adrenergic alpha-2 receptor agonist, clonidine, which inhibits release of noradrenaline (NE) by its action on inhibitory autoreceptor, reversed the deficit in acquisition of a spatial working memory task in rats with fornix lesions (Sara, Amassari and Maho, 1987, Neurosci. Abstr.). To assess to what extent this facilitation was due to clonidine's inhibition of NE release, we lesioned the fornix and then injected the specific NE neurotoxin DSP4. Rats with fornix lesion alone were impaired, while those with both NE depletion and fornix lesion performed as well as controls. Neurochemical analysis of the hippocampus of these rats showed a unilateral decrease of 50% in choline acetyl transferase activity, along with 50% decrease in NE content and a slight increase in the NE metabolite MHPG, indicating a hyperactivity of NE in fornix lesioned rats. DSP4-treated rats showed low levels of both NE and MHPG. This hyperactivity of the NE system after fornix section, appears to exacerbate the behavioral deficits due to cholinergic deficits.

The interaction between NE and Ach requires an appropriate balance between the two for adequate cognitive function.

288.12

ENHANCED BEHAVIOURAL CONDITIONING TO CONTEXT BUT UNCHANGED CORTICOSTERONE RESPONSE IN NORADRENALINE DEPLETED RATS. N.R.W. Selden*, T.W. Robbins, and B.J. Everitt[1]. Departments of Experimental Psychology and [1]Anatomy, University of Cambridge, Cambridge, U.K. CB2 3EB

This study tested the hypothesis that cortical noradrenaline (NA) depletion induced by 6-hydroxydopamine widens attentional span, impairing the acquisition of conditioning to a punctate stimulus (CS), while enhancing conditioning to contextual surroundings. A conditioned suppression paradigm was used involving baseline lever pressing for food reward, in an operant chamber, and the conditioning of a clicker stimulus predicting shock, in a separate place-preference apparatus (PPA). After conditioning, NA depleted animals showed a longer latency to enter the shock side and greater preference for the safe side of the PPA, indicating greater contextual conditioning, as predicted by attentional theory. However, NA depleted rats also suppressed operant responding more than controls during CS presentation. Plasma corticosterone was increased after exposure to the PPA and to the CS, but was unaffected by NA depletion. Lesioned animals may have shown increased suppression to the nominal CS because of a deficit in temporal processing, causing them to treat the shock as temporally unrelated to CS onset. In this case, the CS would constitute another element of the context, and enhanced conditioning would further support an attentional interpretation.

288.13

EFFECTS OF VAGOTOMY ON THE FACILITATION OF LEARNING AND MEMORY PRODUCED BY 4-OH AMPHETAMINE. C.L. Williams* and R.A. Jensen. Department of Psychology, Southern Illinois University, Carbondale, IL 62901.

Previous studies have demonstrated that peripherally administered catecholamines (CA), or agents affecting CA release such as amphetamine or 4-OH amphetamine, modulate brain processes involved in memory storage (Martinez et al., Br. Res., 182:509, 1980). These agents may act indirectly to modulate memory since some (e.g. 4-OH amphetamine) do not easily cross the blood-brain barrier.

To determine the route by which these substances exert their effects, rats received either subdiaphragmatic vagotomies, sham surgeries, or no surgery. After recovery from surgery, all animals were trained in an inhibitory avoidance task with a 0.92 mA, 0.5 sec footshock. Immediately after training 2.0 or 4.0 mg/kg 4-OH amphetamine or saline was injected i.p. Retention was tested 24 hours later. Preliminary results indicate that the retention of vagotomized rats receiving either dose of amphetamine did not differ significantly from saline-injected controls. Injection of 2.0 mg/kg 4-OH amphetamine to sham operated animals enhanced retention performance compared to the vagotomized rats. At this dose, sham-operated animals did not differ from control rats. These findings suggest that vagotomy may attenuate the effects of peripherally acting agents that modulate memory and that vagal afferents may carry information about peripheral events that modulate memory storage processes.

288.14

ENHANCEMENT OF MEMORY FOR AN APPETITIVE LEARNING TASK BY GLUCOSE IN MICE. C. Messier and C. Destrade, Lab. Psychophysiologie, UA CNRS 339, Univ. Bordeaux I, 33405 TALENCE FRANCE.

Experiments have shown that a post-training glucose injection can retroactively and non-contingently improve the retention of a previously learned association (Messier and White, Physiol. Behav., 32, 195-203, 1984; Behav. Neural Biol., 48, 104-127, 1987). However this memory-improving action of glucose has only been shown in rats and for aversively motivated tasks. The present experiments sought to generalize these previous findings by demonstrating the memory improving action of glucose in an other species (BALB/c mouse) and for an appetitively motivated task (an operant bar pressing response to obtain a food reinforcer). The results show that a post-training 3 g/kg or 4 g/kg glucose injection could retroactively and non-contingently improve the retention of this task 24 h later while 2 or 5 g/kg glucose injections were ineffective. Post-training glucose did not produce any effect on the retention of yoked animals for which food reinforcement was not contingent on bar pressing, but were otherwise exposed to all the stimuli of the training situation. The lack of effect of glucose in the yoked animals suggests that the effect of glucose in the trained animals was dependent on the existence of an association between the act of bar pressing and the availability and consumption of the reinforcer and that glucose improved the memory for this association.

288.15

MEMORY FACILITATION BY GLUCOSE IN AGED HUMANS. C.A. MANNING, C.L. JONES, J.L. HALL and P.E. GOLD. Dept. Psychology, University of Virginia, Charlottesville, VA 22903 (SPON: J. HAHN).

Recent evidence suggests that the effects of epinephrine on memory in rodents may be mediated, in part, by the hormone's hyperglycemic actions. Consistent with these findings, glucose also enhances performance on some memory tests in elderly people. In addition, poor glucose regulation was related to poor memory performance in individual subjects. This study investigated the role of glucose on additional memory tests as well as on other measures of cognitive and motor functioning.

Subjects, aged 62-84 (n=17), were tested on two consecutive mornings. Subjects took a series of tests assessing memory, IQ, attention, and motor function after ingesting either 50 g of glucose or 32 mg of saccharin. Plasma glucose was measured at several times during testing. On Day 2, subjects received alternate treatments and test forms.

Glucose, but not saccharin, increased circulating glucose levels significantly. Performance on memory tasks, but no other tasks, was enhanced by glucose. Thus, the glucose effects appear to be selective to memory. As seen before, glucose control was related to memory in individual subjects. Thus, these findings provide additional evidence that glucose enhances memory, and that poor glucose regulation is related to poor memory, in elderly humans. (Supported by MH 31141, ONR N00014-85-KO472, and the American Diabetes Association, and training grant MH 18411-01).

288.16

REGIONAL BRAIN [^{3}H]2-DEOXYGLUCOSE UPTAKE IN MICE: EFFECTS OF AMNESTIC AND MEMORY-ENHANCING DRUGS. W.S. STONE and P.E. GOLD. Dept. Psychology, University of Virginia, Charlottesville, VA 22903.

Scopolamine (SCOP)-induced amnesia in mice can be attenuated with several non-cholinergic treatments, including epinephrine and glucose (GLU). On the basis of growing evidence that GLU regulates memory storage, we examined the effects of an amnestic dose of SCOP on regional brain [^{3}H]2-deoxyglucose (2DG) uptake, alone and in combination with drugs that attenuate SCOP-induced memory deficits.

Mice received combined injections (IP) of SCOP (3 mg/kg) and saline, or SCOP and GLU (100 mg/kg). Ten min later, animals received 2DG (10uCi/animal, SC) and were sacrificed 40 min after the 2DG injections. Eight brain regions were then dissected, solubilized, and assessed for specific activity.

SCOP reduced 2DG uptake in several areas, with maximal decreases (21-50%) seen in hypothalamus, hippocampus, septum and striatum. Preliminary data also suggest that peripheral GLU injections may attenuate SCOP-induced reductions in 2DG uptake in these areas, consistent with the view that GLU may enhance CNS GLU utilization and perhaps cholinergic function. (Supported by MH 31141, ONR N00014-85-K0472, the American Diabetes Association, and NIA Postdoctoral Fellowship AG 05408).

288.17

MEDIATION OF ADRENALECTOMY MEMORY EFFECTS BY CIRCULATING GLUCOSE. J.L. Hall & P.E. Gold. Dept. Psychology, University of Virginia, Charlottesville, VA 22903.

Epinephrine released from the adrenal medulla, modulates memory storage. Recent evidence suggests that these hormonal effects are mediated, in part, by increases in blood glucose (BG) levels. This study examined the possible contribution of BG to memory changes seen in rats after adrenalectomy (ADX).

Rats exhibited a transient impairment in retention of an inhibitory (passive) avoidance task after ADX. The deficit was most pronounced 48 hr after surgery; retention 8 days after surgery was comparable to that of controls. Baseline BG was decreased 1 and 2 days after ADX but had recovered after 8 days. Compared to the training-related increase in BG levels seen in sham-operated rats, ADX rats had a delayed BG increase after 1 Day, a BG decrease at 2 Days, and no change 8 days after surgery.

Preliminary studies indicate that BG increases after glucose injection are attenuated 2 days after ADX. By using a dose which produces a BG response comparable to that accompanying glucose memory enhancement in intact animals, posttraining glucose attenuated the ADX-induced memory deficit.

These findings suggest that altered BG baseline levels and responses to training may contribute to memory impairments and recovery after ADX. Additionally, the results further implicate blood glucose in the regulation of CNS information processing systems. (Supported by MH 31141, ONR N00014-85-K0472, and the American Diabetes Association).

288.18

COCAINE ENHANCES RETENTION OF AN ACTIVE AVOIDANCE TASK IN RATS. P.H. Janak and J.L. Martinez Jr. Dept. of Psychology, University of California, Berkeley, CA 94720.

Earlier work showed that posttraining intraperitoneal administration of d-amphetamine enhanced retention of a one-way active avoidance task in rats, and that these effects depend upon peripheral catecholamine stores (Martinez et al, Brain Res., 182:157-166, 1980). The current experiments show that posttraining administration of cocaine, which shares many pharmacological actions with amphetamine, also enhances retention of an active avoidance task in rats.

Male Sprague-Dawley rats were given cocaine HCl or saline i.p. immediately following 2 training trials in a one-way active avoidance task. The animals were given 10 s to avoid delivery of a 600 uA shock by crossing to a safe chamber. Rats were tested 24 h later; the number of avoidances made in 8 trials was used to measure retention. A posttraining 5.0 mg/kg cocaine dose enhanced retention of the avoidance task 24 h after training ($t=3.07$, $p<.01$); both a lower dose (2.5 mg/kg) and a higher dose (7.5 mg/kg) were without effect. Lidocaine was without effect indicating that local anesthesia does not account for cocaine enhancement of active avoidance performance. Cocaine's effect was time dependent such that it was only effective when administered immediately after the training trials.

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288.19

THE EFFECTS OF THE ALPHA-2 AGONIST CLONIDINE ON DELAYED NONMATCH-TO-SAMPLE PERFORMANCE IN AGED RHESUS MONKEYS A.F.T. Arnsten and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale Medical School, 333 Cedar St., New Haven, CT 06510

The alpha-2 adrenergic agonist clonidine significantly improves the spatial working memory abilities of aged monkeys performing the delayed response task (Science 230:1273-1276, 1985). This type of memory is subserved by the principal sulcal prefrontal cortex (PS-PFC) and pharmacological data from 6-OHDA-lesioned monkeys suggest that clonidine's beneficial actions may occur at least partially in this brain region (ibid). The present study examined whether clonidine might improve another type of memory that is not dependent on the PS-PFC, visual object recognition memory, as tested by the delayed nonmatch-to-sample task (DNMS). Aged monkeys were trained on DNMS according to the methods of Presty et al. (Neurobiol. Aging 8: 435-440, 1987), and a wide range of clonidine doses was explored (0.00001-0.1 mg/kg). As with the delayed response task, clonidine improved performance on DNMS. However, the effective dose range was more limited, and the improvement less robust than with delayed response. These data suggest that clonidine's effects on cortical function are not limited to the PS-PFC, and that the beneficial effects of alpha-2 agonists on cognitive function are not restricted to spatial working memory.

288.20

SPATIAL WORKING MEMORY ON THE PLATFORM MAZE IS NOT IMPAIRED BY A NORADRENERGIC ANTAGONIST INJECTED INTO THE HIPPOCAMPAL DENTATE GYRUS. S. Benloucif, A. Packer*, E.L. Bennett and M.R. Rosenzweig. Dept. of Psychology, University of California, Berkeley, CA 94720.

Long-term potentiation in the dentate gyrus of the hippocampus is enhanced by application of norepinephrine (NE) and blocked by either NE depletion or beta-receptor antagonists. The integrity of hippocampal functioning is necessary for learning and performance of spatial working memory. We examined whether beta-receptor antagonism in the dentate gyrus would impair retention of a spatial working memory problem. Rats were trained on a working memory version of the Barnes' platform maze which required the animals to return to an escape hole they had found two hours earlier. The rats received two training trials followed by a 2 hr delay and a single test trial. Rats had to choose from among 12 goal locations spaced evenly around the outer edge of a 4 ft diameter circular platform. The goal location changed each day. After 20 days of training subjects were implanted with indwelling injection cannulas. Following recovery the rats were trained for an additional 8 days, then received pre- or post-training injections of one of 3 doses of propranolol or vehicle every third day for 24 days. At the conclusion of the experiment rats were divided into dentate gyrus (DG, N=7) or non-dentate (N=4) groups based on cannula placement. Propranolol did not impair retention in comparison with non-drug days. However, both cannula placement and injections increased errors and variability on non-drug days in the DG group that may have masked a more subtle impairment of performance with beta receptor blockade. Additional work with other amnestic agents (anisomycin, lanthanum chloride, glutamate) also indicates that retention of this task may not be susceptible to drugs injected into the dentate.

288.21

SUPPRESSION OF DRINKING IN RATS BY AMPHETAMINE-CONDITIONED STIMULI. P.M. Duncan . Psychology Dep't, Old Dominion Univ., Norfolk, VA 23508.

Pavlovian conditioning of d-amphetamine sulfate (DA) effects on suppression of water-drinking was investigated in two experiments. Rats (24-hrs water-deprived) were adapted to drinking from a touch-sensitive spout in the test-box over a 3-4 day period. A conditioning day followed, on which experimental groups were injected IP with DA 15-min prior to 20 presentations of a 10-sec, 5-hz, 75-db clicking stimulus (CS) which initially inhibited drinking. Habituation was indicated by less suppression (by nondrugged control rats:ND) to subsequent CS presentations both during this session, and 72-hours later during a test session. No-stimulus controls (NSC) had no CS exposure until the test day, and delayed-drug controls (DD) were not DA-injected until two hrs post "conditioning" in the home cage. DA produced dose-related drinking-impairment and greater suppression during the conditioning session. Suppression ratios (SR: 100=no drinking during CS, 0=no change in lick-rate) recorded on the test day (all groups non-drugged, and 24-hrs water-dep) indicated dose-related suppression in DA-groups, compared to ND and DD controls. Median CS presentations to SR<20, DA dose (mg/kg) and n() for each group: Exp#1; ND(11)=3, DA6(10)=12*, DA2(10)=12*, NSC(7)=12*. Exp#2; ND(10)=1, DD(7)=1.5, DA2(8)=18*, DA.6(8)=2, NSC(7)=5* (*=different ($p<.05$) from ND). Similar differences were indicated by ANOVA of SR-values. These results show that the adipsic effects of DA can be conditioned to environmental stimuli during one drug treatment at a dose of 2, but not .6 mg/kg. Suppression of drinking may have been elicited by overall test-situation cues as well as by the auditory stimulus.

288.22

ALPHA-2 ADRENOCEPTOR ANTAGONISTS DECREASE SCOPOLAMINE-INDUCED SWIMMING ACTIVITY WITHOUT ALTERING ACTIVITY PATTERNS. W. Lipinski*, M. Smith* and R.E. Davis (SPON: J.G. Marriott). Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

In previous work we have shown that the anticholinergic scopolamine alters two components of swimming activity eliciting excessive and stereotyped patterns of activity. Cholinesterase inhibitors and direct muscarinic agonists reverse both the total amount and stereotyped patterns of activity elicited by scopolamine. However, these two components of scopolamine-induced swimming activity may be independent and dissociable processes. In an attempt to demonstrate pharmacological dissociation of these two process, we examined the ability of a series of alpha-2 antagonists, compounds which are known to decrease locomotor activity, to differentially alter the total amount and stereotyped pattern of activity elicited by scopolamine.

To accomplish this, scopolamine (0.32 mg/kg, IP) was administered in combination with subcutaneous injections of vehicle (0.9% saline) or one of the following alpha-2 antagonists: yohimbine (Sigma), idazoxan (Reckitt and Coleman), WY 26902 (Wyeth, UK), or L-654,284 (Merck Sharp and Dohme) to male Long-Evans rats. Thirty minutes later these animals were placed in a white plastic enclosure filled to a depth of 33 cm with water (22° C) made opaque by the addition of powdered milk. Swimming activity was monitored continuously for the next 5 mins using a computerized system. Total distance traveled and several metrics for quantifying swimming activity patterns served as the dependent measures.

All alpha-2 antagonists tested decreased total distance traveled by scopolamine-pretreated rats at doses that did not decrease spontaneous swimming activity. In contrast, these alpha-2 antagonists did not alter the stereotyped patterns of activity induced by scopolamine. This suggests that these two components of scopolamine-induced swimming activity are pharmacologically dissociable and may be independent processes.

288.23

THE C57BL/10j MOUSE AS A MODEL OF IMPAIRED COGNITION: COMPARISON OF THE EFFECTS OF REFERENCE AGENTS ON WATER-MAZE PERFORMANCE. C. Bay-Gemmill*, M. Smith*, J. Kinsora*, M. Dickerson*, W. Lipinski* and R.E. Davis. Parke-Davis Pharmaceutical Research Div., Warner-Lambert Co., Ann Arbor, MI 48105.

Previous work has shown that mice with a small hippocampus (C57BL/10j, B10) have greater difficulty acquiring a spatial water maze task than mice with a large hippocampus, (NZBXNZW F1 strains). The performance deficits of B10 mice can be ameliorated by admininistration of some cholinomimetics suggesting that there may be a cholinergic deficiency contributing to the poor performance of B10 mice in the water maze. However, this does not exclude the possibility that pharmacological manipulation of other neurotransmitter systems might also improve the water-maze performance of B10 mice. Because of this, selected agonist and antagonists from several classes of drugs were studied for their ability to improve the water-maze performance of B10 mice.

To accomplish this B10 mice were tested in a square water maze with a small moveable platform hidden below the surface of opaqued water (21-23°C) and located in the center of one of four quadrants of the pool. Four trials were conducted each day over two consecutive days. Latency to find the hidden platform served as the dependent measure. Drugs were administered on both test days 30 minutes prior to testing.

Of the classes of agents tested most impaired or failed to improve the performance of B10 mice in this test. These included agonists and antagonists interacting with dopaminergic, alpha-1 adrenergic, serotonergic systems and monoamine uptake inhibitors. The exceptions were ±3-PPP, a dopamine autoreceptor agonist, and yohimbine, an alpha-2 adrenergic antagonist, both of which improved the water-maze performance of B10 mice. These findings suggest that most agents which alter classical neurotransmitter function do not improve the water-maze performance of B10 mice. However, agents representative of 3 classes of drugs appear to improve the performance of B10 mice in the water-maze, alpha-2 antagonists, dopamine autoreceptor agonists and cholinomimetics.

288.24

EFFECTS OF CHRONIC SEROTONIN RECEPTOR ANTAGONIST ADMINISTRATION ON ACQUISITION OF A COMPLEX SPATIAL DISCRIMINATION TASK IN YOUNG AND OLD RATS. H.J. Normile, M.P. Galloway and H.J. Altman, Department of Psychiatry, Wayne State University, Detroit, MI 48207

Previously research from this laboratory has shown that p-chloroamphetamine-induced chronic reductions in brain serotonin (5-HT) facilitated the performance of young and old rats in a complex spatial discrimination task (Stone 14-unit T-maze). The present experiment examined the effects of chronic 5-HT receptor antagonist administration on acquisition of the same task in both young and old rats. The rats (male Sprague-Dawley) were trained one trial per day for a total of 25 days. Ketanserin (1.0, 10.0 mg/kg) and mianserin (0.1, 1.0 mg/kg) were administered (ip) using a variety of treatment schedules, including a 14 day pretreatment period and daily injections commencing with the first maze trial. Analysis of the data (errors over days, days to criterion) suggests that the drugs interfered with the ability of the animals to acquire the task. Therefore, in contrast to the facilitative effects observed following cytotoxic destruction of 5-HT neurons, chronic blockade of post-synaptic 5-HT receptors appears to impair acquisition in the 14-unit T-maze.

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288.25

FACILITATION OF COMPLEX MAZE LEARNING IN RATS FOLLOWING SELECTIVE 5-HT DEAFFERINATION OF THE DORSAL HIPPOCAMPUS. H.J. Altman[1], H.J. Normile[1], M.P. Galloway[1], T. Ramirez[2] and E.C. Azmitia[2]. [1]Dept. Psychiatry, Wayne State University School of Medicine, Detroit, MI, [2]Dept. Biology, New York Univivesity, New York, N.Y.

Recent evidence from this laboratory indicates that PCA-induced 5-HT depletion of the brain significantly enhances the performance of rats trained in the Stone 14-Unit T-maze. Although PCA depletes brain 5-HT indiscriminantly, the most profound reductions are observed in the hippocampus (HIPP) - an area repeatedly shown to play an important role in learning and memory. To determine a more specific locus of 5-HT action, the performance of rats trained in the Stone maze was determined following injection of the selective serotonergic cytotoxin 5,7-DHT into the fimbria-fornix which results in selective destruction of HIPP 5-HT terminals. The behavioral results indicated that the performance of the 5,7-DHT-lesioned rats was significantly better than that of both sham-operated and vehicle-injected controls (both in terms of mean number of errors and trials to criterion). The performance of the sham-operated and vehicle-injected controls did not differ. Neurochemical determinations revealed that HIPP, but not striatal, 5-HT was profoundly decreased following 5,7-DHT administration. The results of the present experiment are important because they extend our understanding of the role 5-HT plays in the processes underlying learning and memory.

289.1

PARTICIPATION OF THE RAT'S PELVIC, PUDENDAL AND GENITOFEMORAL NERVES IN SOME PERINEAL MUSCULAR REFLEXES. M. Martínez-Gómez, J. Manzo-Denes*, C. Beyer*, B.R. Komisaruk and P. Pacheco. Univ. Aut. de Tlaxcala, Méx.; Univ. Veracruzana, Xalapa, Ver., Méx.; CINVESTAV-Tlax., Méx.; Rutgers Univ., Newark, NJ, USA; and IIB-UNAM, Méx., D.F..

The iliococcygeus (icm) and pubococcygeus (pcm) muscles can be reflexively contracted by contralateral tail displacement, and by perineum, clitoral sheath and distal vagina stimulation. This reflexive contraction was abolished by gentle cervical stimulation while the psoas muscle (psm) was reflexively activated by the same stimulation. The icm and pcm contraction had a duration related to the origin of stimulation. Perineal skin stimulation is more effective than clitoral and both of them more than vaginal. When clitoris was held, the pudendal nerve's (PuN) clitoral receptors and the genitofemoral nerve's (GfN) groin skin receptors also initiated icm and pcm reflex contraction. Both cervical inhibitory action upon icm-pcm contraction and psm activation effect were partially abolished during the ipsilateral transection of the pelvic nerve's (PeN) viscero-cutaneous branch, and completely abolished after bilateral transection. In male rats PuN was activated by scrotal skin and thigh inner surface stimulation; PeN by perianal skin; and GfN by groin skin. When electrical stimulation was applied, PuN provoked contraction of the coccygeus muscle, external anal sphincter and the cremaster area closer to the epididymis' tail; PeN evoked icm and pcm contraction; and GfN contracted the cremaster that covers the testis. CONCLUSIONS: In male rats both PuN and GfN innervate the cremaster, PuN the scrotal skin, and PeN the perianal skin. In female rats cervical stimulation provides facilitatory and inhibitory reflex mechanisms involved in reproductive activities.

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289.2

OLFACTORY AND VISCERAL PROJECTIONS TO THE NUCLEUS OF THE SOLITARY TRACT. R. Guevara-Aguilar, D.E. García-Díaz, L.L. Jiménez-Montúfar* and M.J. Wayner. Depto. de Fisiología, Facultad de Medicina, U.N.A.M., Apdo. Postal 70-250, 04510 México, D.F., and Division of Life Sciences, University of Texas at San Antonio, Texas 78285, U.S.A.

Electrophysiological studies were performed to determine if neurons of nucleus of the solitary tract (NTS) which receive inputs from the stomach via vagal afferents also respond to olfactory bulb (OB) stimulation. Experiments were conducted on twenty Wistar rats weighing between 230-280 g. The frequency of neuronal activity of the rostral ventral portion of the NTS was increased by gastric distension (GD). The evoked potentials in the same site due to vagal stimulation displayed short latencies; whereas, the evoked potentials in the dorsomedial part of the NTS due to vagal stimulation had considerably longer latencies. Gastric distension decreased neuronal activity in the dorsomedial NTS. Evoked potentials and increases in neuronal activity were also observed in the dorsomedial NTS due to electrical stimulation. In the dorsomedial NTS, OB stimulation enhanced the decrease in neuronal activity due to GD. Olfactory and visceral functions apparently interact in the NTS in modulating taste mechanisms involved in food selection and ingestion.

289.3

RESPONSES OF SPINE-ASSOCIATED PENILE MECHANORECEPTORS TO TANGENTIAL MECHANICAL STIMULATION. R.D. Johnson and V.P. Dugan*, Dept. of Physiological Sciences, University of Florida, College of Veterinary Medicine, Gainesville, FL 32610.

Single unit activity from penile mechanoreceptors were recorded from a teased fiber preparation of the dorsal nerve of the penis in barbiturate anesthetized male Wistar rats. By utilizing a modified Chubbuck mechanostimulator which provided "swept" tangential stimulation of the spiny penile surface, mechanoreceptors preferentially responding to these stimuli were studied. These receptors, classified as 'spiny RA units', were characterized by (1) 2 to 4 discrete receptive fields; each under a single proximally directed spine or spine cluster and separated by insensitive spines and interspinous skin (up to 4.5 mm spacing), (2) myelinated branching to each RF from a single myelinated parent axon as confirmed by collision techniques, (3) an exquisite vibratory sensitivity up to 1200 Hz as determined with 10 cycle entrainment, (4) a relative insensitivity to punctate vertical skin movement, and (5) greater responses to distal movements (as in copulatory withdrawal motion) than to proximal movements. Computer analysis of discharge patterns revealed coding of displacement and velocity components of stimuli swept over the receptive area.

The direction sensitivity and spatial coding ability of these mechanoreceptors even when only the spines were deflected suggests a sensory role for the penile spines of the rat in copulatory behavior.

289.4

AN INTRACRANIAL, PEPTIDERGIC, SENSORY MICROGANGLION IS ASSOCIATED WITH CRANIAL NERVE XI IN THE RAT C. J. Wetmore and R. P. Elde Dept. of Cell Biol. and Neuroanatomy and Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

Cranial nerve XI (CnXI) is considered to be a purely motor nerve. However, a small ganglion (250-260 µm) associated with the arachnoid near the vertebral artery and the spinal trunk of CnXI on the ventrolateral aspect of the rat brainstem was noted in over 30 Sprague-Dawley rats. The ganglion was found to have 3-5 processes extending into the arachnoid, one of which joins the spinal trunk of CnXI. Exposure of the proximal stump of the severed peripheral CnXI to Fluoro-Gold (FG) resulted in fluorescent staining of all neurons within the ganglion, as well as several bipolar, intrafascicular neurons within CnXI.

Immunohistochemical studies were conducted on whole mounts of this ganglion with antisera to calcitonin gene related peptide (CGRP) and substance P (SP). Virtually all neurons were intesely immunoreactive for CGRP and many indicated some degree of SP immunoreactivity. Not suprisingly, two-color immunoflourescence analysis demonstrated doubly labeled neurons, indicating coexistence of SP and CGRP in over half of the cells. The morphology of these neurons resembled the small- and medium-diameter pseudounipolar primary afferent neurons in dorsal root ganglia. Support for the sensory, rather than sympathetic character of this ganglion was found in that neonatal capsaicin treatment significantly diminished the intensity of SP and CGRP immunoreactivity whereas, neonatal guanethidine treatment failed to alter the staining or morphology of the cells. Thus, this microganglion appears to have morphological and neurochemical properties similar to primary afferent, nociceptive neurons and may have intracranial as well as extracranial targets of innervation. Supported by DA 02148

289.5

MORPHOLOGICAL DIFFERENCES BETWEEN FINE NERVE FIBERS IN THE KNEE JOINT. R.F. Schmidt, B. Heppelmann*, K. Meßlinger*, and W.F. Neiss*. Inst. of Physiology and Inst. of Anatomy, Univ. of Würzburg, D-8700 Würzburg, Fed.Rep.Germany

In electrophysiological studies fine afferent joint units (group III and IV) revealed marked differences in their threshold and response patterns to passive movements: some units responded forcefully to movements in the normal working range of the joint, others only to noxious stimuli, still others to no stimuli at all (H.-G. Schaible and R.F. Schmidt, J. Neurophysiol., 49: 1118, 1983). To answer the question, whether there exist any structural correlates to the various functional types, we started a morphological examination of fine afferent nerve fibers in the cat's knee joint at the light and electron microscope level.

Here we report on the examination of fine nerve fibers in joint tissue of sympathectomized cats. The fibers were reconstructed and analyzed in serial sections over a distance of up to 100 µm using an electron microscope (Zeiss EM 902).

In their terminal parts (length up to several 100 µm) the fibers split up into several branches. Preterminal bulges in the whole terminal parts and true endings of the fibers show axonal membrane not covered by Schwann cell and accumulations of mitochondria, glycogen particles and vesicles. The number of these organelles, however, greatly varies between different fibers. This may reflect differences in the metabolic activity. On the assumption that the metabolic activity is correlated with the nerve fiber activity, it may be speculated that units which are activated by normal joint movements contain a high amount of mitochondria and glycogen particles. Fibers with a low content of organelles may be considered as nociceptive units only activated under extreme conditions.

289.6

DIFFERENTIAL DISTRIBUTION OF PEPTIDERGIC VISCERAL AFFERENT PROJECTIONS FROM THE NODOSE GANGLION TO THE LARYNX AND STOMACH. K.M. Hill* and C.J. Helke. (SPON: E. Frey) Uniformed Services Univ. of Health Sci., Bethesda, MD.

Several neuropeptides were localized in cells in the inferior vagal (nodose) afferent ganglion of the rat (Helke and Hill, Neurosci. in press). Substance P(SP), calcitonin gene related peptide (CGRP), and cholecystokinin (CCK)-ir cells were more numerous in the rostral compared to the caudal nodose ganglion. To determine if this chemotopic organization reflected a somatotopic organization, we studied peptide-ir in organ specific projections to a caudal (stomach) vs a rostral (larynx) vagal target. Fluoro-Gold (FG) was applied to the cut end of the anterior gastric vagus nerve (AGN) and the superior laryngeal nerve (SLN), respectively. FG application to the AGN labeled numerous cells in the caudal (main) nodose ganglion whereas FG transported by the SLN labeled more rostral and fewer caudal nodose neurons. Transection of the nerve just rostral to the FG application in control experiments eliminated ganglionic labeling. Peptide-ir of FG-labeled cells was detected with immunofluorescence. SP, CGRP, and CCK-ir nodose ganglion neurons projecting to the AGN were extremely rare. SP- and CGRP-ir (but not CCK-ir) neurons projecting to the larynx were found in the rostral and to a lesser extent caudal nodose ganglion. These studies demonstrate SP and CGRP vagal afferent projections in the SLN, and suggest a loose intraganglionic organization of projection and transmitter specific neurons. (NIH NS20991).

289.7

BRAINSTEM ACTIVITY EVOKED BY ABDOMINAL VAGAL STIMULATION IN FERRET. G.E. Lucier, K.A. Sharkey*, R. Egizii* and J.S. Davison*. Dept. of Physiology, University of Calgary, Calgary, Alta., T2N 4N1 Canada.

Electrical stimulation of the vagal communicating branch (VCB) elicits vomiting in anaesthetized ferrets. Neurophysiological mapping of the projection sites of the VCB in the region of the nTS is an essential preliminary to analysis of the central mechanisms involved in emesis. Adult male ferrets were anaesthetized with halothane. A cuff electrode was placed on the VCB and tested by inducing a single emetic episode, after which the animal was paralyzed. Hypertonic saline was infused into the stomach as an alternative stimulus for inducing emesis. Brainstem evoked activity was recorded using stereotaxically placed tungsten microelectrodes. Responses to either stimuli were obtained in units that were either previously silent or exhibiting low frequency discharge. Latencies to electrical stimulation were usually around 200 msec (range 75 - 300 msec). These units were found to lie in nTS, 2.0 mm rostral and 2.5 mm caudal to obex and from midline to 1.0 mm lateral at the level of obex. In the rostral nTS, they were found to be primarily ventral and medial to respiratory and cardiovascular units. In the caudal nTS, only evoked responses were found, indicating some degree of viscerotopic organization. (Supported by MRC Canada)

289.8

VISCERO-SOMATIC CONVERGENCE AT TWO LEVELS OF THE RAT SPINAL CORD. E.W. Akeyson* and L.P. Schramm (SPON: R. Taylor), Depts. of Neuroscience and Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

We are studying viscero-somatic convergence at two spinal cord levels in chloralose-anesthetized rats. Splanchnic responsive (SPL) neurons at T_8-T_{11} and C_2-C_5 were tested with greater splanchnic (GSN) and ilio-hypogastric nerve (IHN) stimulation and mechanical stimulation of the skin. Thoracic SPL neurons responded to GSN stimulation with one or two excitatory bursts. IHN evoked responses were as long as GSN evoked responses, but much earlier in onset. Most neurons had cutaneous receptive fields (RF) located on the ipsilateral lower chest and upper flank regions. Two-thirds of the neurons were excited by light touch and intense, noxious stimulation (WDR type). The remaining neurons responded only to high intensity, noxious stimuli (HT type). Half of cervical SPL neurons are inhibited by GSN stimulation. Cervical SPL neurons displayed RF's on the ipsilateral shoulder and forepaw, which often extended to the contralateral side. Two-thirds of cervical neurons responded only to high intensity stimulation of the skin (HT type), but a few responded only to light touch of the skin (LT type). One-third of cervical neurons also responded to high intensity stimulation of the RF's of thoracic neurons. This data may suggest specificity of abdominal visceral and somatic inputs to cervical neurons. Supported by NIH grants HL16315 and 5-T32-GM07309-12.

289.9

SPINAL CORD DISTRIBUTION OF VISCERAL SENSORY AXONS FROM THE BLADDER AND LOWER COLON OF THE CAT. C. W. Morgan, W. C. deGroat, and I. Nadelhaft, Department of Anatomy and Cell Biology, Eastern Virginia Medical School, Norfolk, VA 23501; Department of Pharmacology, University of Pittsburgh Medical School and VA Medical Center, Pittsburgh, PA 15261.

Visceral sensory axons in the cat pelvic nerve play a major role in the micturition and defecation reflexes. Understanding their spinal distribution, established a number of years ago, has contributed to our continuing analysis of the mechanisms underlying these reflexes, partly by identifying areas of the spinal cord where interneurons may be found. Using the transganglionic HRP method, the present study has continued this analysis by comparing spinal distributions of branches of the pelvic nerve supplying the bladder with those branches to the lower colon. The bladder n. experiments were similar in pattern to earlier pelvic n. experiments, labelling Lissauer's tract, the lateral collateral pathway and the dorsal commissure region of sacral segments 1,2 and 3. Notably missing in the colon nerve experiments was the dorsal commissure terminal field while significant label appeared in Lissauer's tract and the lateral collateral pathway. These data suggest an organization of afferents corresponding to the different reflexes of these organs.

289.10

NEUROPEPTIDES IN DORSAL ROOT GANGLION CELLS INNERVATING THE COLON AND UROGENITAL ORGANS IN THE RAT AND CAT. J.R. Keast, A.W. Yau* and W.C. de Groat. (SPON: B. Dixit) Depts. Pharmacology and Behav. Neurosci., Univ. Pittsburgh, Pittsburgh, PA 15261.

Afferent neurons (AN) which supply three pelvic organs of the male rat and the colon of the cat were identified by injecting retrogradely-transported dyes into the colon, bladder or penis; 3 days to 6 weeks later dorsal root ganglia (L5-S2) were removed, incubated overnight in culture medium containing colchicine, fixed and processed for immunohistochemical localization of substance P (SP), calcitonin gene-related peptide (CGRP), leu-enkephalin (LENK), vasoactive intestinal peptide (VIP) or somatostatin (SS). SP and CGRP were present in many AN supplying these organs and were often colocalized. In rats there were fewer SP AN supplying the penis (15-20%) than the colon or bladder (25-50%). The proportion of VIP AN supplying different organs also varied. In cats, 20-30% of colonic AN contained VIP, whereas in rats $\leq$5% of these AN contained VIP. In rats there were slightly more VIP bladder AN (5-20%) but no VIP penile AN. Many VIP neurons contained SP. Dye-labelled neurons rarely contained LENK and none contained SS. These studies indicate that there are many similarities, but also some differences, in the populations of peptide-containing neurons supplying pelvic viscera. (J.K. is supported by an NH&MRC C.J. Martin Fellowship.)

289.11

SENSORY INPUT FROM CONTRACTIONS OF THE UTERUS IN THE RAT. K.J. Berkley, A. Robbins* and Y. Sato*. Dept. of Psychol., Florida St. Univ., Tallahassee, FL 32306; Dept. of Physiol., Tokyo Metropol. Inst. of Gerontology, Tokyo 173, Japan.

Although both the hypogastric and pelvic nerves contain afferent fibers innervating female reproductive organs, it is unknown how (or whether) information about uterine contractions is conveyed to the CNS. To address this question, the relationship between uterine contractions and activity of afferent fibers in the two nerves was studied in adult virgin anesthetized rats. Although fibers in both nerves readily responded to mechanical stimulation of reproductive organs, activity in the pelvic nerve was never associated with uterine contractions. Such a relationship was observed in about 50% of the cases for hypogastric nerve fibers, but only near the end of the experiment (after the uterus had been subjected to considerable mechanical stimulation). These results suggest that information about uterine contraction is conveyed to the CNS by fibers in the hypogastric, but not the pelvic nerve only under certain unknown conditions of uterine "sensitization."

Supported by NIH grant RO 1 NS 11892 and the Japanese Society for the Promotion of Science.

290.1

DEVELOPMENT OF METHYLMERCURY-INDUCED MOVEMENT AND POSTURAL DISORDERS IN NEONATAL RATS IS CORRELATED WITH ABNORMAL ACTIVITY OF MESENCEPHALIC NEURONS DEMONSTRATED BY CYTOCHROME OXIDASE HISTOCHEMISTRY. R. H. Dyck and J. R. O'Kusky. Dept. of Pathology, Division of Medical Microbiology, and the Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B.C., Canada.

The neurotoxicity of methylmercuric chloride (MeHg, 5 mg/kg/day), administered to neonatal rats beginning on postnatal day (PND) 5, produces movement and postural disorders which appear during the fourth postnatal week. In MeHg-treated animals at the onset of neurological impairment, an increase in the activity of an anomolous population of small neurons within the magnocellular red nucleus and interrubral mesencephalon was demonstrated using cytochrome oxidase histochemistry. Intensely stained neurons were observed beginning PND 17, 4-9 days before onset of the motor impairment. Hemi- or complete decortication on PND 3 or following five days of MeHg administration (PND 10) had no effect on the appearance of this anomolous population, while hemicerebellectomy on PND 10 resulted in fewer neurons within the contralateral rubral area. An increase in the number of intensely stained neurons was observed following thoracic spinal cord transection. The results indicate that the histochemical detection of abnormal neuronal activity precedes the onset of neurological impairment. Furthermore, the movement and postural disorders appear to be mediated via cerebellar but not neocortical afferents to the red nucleus.

290.2

REGIONAL EFFECTS OF DSP-4 TREATMENT ON NOREPINEPHRINE (NE) IN THE NEONATAL RAT. C.J. Gibson and C.C.G. Naus. Depts of Pathology and Anatomy, The University of Western Ontario, London, Ont. N6A 5C1.

The selective noradrenergic neurotoxin, DSP-4, was given to Sprague-Dawley rat pups 12 to 24 hr after birth (50 mg/kg, s.c.). Brain regions were examined neurochemically (for NE) and immunohistochemically (using antiserum to dopamine-β-hydroxylase (DBH)) at varying intervals postnatally. Two weeks after DSP-4 treatment cortical NE level was severely reduced (-94%); hypothalamic NE was unchanged (-9%); and pons-medulla (+40%) and cerebellar NE (+80%) were significantly increased compared to controls. Immunostaining of cortex and cerebellum indicated an initial reduction of DBH positive fibres 1 day after DSP-4 injection which persisted in cortex through postnatal day 15. In cerebellum, DBH staining and NE levels gradually increased to exceed control values from day 9 onward. Thus, the increase of cerebellar NE is due to an increased number of NE terminal fibres (the "pruning effect"; Jonsson, G., Hallman, H. and Sundstrom, E., Neurosci. 7:2895,1982). The effect of NE depletion on the development of intrinsic cortical neurons (e.g. somatostatin) is being investigated.

290.3

6-HYDROXYDOPAMINE LESIONS AND ROTATIONAL BEHAVIOR IN LONG-TAILED MACAQUES Richard H. Schmidt, Mark Dubach, Douglas M. Bowden. Neurological Surgery, Psychiatry and Behavioral Sciences, and Regional Primate Research Center, University of Washington, Seattle WA 98195.

We have devised a protocol of unilateral intranigral 6-OHDA injections in long-tailed macaques, producing degeneration of SN-derived striatal terminals and, consequently, asymmetric behavior measurable by automated rotometer (J Neurosci Meth, in press).

Lesions produced marked (70-85%) chronic rotational behavior, as well as contralateral akinesia and tremor. Contralateral turning that did occur may have been due in part to receptor supersensitivity, surviving innervation, or accumulation of small increments due to postural adjustments. In addition, however, contrary to expectations, the favored direction for two well-lesioned animals was actually toward the contralateral side, keeping the intact (ipsilateral) side near the wall. Similar paradoxical turning has been observed in rodents in related models, under conditions designed to emphasize wall-oriented behavior (Wise and Holmes, Brain Res Bull 16:267; Steiner et al., Exp Neurol 99:556).

Treatments in all monkeys caused massive depletion of ipsilateral striatal tyrosine hydroxylase-like immunoreactive terminals, and correspondingly diminished intensity measured densitometrically. Catecholamine cells in the striatum, on the other hand, had a more extensively ramified neuritic field on the lesioned side, suggesting the occurrence of sprouting (Dubach et al., current volume). (USPHS grants NS25724 and RR00166).

290.4

ASTROCYTE LESIONS IN RAT NEOSTRIATUM. X. Ou*, M.N. Gordon and D.G. Morgan, Dept. of Biological Sciences and Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089-0191.

We have attempted astrocyte (AC) specific lesions in rat brain by coinjecting 2 toxins which are selective for ACs; alpha-aminoadipic acid (alpha-AAA) which is gliotoxic in culture (Huck et al, Neurosci 12:783-791, 1984), and fluorocitrate (FC) which selectively impairs AC energy metabolism (Paulsen et al, J Neurochem 48:1377-1385, 1987).

Male F344 rats were anesthetized with pentobarbital and injected directly into the neostriatum using a 30 gauge needle at 0.2 ul/min. For all injections the FC concentration was 0.8 ug/ul. L-alpha-AAA was varied from 0.048 to 2.4 ug/ul. The contralateral neostriatum was injected with the PBS vehicle to control for nonspecific damage.

All 1 ul injections failed to produce significant AC damage, except immediately around the injection site. A moderate to strong reactive astrocytosis occurred on both sides of the brain, which was slightly stronger on the toxin-injected side 2-7d post-injection. However, 2 ul injections of the highest concentration of L-alpha-AAA produced a complete loss of ACs (assessed by GFAP immunoreactivity) in most of the neostriatum, and the ventrolateral quadrant of the cortex 4 days later. Only a thin strip of ACs were apparent in lamina I of cortex, and these appeared to radiate their processes in toward the astrocyte lesion, instead of towards the glia limitans. Nissl-stains of the AC-free zone revealed some degenerating neurons, many healthy neurons, and a unique arrangement of neuronal profiles around what appeared to be blood vessels. Studies of the time course of this response are in progress. Supported by the John Douglass French Foundation for Alzheimer's Disease, and the Anna Greenwall Award from the AFAR (to DGM).

290.5

INTERACTION OF CADMIUM AND LEAD: EFFECTS ON SCHEDULE-CONTROLLED RESPONDING; DOPAMINERGIC AND SEROTONERGIC NEUROTRANSMISSION. G.D. Frye, J. Nation*, J. Von Stultz*, J. & G. Bratton*. Texas A&M Univ., Depts. of Med. Pharmacol., Psychol. & Vet. Anat., College Station, TX 77843

Operant responding of adult Sprague-Dawley rats was examined after being fed for 60 days either standard rat chow or chow containing lead (Pb, 500 ppm) and/or cadmium (Cd, 100ppm) on a fixed interval 1 min schedule for food. Both metals alone increased total lever presses without changing distribution, however, combined Cd and Pb did not change response rates. After 21 operant test sessions frontal cortex, n. accumbens, striatum, olf. tubercle and brain stem contents of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) were measured. Both metals changed amines, metabolites and/or metab./amine ratios in all areas with most prominent effects after lead alone. However, there was a striking non-additive blunting of lead effects on DA and 5-HT metabolism when Cd was co-administered with Pb. These results suggest that behavioral and biochemical effects of Pb and Cd, when combined, are not simply additive but may be antagonistic. (Supported in part by PHS AA06322, AA00101 & TEES 90470)

290.6

NEUROTOXIC EFFECTS OF ALUMINUM: AN ELECTROPHYSIOLOGICAL STUDY. H. Meiri, M. Roll* and E. Banin*. Deptartment of Physiology, Hebrew University - Hadassah Medical School, Jerusalem, ISRAEL.

While the possible role of aluminum in various dementive disorders is still highly controversial, it's neuropathological influence is well established in experimental animals. In this work the influence of aluminum on neuronal function was examined using electrophysiological techniques and neuroblastoma clone cells, which offer a convenient model of prolifferating, differentiating and fully excitable cells. Two specific questions were addressed: (1) Can differentiated active cells maintain their normal excitable function when exposed to aluminum? (2) Can proper development of excitable properties be achieved in its presence?

We report that aluminum caused premature onset of deterioration in differentiated cells. Within 4 - 6 days they depolarized from the mean value of -29.3 ± 0.9 mV, to levels lower than -15 mV; compound polyphasic action potentials which could be evoked in 53% of control cells were gradually replaced by slow monophasic spikes that preceded the final loss of excitable properties and the onset of morphological deformations. A similar sequence of changes also characterized deteriorating control cultures but at a later time; the average lifespan of control cultures was 20.5 ± 1.4 days as compared to 15.7 ± 1.9 days in cultures exposed to aluminum on day 10. Developing cells followed the normal pattern of differentiation in the presence of aluminum: within 7 days they exteded neurites, hyperpolarized and 40% of the examined cells exhibited polyphasic spikes. However, this highly developed state could not be maintained for long and deterioration soon became the dominant process limiting the lifespan of these cultures to 10.7 ± 2.1 days. Developing cells did however survive more days of aluminum exposure than similarly treated "mature" - differentiated cultures.

These results show that neuroblastoma cells are apparently less susceptible to aluminum's toxicity during the process of development than after differentiation.

290.7

GROWTH OF EMBRIONC DOPAMINERGIC NEURON GRAFTS IN MICE AFTER SEVERE DOPAMINERGIC DAMAGE BY ACETALDEHYDE AND MPTP

A. Zuddas, G.U. Corsini*, I.J. Kopin, J.L. Barker^ and U. di Porzio^. Clinical Neuroscience Branch and ^Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892.

Ethanol and acetaldehyde (ACE) potentiate MPTP toxicity in mice; striatal dopamine (DA) is depleted and loss of dopaminergic neurons in the substantia nigra is extensive (Life Scie. 40:827, 1987; Soc. Neurosci. Abst. 13:71,1987). Here we show that after treatment with MPTP and ACE, but not MPTP and ethanol, striatal MPP^+ levels remain elevated for at least four hours, whereas MPP^+ accumulated in the striatum after treatment with MPTP alone rapidly decreases. No significant differences in MPP^+ content in the whole brain are detectable between the ACE-MPTP and the MPTP treated mice. These data suggest that the potenciating effect of ACE on MPTP is, at least in part, related to the clearance of MPP^+ from the striatum. *In vitro*, neither ethanol nor ACE alter MPP^+ toxicity on DA neurons in mesencephalic primary cell cultures from mouse embryos. Striatal DA content remained at about 7% of controls for several months after this combined treatment. Five weeks after treatment, apomorphine (0.25mg/Kg) induced a marked increase of motor activity in the lesioned animals but not in control animals. Apomorphine-responsive mice and normal controls were injected with $3x10^6$ cells from E13 ventral mesencephalon in the right striatum. Forty days after the implant, amphetamine (2mg/Kg) induced a marked turning, contralateral to the side of the graft. Two months after the implant, immunohistochemical studies showed the presence of clusters of tyrosine hydroxylase positive cells in the right striatum. These data indicate that the combined ACE and MPTP treatment offers a useful animal model to study new therapeutic approaches to parkinsonism.

290.8

BEHAVIORAL DEFICITS IN RATS WITH MINIMAL CORTICAL HYPOPLASIA INDUCED BY METHYLAZOXYMETHANOL (MAM) ACETATE

M. Mercugliano*, S.L. Hyman*, M.L.Batshaw. Dept Pediatr & Kennedy Inst, Johns Hopkins Med Inst, Balto MD 21205

MAM, a short-acting antimitotic agent, produces cortical hypoplasia (45-67%) in fetuses. Offspring also have increased cortical concentrations of biogenic amines, hyperactivity and learning deficits. We studied rats with a lesser degree of MAM-induced cortical hypoplasia to see if these abnormalities persisted. Sprague Dawley pregnant rats were injected i.p. on d.15 gestation with either 25 mg/kg MAM acetate or saline. Total brain weight was reduced by 12% (1.35±.06 vs 1.54±.08, p<.001) and cortical slab weight by 28% (.127±.015 vs .177±.022, p<.001). Experimental animals were more active than controls on total distance (p<.05), average speed (p<.05), average distance (p<.005), and vertical activity (p<.05). There was a trend towards slower learning on a swim maze (p=.05). Experimental animals had increased cortical concentrations of norepinephrine (pmol/mg tissue) (3.95±.56 vs 3.35± .19, p=.01), 5-HIAA (4.68±.60 vs 3.45±.45, p<.001), and glycine (nmol/mg tissue) (1.19±.18 vs 1.04±.04, p<.001). There was no significant difference in serotonin, GABA, aspartic acid, glutamic acid and glutamine. MAM-lesioned animals with mild cortical hypoplasia and elevated norepinephrine remain measurably hyperactive. Further study of subtle lesions may clarify the neurochemical basis of the behavioral deficit.

290.9

THE ULTRASTRUCTURAL LOCALIZATION OF MAOB-ENZYME ACTIVITY ALONG THE NIGROSTRIATAL SYSTEM IN PRIMATE.

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As an extension of the previous study (Nakai et al.Soc.Neurosci.Abst.13:787,1987) which demonstrated a strong monoamine oxidase B (MAOB) activity in the substantia nigra pars reticulata(SNr) and the white matter area dorsal to the substantia nigra(SN),an ultrastructural distribution of MAOB activity was investigated under the electron microscope for further clarification of the neurotoxicity of MPTP. Macaca fascicularis(5-10kg) were transcardially perfused with aldehyde mixture under deep anesthesia with Nembuthal. 50-micron-thick Vibratome sections of the brains including nigrostriatal system were processed for the enzyme histochemistry for MAOB, followed by the osmication for electron microscopy.

Most of the MAOB activities in the white matter dorsal to SN and in the striatum were found only in cell somatas of astroglia. We found two kinds of MAOB activity in SNr. One of them belonged to neuronal structures probably originated from hypothalamic neurons, the other appeared to be within thin structures surrounding the dendritic processes and corresponded to the diffuse staining in SNr under the light microscope. In conclusion, the MAOB activity either in the astroglial processes or in the extracellular space in SNr may be the most important MAOB activity in the pathogenesis of MPTP-induced parkinsonism.

290.10

LOCUS COERULEUS DEVELOPMENT IN RATS FOLLOWING PRENATAL EXPOSURE TO MORPHINE OR CLONIDINE.

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Several lines of evidence provide support for the concept of interactions between endogenous opioid systems and noradrenergic (NE) neuronal systems during growth and maturation of the nervous system. In the present study, drug delivery devices containing morphine sulfate (MS) or the α-2-adrenergic agonist, clonidine (CL), were implanted s.c. in rats on gd 14 to provide continuous delivery of drugs during pregnancy. Observations of litters revealed prenatal MS and CL both produced increased neonatal mortality, neonatal abstinence, transient growth retardation, and decreased nociception in rats up to 4 mo. old. As a first approach in the search for the substrate of these functional alterations, we assessed serial histological sections of the nucleus locus coeruleus (LC) from rats at different stages of postnatal development (5-,10-, or 30-days of age). A glyoxylic acid histofluorescent method was used to assess NA neurons. A marked reduction of histofluorescence in LC neurons and processes was shown for both MS & CL groups during early development. Diminished histofluorescence, albeit slight, was seen in LC, particularly the dorsal portion, of 30 day-old animals. However, the most impressive difference between both MS and CL rats and controls at 30 days was an increase in histofluorescent fibers projecting from the LC, particu-larly the ventral-posterior portion. These projections may represent a reinnervation response following drug-induced inhibition or degeneration which actually progresses to a state of hyperinnervation. Since descending NA axons of supraspinal origin have been suggested to inhibit nociception at the spinal level, hyperinnervation from the response seen in MS and CL animals may be involved in the enduring decreased nociception exhibited by these animals.

290.11

DEVELOPMENTAL NEUROTOXICITY OF ORAL ALUMINUM IN MICE.

M.S. Golub, J.M. Donald*, C.L. Keen*, M.E. Gershwin*. Depts. Internal Medicine and Nutrition, Univ. California, Davis, CA 95616.

Swiss-Webster mice were fed semipurified diets with 25, 500 or 1000 ppm Al as aluminum lactate from conception through weaning (21 days postnatal). Male and female pups were tested for neurobehavioral development [Wahlsten index (Wahlsten, D, Brain Res 72:251,1974), days 8-18, 2 pups/litter] and neurotoxicity [foot splay and NIEHS test battery (Pryor, GT et al, Neurobehav Toxicol Teratol 10:31,1982.), 21-25 days, 4 pups/litter]. Mortality and weight gain of dams and pups were not affected by Al (10 litters /group). Neurobehavioral maturation was unaffected but performance in the screen climb task was impaired in 1000 ppm pups. In weanlings, average foot splay distance was greater in both 1000 (N=28) and 500 (N=22) ppm groups than in 25 ppm controls (N=32) (28.5±0.7, 27.2±0.7, 25.2±0.6 mm respectively, mean±SE). Hind limb grip strength was greater in the 1000 ppm group than in controls (48.5±2.2 vs 37.6±3.1 g) and heat sensitivity was less (tail withdrawal latency 3.9±0.3 vs 3.0±0.2 sec). Negative geotaxis was slower in the 500 ppm group (9.4±1.2 vs 13.7±1.7 sec). Startle responses were unaffected in both treated groups. Thus dietary Al exposure can affect certain neurotoxicity indices during development. Supported by ES04190.

290.12

ETHYLENE OXIDE RETAINED IN HEMODIALYSIS MEMBRANES IS NEUROTOXIC IN VITRO

A. J. Windebank and M. D. Blexrud* Mayo Clinic, Department of Neurology, Rochester, MN 55905

Ethylene oxide (EO) gas is used to sterilize plastic medical equipment including capillary flow dialysis membranes. In order to test whether EO retained in the dialysers might be neurotoxic, tissue culture medium (Eagles Minimal Essential Medium supplemented with 15% calf serum, L-glutamine and 7S NGF) was incubated in the blood compartment of dialysers for periods of 1-12 hours. E15 rat dorsal root ganglion neurons (DRG) were then incubated in this medium for up to five days. During the first 24 hours, neurite outgrowth occurred at a normal rate. During the next 24 hour period, varicosities appeared on distal neurites followed by degeneration of neurites and finally, after four days by death of neuron cell bodies. The pattern of degeneration was identical to that observed when cultures were exposed to an atmosphere containing 1 p.p.m. EO gas. The degeneration produced by incubation in the dialysers was still present after routine prerinsing of the dialysers. The toxic effect was partially abolished by prerinsing with 5 liters of normal saline but was still not completely abolished by a 10 liters rinse. The toxic effect was not seen when identical dialysers sterilized by -irradiation were used.

Since EO is toxic to the human peripheral nervous system, it is proposed that retained EO in dialysers may contribute to the progressive neuropathy observed in patients on long term hemodialysis.

290.13

BEHAVIORAL CHANGES IN OFFSPRING OF RATS EXPOSED TO METHYL MERCURY DURING PREGNANCY. R. Cagiano, M. Colonna, M.A. De Salvia, E. Tortella, Z. Annau* & V. Cuomo (SPON: A. Hyndman). Inst. Pharmacology, Univ. of Bari, 70124 BARI, Italy and *Dept. of Environ. Health Sci., The John Hopkins University, Baltimore, MD 21205, U.S.A.

On day 15 of gestation, pregnant Sprague-Dawley rats were intubated with 8 mg/kg of methyl mercury (MMC) or vehicle. This dose produces no effect on neonatal birthweight or weight gain. Prenatal exposure to MMC did not significantly affect ultrasonic vocalization in rat pups removed from their nest, but open field-hyperactivity elicited by amphetamine (1 mg/kg i.p.) was significantly increased in 14 day old MMC-treated rats. Subjects were also given a passive avoidance task at 60 days. Approach latencies in the first and second trials were not influenced by MMC exposure; however, the avoidance latency of the MMC- pretreated group was significantly shorter than in controls. A comparison of these results with our previous findings (Cuomo et al., 1984; Cagiano et al., 1988) shows that the behavioral effects of a single prenatal dose of MMC depends upon the day of exposure. While the neuronal bases for these differences remain to be determined, our results confirm that prenatal exposure to MMC produces short- and long-term behavioral alterations in the absence of overt signs of neurotoxicity.

290.14

POTENTIAL NEUROTOXICITY OF TRYPTOPHAN: STRIATAL QUINOLINIC ACID MONITORED BY MICRODIALYSIS. M.J. During, A. Freese, M.P. Heyes*, K.J. Swartz, S.P. Markey, J.B. Martin, R.H. Roth. Dept. Pharm., Yale Univ. Sch. Med., New Haven, CT; Lab. Clin. Sci., NIMH, Bethesda, MD; Dept. Neurol., Mass. Gen. Hosp., Boston, MA.

The relationship of the precursor amino acid L-tryptophan to serotonin (5-HT), is well established. Much less is known about an alternative tryptophan metabolic route in brain - the kynurenine pathway. Several intermediates in this pathway are neuroactive, but perhaps of greatest importance is quinolinic acid (QUIN). This dicarboxylic acid is an endogenous ligand of the NMDA receptor, and acts as a potent excitotoxin. The compartment of most relevance to QUIN's neurotoxicity is the brain extracellular fluid (ECF). In rats, intrastriatal microdialysis was used to monitor ECF tryptophan, QUIN, 5-HT, and its major metabolite 5-HIAA, following an acute tryptophan load. At i.p. tryptophan doses ranging from 50 to 250 mg/kg, tryptophan levels in striatal dialysates increased by 4-17 fold; 5-HT by 50-90%; and 5-HIAA by 35-60%. QUIN levels were markedly elevated from 60 nM to 2-14 uM, corresponding to increases ranging between 40 and 230 fold. These results suggest that: (1) The kynurenine pathway in brain appears to be markedly precursor responsive; (2) ECF QUIN reaches potentially neurotoxic levels following a systemic tryptophan load. (3) Pharmacological effects of tryptophan may in part be mediated through intermediates of kynurenine and in particular QUIN.

290.15

FUNCTIONAL EFFECTS OF COCAINE GIVEN DURING CRITICAL PERIODS OF DEVELOPMENT L.A Freed *, T.H Milhorat and D.L. Dow-Edwards (spon. E.Elowitz); Laboratory of Cerebral Metabolism,, State Univeristy of New York, 450 Clarkson Ave, Brooklyn , N.Y 11203

At least one baby each day is born to a cocaine abusing woman in a single large N.Y metropolitan hospital. The affected children show neurobehavioral abnormalities. Direct effects of cocaine on brain development are suggested by recent animal studies. We have found that there are lasting changes in glucose metabolic activity in several brain regions following exposure to cocaine during the first 10 postnatal days in the rat (Dow-Edwards, et al. Dev. Brain Res., in press). We have now identified glucose metabolic changes in brain following late postnatal (days 11-20) exposure to cocaine. These changes are correlated with patterns of dopamine ligand binding in the same animals.

Sprague-Dawley rats were mated in our animal facility and housed under standard laboratory conditions until day of parturition. Litters were culled to 8 and pups assigned to receive cocaine or vehicle injections. The treated groups received 50 mg/kg cocaine sc each day between days 11- 20 and the controls received an equivalent volume of vehicle.

When the animals were 60-65 days of age, local rates of glucose utilization were determined using the deoxyglucose method of Sokoloff et al. (J. Neurochem. 28:897, 1977). Selective tritiated D1 and D2 ligands were used to quantify dopamine receptor concentrations autoradiographically in regions showing altered glucose metabolic activity.

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DIFFERENTIATION AND DEVELOPMENT IV

291.1

HOMOPHILIC BINDING PROPERTIES OF THE NEURAL CELL ADHESION MOLECULE. A.K. Hall and U. Rutishauser* (SPON:R.J. Lasek). Neuroscience Center, Dept. Dev. Genetics & Anatomy, Case Western Reserve Univ. Sch. Med., Cleveland, OH 44106.

The neural cell adhesion molecule (NCAM) is a cell surface glycoprotein that mediates adhesion between cells. Previous studies have shown that purified NCAM can bind to itself in solution as well as when inserted in lipid membranes (Rutishauser et al., 1982. P.N.A.S. 79:685). The question remained as to whether NCAM is also the primary receptor for itself among the full complement of cell surface proteins.

An assay has been developed which allowed the identification of such components in a detergent extract of brain plasma membranes. This assay takes advantage of species-specific anti-NCAM reagents in combination with radio-iodination, such that after chicken and iodinated frog membranes were allowed to coaggregate, and the membranes solubilized, the chicken NCAM could be specifically immuno-precipitated. The iodinated frog proteins which were co-precipitated with chicken NCAM were subjected to one- and two-dimensional electrophoretic analysis in combination with Western immunoblots. Using these procedures, the radiolabelled frog proteins isolated by this assay were identified as NCAM polypeptidesm suggesting that the major cell surface receptor for NCAM is NCAM itself.

291.2

IDENTIFICATION OF AN mRNA ENCODING A SECRETED N-CAM ISOFORM. F.S. Walsh, H.J. Gower*, C.H. Barton*, V.L. Elsom*, J. Thompson*, S.E. Moore* and G. Dickson* Institute of Neurology, Queen Square, London WC1N 3BG, England.

A number of different membrane associated isoforms of the neural cell adhesion molecule (N-CAM) have previously been identified and have been found to be generated by specific alternative splicing pathways. We have identified a novel secreted isoform of N-CAM by analysis of a cDNA corresponding to an N-CAM mRNA from human skeletal muscle. The mRNA incorporates a novel sequence block into the extracellular domain, which introduces an in-frame stop codon and thus prematurely terminates the coding sequence. The polypeptide encoded by this RNA represents a truncated N-CAM form lacking a hydrophobic domain and is incapable of insertion into the plasma membrane. Analysis of genomic clones indicates that the inserted sequence is present as a discrete exon within the human N-CAM gene, and Northern analysis shows it to be associated specifically with a 5.2Kb mRNA species from skeletal muscle and brain. In addition, muscle cells were found to exhibit release of a 115Kd N-CAM isoform into culture media. These data suggest that in addition to mediating cell-cell adhesive events N-CAM may function as a recognition molecule in interactions between the cell and the extracellular matrix.

291.3

EXPRESSION OF MYELIN ASSOCIATED GLYCOPROTEIN (MAG) IN HUMAN NEUROBLASTOMA. J.D. CHAPMAN* AND R.C. McGARRY, Dept. of Paediatrics; University of Calgary, Calgary, Alberta, Canada

While MAG is defined histochemically as being myelin associated, we present evidence here that suggests it is expressed in human neuroblastoma (NB). FACS analysis shows that the monoclonal antibodies Gen S3 and B11F7 recognize the NB cell line SMS-KAN. Both of these antibodies recognize epitopes on the peptide backbone of MAG. As well, HNK-1 and F7F7 which recognize carbohydrate moieties of MAG react with SMS-KAN. Retinoic acid (RA) induced differentiation resulted in decreases in reactivity with GenS3, in contrast to an increased reactivity with HNK-1. These changes correlated with morphological differentiation to a neuron-like phenotype. To correlate peptide expression with mRNA levels we probed SMS-KAN RNA with a cDNA for MAG (clone 6.1, Arquint et al, PNAS, 84:600, 1987). Preliminary results from slot-blots indicate a titratable decrease in cDNA hybridization to RNA isolated from RA treated cells. Thus, we suggest that the MAG peptide is expressed in human NB in vitro and differentiation induction results in a decrease in MAG expression in this cell line. (Supported by the NCI of Canada).

291.4

THE APPEARANCE OF THY-1 IN THE RETINOTECTAL SYSTEM OF THE CHICK. A.M Sheppard*, M. Konopka* and P.L. Jeffrey. Children's Medical Research Foundation, Camperdown, Australia, 2050.

Thy-1 is expressed exclusively by the ganglion cell population of the avian retina. Work from this laboratory has shown that Thy-1 appearance during the latter phases of chick retinal development correlates with the formation of the inner plexiform layer (Sinclair, C.M., Greig, D.I. and Jeffrey, P.L., Dev. Brain Res. 35:43-53, 1987). In the present study we have employed a more sensitive immunohistochemical procedure to investigate the expression of Thy-1 during early retinal development. As this includes the time at which ganglion cell axons grow out to form the retinotectal projections (Rager, G.H., Development of the retinotectal projection in chicken, Springer-Verlag, Berlin, 1980), we also investigated the appearance of Thy-1 in the tectum. Our study demonstrates that Thy-1 is being made by the ganglion cells at or shortly after they cease dividing and begin to differentiate. It is first detected in the optic fibre layer at E6. The expansion of this layer that occurs as more and more axons grow out is reflected by an increase in immunoreactivity to Thy-1. By the time the majority of fibres have left the eye and are projecting to the tectum (about E9) high levels of Thy-1 are observed at the optic disk. As the layer of retinal axons on the tectal surface expands a corresponding increase in immunoreactivity occurs. At E12 three further tectal layers independently express Thy-1. Subsequently, from E14 Thy-1 expression extends across the entire tectum. From our data we speculate that Thy-1 may contribute to the formation of the highly ordered system of retinotectal projections.

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291.5

EXPRESSION OF AN OX-2 HOMOLOGUE, GP39, IN AVIAN NERVOUS SYSTEM. A.M. Cunningham* and P.L. Jeffrey (SPON: J.A.P. Rostas). Children's Medical Research Foundation, Camperdown, Australia 2050.

A group of related molecules including Thy-1, OX-2 and N-CAM, have been found to possess some sequence homology, are members of the immunoglobulin gene superfamily, and are postulated to function in cell recognition or binding interactions. The OX-2 surface antigen was initially identified in rodents and the human gene for the molecule has been characterised.

A panel of monoclonal antibodies against chick neuronal membrane components was produced by immunisation of mice with a glycoprotein enriched fraction from chick brain. A Mab recognising an antigen with an apparent mwt of 39kDa was identified. This glycoprotein, GP39, is abundently distributed throughout the CNS. Developmentally, it is apparent from embryonic day 9 (ED9) in forebrain, cerebellum and tectum, and from ED10 in retina with levels rising to a maximum post-hatch. Immunohistochemical localisation studies from early embryogenesis to maturity reveal a distinctive pattern of distribution on certain neuronal populations and concentration in synaptic rich areas. In addition to surface localisation, some cell types eg Purkinje cells and retinal photoreceptors, show intracytoplasmic accumulation of antigen, presumably at the site of biosynthesis. GP39 is not unique to CNS, being also found at the myoneural junction in skeletal muscle and in adrenal medulla.

The antigen has been purified by monoclonal antibody affinity chromatography and further characterised. Avian GP39 and Thy-1 studied together may provide a means of further defining the role of cell surface interactions in neuronal development.

Supported by the National Health and Medical Research Council of Australia.

291.6

CLONING AND EXPRESSION OF A SOLUBLE 14 kDa ß-GALACTOSIDE BINDING LECTIN IN RAT NERVOUS SYSTEM. M.A. Hynes[1,2], L.B. Buck*[2], F.I. Casano*[2], K.K. Huang*[2], S.H. Barondes[3] and T.M. Jessell[1,2]. Center for Neurobiology[1] and Howard Hughes Medical Institute[2], Columbia University, New York, NY 10032, and Dept. of Psychiatry[3], UCSF, San Francisco, CA 94123.

Protein-glycoconjugate interactions have been suggested to play a role in neural cell recognition. Subsets of dorsal root ganglion (DRG) neurons express lactoseries oligosaccharides on their cell surface. These neurons also express two ß-galactoside binding lectins RL 14.5 and RL 29. To investigate further the role of lectins in neural development we have isolated a cDNA from rat nervous tissue that encodes RL 14.5. Sequence analysis reveals that RL 14.5 shows high homology to 14 kDa ß-galactoside binding lectins isolated from other vertebrate species.

Using RL 14.5 cDNA as a probe in Northern blot analyses, an mRNA of estimated size 0.75 kb was detected at high levels in poly A+ RNA isolated from dorsal root ganglion, lung, and heart in embryonic, postnatal and adult animals. Lower levels of this mRNA were found in spinal cord and brain and this mRNA was barely detectable in liver. By in situ hydridization, in adult animals RL 14.5 mRNA was localized to DRG neurons, and to spinal and brain stem motor neurons. RL 14.5 mRNA was detected in DRG neurons from embryonic day 13 and was expressed in a large proportion of DRG cells in embryonic, newborn and adult animals. These results are consistent with the distribution of RL 14.5 detected previously by immunochemistry. No hybridization to RL 14.5 mRNA was detected in neurons in the dorsal spinal cord or in forebrain structures. Expression of this lectin therefore appears to be highly restricted in the nervous system, and confined to neurons with peripheral projections. Antisera to synthetic peptides corresponding to different regions of the protein predicted by the RL 14.5 cDNA recognize the native lectin and may be useful in blocking carbohydrate binding function.

291.7

A GROWTH ASSOCIATED ANTIGEN IN THE ADULT REGENERATING NERVOUS SYSTEM OF THE COCKROACH. J.L. Denburg. Biology Dept., University of Iowa, Iowa City, IA 52242

Hybridoma techniques have been used to obtain a library of monoclonal antibodies (MAb) that bind to the cockroach embryonic nervous system and not to that of the adult. These MAbs were screened for binding to adult neurons undergoing axonal regeneration as a step towards identifying the role these antigens play in the development of the nervous system. One of the MAbs binds to all embryonic neurons and is absent from all but a small subset of adult neurons. After crushing the nerve containing the axons of motor neurons innervating leg muscles and of sensory neurons in the leg, this antigen reappears in these neurons. The time course of the appearance and disappearance of the antigen during axonal regeneration was determined and indicates a correlation between the presence of the antigen and the occurrence of axon growth. In this respect the antigen is similar to the growth associated proteins detected in vertebrate neurons undergoing axonal regeneration. However, unlike these proteins this antigen is a cell surface glycoprotein. These results support the view that axonal regeneration in adult insects is a good model for events occurring during embryonic development and indicates that studies in the adult nervous system will lead to an understanding of the mechanisms regulating the expression of developmentally important genes.

291.8

LOCALIZATION OF THE S-7B8 ANTIGEN DURING SYNAPTOGENESIS. L.M. Cabalka, T.C. Ritchie, and J.D. Coulter. Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

The S-7B8 antibody binds to a membrane protein in select nerve terminals of the adult rat CNS. The S-7B8 antigen co-localizes extensively, but not exclusively, with nerve terminals thought to use excitatory amino acid transmitters. Previous studies in developing rat spinal cord have shown the S-7B8 antigen to be present in elongating nerve fibers, becoming localized to nerve terminals during synaptogenesis. Present studies demonstrate the distribution of the S-7B8 antigen in several developing pathways in the rat brain. S-7B8 staining in developing fiber tracts and nuclei of the auditory and olfactory systems was found to correlate with their individual timecourse of synaptic development, ie, the S-7B8 antigen becomes localized to the nerve terminals during synaptogenesis. In the dentate gyrus, it has been previously demonstrated that the S-7B8-positive nerve terminals in the outer two thirds of the molecular layer arise exclusively from the superficial layers of the entorhinal cortex. In development, the perforant path fibers grow into the molecular layer during the first postnatal week, while synaptogensis is delayed by 2 to 3 days after arrival of the fibers. By P10, S-7B8 immunoreactivity is localized to nerve terminals in the molecular layer of the dentate gyrus. Studies of these developing systems indicate the S-7B8 antigen is developmentally regulated. Its localization in nerve terminals during development encourages further investigation into the possibility that the antigen may be involved in synaptogenesis. Supported by NIH grant NS23783.

291.9

DIFFERENCES IN MAb 1 A-6 REACTIVITY IN OLFACTORY EPITHELIUM OF NORMAL AND BULBECTOMIZED RATS. V.McM.Carr and A.I. Farbman. Dept. Neurobiol. and Physiol., Northwestern Univ., Evanston, IL 60208.

MAb 1 A-6 has been shown to react with olfactory receptor cell(ORC) surfaces and cilia and supporting cell(SC) surfaces and microvilli in olfactory epithelium(OE) of rat embryos (Neurosci.Abs.13:1410,1987). This reactivity disappears from ORC surfaces but remains on SCs into adulthood. Olfactory nerve(ON) bundles also stain more intensely in E14-E18 embryos than in older animals. To see if re-expression of 1 A-6 reactivity on ORCs is induced by olfactory bulbectomy(OB-X), which causes ORC degeneration and regeneration, adult rats were unilaterally OB-Xed. No re-expression of 1 A-6 reactivity occurred on ORCs 9-17 days after OB-X. However, increased ON staining, as at E14-E18, was seen. Western blots show that 1 A-6 reacts with material having Mr's of 77 and 52kD and 85 and 50kD in OE membranes and cytosol fractions, respectively. Con A is reactive with the 77kD component of the membrane fraction.

These findings suggest differences in glycoprotein expression may occur between initial OE development and the large-scale turnover after OB-X. Such results are especially interesting in light of observations by Brockes et al. (e.g.,TINS 10:364,1987) of differences in antigenic expression in preinnervated or aneurogenic regenerating limbs versus normal regenerating limbs in urodeles.

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291.10

A DEVELOPMENTAL ANTIGEN IN EXTERNAL GRANULAR LAYER OF POSTNATAL MOUSE CEREBELLUM

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We have described a rat monoclonal antibody OZ42 that defines a differentially expressed antigen in sections of early postnatal mouse cerebellum. Staining was observed from postnatal day 3 (P3) to P15 associated with post-mitotic, premigratory granule cells (GC) in the deep external granular layer (EGL), adjacent to the developing molecular layer (ML). There was no staining in the external proliferative zone, ML or internal granular layer. Weaver mutant mice, which have a defect in migration of GC showed differences in expression of the OZ42 antigen consistent with their abnormal morphology.

The OZ42 antigen appears to be specific to developing neural tissue. It was found in neonates (P0-P3) in the GC region of the hippocampus dentate gyrus. The anterior commissure and regions of corpus callosum were stained with a punctate pattern from P0 to P5. Mouse embryo staining was restricted to the nervous system including the CNS especially at the external margins of cellular areas, spinal cord, dorsal root ganglia, cranial nerve bundles and motor and sensory roots adjacent to the developing spinal cord and vertebrae. There was a gradual decrease in intensity of staining of these neural tissues with E10 > E13 > E17.

Preliminary western blot analysis of the antigen has shown that OZ42 specifically recognizes a protein of molecular weight of 120-140kD in P10 cerebellum while a second band of 60-70kD was also found under reducing and non-reducing conditions in P10 cerebellum and cerebrum. The restriction of the antigen to the described regions suggests an association with early axonal elongation. Further biochemical and localization studies are in progress.

Supported by the Weingart Foundation.

291.11

GLYCOPROTEIN BIOSYNTHESIS BY CULTURED GRANULE CELLS
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The effect of development on the oligosaccharide composition of glycoproteins (gp) synthesized by rat cerebellar granule cells in primary tissue culture was investigated. Cultures were incubated with [^{3}H]mannose for 48 hours and oligosaccharides associated with cellular and secreted gp analyzed by con A-affinity and anion-exchange chromatography. The incorporation of [^{3}H]mannose into gp increased 3 to 4-fold between 4 and 10 days in vitro (DIV). Of the total [^{3}H]mannose associated with the oligosaccharides of cellular gp, 36 to 41% bound to Con A at all ages tested between 4 and 14 DIV. In the case of secreted gp con A$^+$ oligosaccharides accounted for 10.3 +/- 0.9% (n=16) of the radiolabel between 4 and 9 DIV, increased to 20.4 +/- 2.1% (n=10) at 10 DIV and then decreased to < 10% by 14 DIV. QAE ion exchange chromatography resolved con A$^-$ oligosaccharides into neutral and negatively charged species containing one, two, three or more sialic acid residues. The relative proportion of the more negatively charged species decreased with increasing time in vitro. These findings indicate that the differentiation of granule cells in vitro is accompanied by changes in the composition of cellular and secreted gp and that a transient increase in the secretion of gp containing con A$^+$ oligosaccharides occurs between 9 and 14 DIV.

Supported by NSERC and MRC.

291.12

A 2-DIMENSIONAL GEL ANALYSIS OF PROTEINS WHICH ARE REGULATED DURING RAT CORTICOGENESIS. D. Geschwind & S. Hockfield, Sect. Neuroanatomy, Yale Med. Sch., New Haven Ct. 06510

At E14 the developing rat cerebral cortex contains predominantly pre-mitotic precursor cells. At E17 and E22, the developing cortex contains large populations of post-mitotic migratory and post-migratory neurons, respectively. By comparing proteins expressed in E14 rat cortical bulbs to those expressed at E17 and E22, we hope to begin to understand some of the molecular events correlated with early cortical developement. To this end, we have run 2D-gels of crude membrane preparations from presumptive E14, E17, and E22 rat cortex labeled *in-vitro* with S^{35} methionine and cysteine. Fluorographs of these gels were then analyzed using a computerized gel scanner.

Gels resolving proteins with molecular weights between 20-240kD and isoelectric points between pH 4 and pH 7, and exposed for approximately 4 x 10^6 cpm x days yielded between 300 to 400 spots corresponding to individual proteins. Most of these proteins showed no significant changes between E14 and E22. We have indentified 15 proteins whose rate of synthesis changes more than three fold from E14 to E22. These can be divided into three major groups: 1.Those that increase between E14 and E22 (n=6). 2.Those that decrease between E14 and E22(n=6). 3.Those that appear *de-novo* between E14 and E22(n=3). The most striking of these age-related changes are three spots which are not present at E14 and appear at E17, growing even more abundant by E22 (MW 29-30kD, pI 5.1-4.6). Judging by the time course of their appearance, they are most probably neural, rather than glial in origin. Also intriguing are 6 proteins that are down-regulated from 3 to 17 fold from E14 to E22. The molecular weight and pI of these molecules suggest that they are previously uncharacterized developmentally regulated neuronal molecules.

291.13

REGULATION OF TISSUE PLASMINOGEN ACTIVATOR IN RAT SYMPATHETIC AND SENSORY NEURONS. A. Repka*, Z. Gajary* (SPON: E. Geller), and R. N. Pittman, Dept. of Pharmacology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

Cultured rat sympathetic and sensory neurons contain tissue plasminogen activator (t-PA) and have a high affinity, high capacity binding site for t-PA on their surface. t-PA has been shown to be an early differentiation marker for the neuronal phenotype in P19S1801A1 embryonic carcinoma cells (Whitford and Levine, Soc. Neurosci. Abstr., 13(pt. 2):892, 1987. In order to investigate the role of tPA in the differentiation of sympathetic and sensory neurons, we have generated monoclonal antibodies (mAbs) and a candidate cDNA for rat t-PA. The cDNA was isolated from a rat heart lambda gt 11 library and has a restriction map similar to that of human t-PA. Characterization of this cDNA is currently underway. Initial studies with mAbs indicate that t-PA immunoreactivity is regulated by tissue culture conditions and length of time in culture. These observations suggest that neuronal t-PA may be under dynamic control by local environmental factors.

Supported by grants from the NIH (NS 22663) and McKnight Foundation.

291.14

SUBCELLULAR LOCALIZATION OF mRNAs IN DROSOPHILA RETINA REVEALED BY LIGHT AND ELECTRON MICROSCOPIC IN SITU HYBRIDIZATION J.A. Pollock, S. Benzer, T. Deerinck*, M.H. Ellisman. Div. Biol. 156-29, Cal.Inst.of Technol.; Dept. Neurosci., Lab. Neurocytol., UCSD.

The subcellular distribution of the mRNAs may be a mechanism for developmental control. We have studied the distribution of the mRNA transcripts for the Drosophila visual system genes, sevenless, chaoptic and ninaE (opsin Rh1) utilizing light microscopic (LM) autoradiographic in situ hybridization for the localization of endogenous mRNAs. Information obtained with LM indicating a differential distribution of mRNAs in both developing and mature photoreceptors stimulated us to develop a new, higher resolution procedure for in situ hybridization. This technique involves mild aldehyde fixation of dissected tissue, sucrose cryo-protection, and cryo-sectioning on an ultramicrotome. Probe DNA labeled by nick-translation with biotinylated-nucleotides is size-selected for optimal probe length. Hybridization and detection is performed on the sectioned tissue. The tissue is then post-treated for EM visualization in a manner similar to immuno-labeling. High voltage electron microscopy (1MeV) enables the use of thicker sections, 0.5-1.0 μm, which provide for greater stability through the subsequent steps of immunodetection and colloidal gold labeling. Using this technique we have obtained evidence that supports the LM observation of a non-uniform distribution of sevenless mRNA in the developing eye imaginal discs, and of the chaoptic mRNA in normal and mutant adult compound eyes. The abundant opsin transcript was also studied as a control in compound eye photoreceptor cells. This technique should aid further investigations into the mechanisms for control of cell polarity that may involve non-uniform distributions of mRNAs.

291.15

DEVELOPMENT OF A PERIPHERAL NERVE DURING METAMORPHOSIS IN DROSOPHILA: PHYLOGENETIC PARALLELS AMONG DIPTERA-MECOPTERA SPECIES. W.J. Costello, Dept. Zool., and Biomed. Sci./College of Osteopathic Med., Ohio University, Athens, Ohio 45701.

The nervous system of a holometabolous insect must undergo profound changes during transition from larva to adult. For a flying insect, these changes encompass modification to motor systems during metamorphosis to convert from the larval crawling form to the adult flying form.

I have conducted a scanning electron microscope study of the pupal nervous system of Drosophila melanogaster as it changes to the adult form. In the larva and prepupa, there is a major peripheral nerve exiting each side of the pre-, meso-, and metathoracic segments. These nerves are, respectively, T1, T2, and T3. T2 is modified during the mid-pupal stage to become two major adult nerves, ADMN and PDMN, providing innervation to the indirect flight muscles (DVM & DLM).

This singular modification of T2 during development has intriguing phylogenetic parallels among dipteran-mecopteran species. A survey of peripheral nerve innervation to the indirect flight muscles shows there are three major variants: 1) one nerve exits theganglion to bifurcate peripherally, providing innervation to DVM and DLM [mecoptera, Calliphoridae], 2) two major nerves, distinctly separate, exit the ganglion, one nerve innervating DVM, the other DLM [Drosophilidae, Muscidae,Sarcophagidae], 3) two nerves exit the ganglion closely together, almost joined [Syrphidae, Tephritidae]. All these variants, though distinct for the species, apparently represent developmental variants which can occur with T2 during metamorphosis. Such developmental changes provide possible restraints on evolutionary changes responding to selection pressures. (Supported by funds from OU-COM and an MBL Fellowship).

291.16

AUTONOMOUS DEFECTS AND FLIGHT MUSCLE CELL LINEAGE IN HEAT PULSED DROSOPHILA MOSAICS WITH TS-SHIBIRE TISSUE. M.R. Hummon and W.J. Costello, Dept. Zool. and Biomed. Sci./ College Osteopathic Med., Ohio Univ., Athens, OH 45701.

Drosophila adults with the ts mutation shibire (shi) exhibit a variety of predictable anomalies following pupal heat pulse (HP: 6h at 30C). The shi defect, revealed at 30C, involves membrane recycling and apparently includes blockage of endocytosis. Adults after HP< 20h pupal development at 25C lack all head and thoracic chaetae, have fused and/or deleted flight muscles (DLM; DVM), and an abnormal thoracic neural structure (neuroma). Using w^{ts} ring-X females and marked shi males ($y\ w\ sn^3$ shi), we are generating shi mosaic pupae and exposing them to HP< 20h. About 4% of F_1 are bilateral thoracic mosaics (30% of all gynanders). After HP, shi cuticle of head and thorax lacks all chaetae; chaetae in w^{ts} tissue are wild-type (WT). Phenotype of the thoracic ganglion matches the ventral cuticle and leg phenotype (behavioral test at 30C, leg paralysis indicating shi). However, phenotype of the dorsal thoracic cuticle (chaetae vs. bare) does not match the phenotype of flight muscles. In WT, non-HP shi, or w^{ts} flies, DLM has 6 fibers, while DVM has 7 fibers arranged in 3 sets; in shi after HP< 20h, DLM has 0-3 fibers and DVM has 0-5 fibers. Of 11 HP flies with bilateral mosaic dorsal thorax, 3 had a completely WT ganglion and flight muscles. Of 8 flies with bilateral ganglion: all muscles were WT (n=1), all muscles were shi (n=2), or both muscle phenotypes occurred, matching the cuticle in 4 of 5 flies. On the same side, DLM fibers were always of the same phenotype (n=16), whereas DVM I could differ from DVM II and III phenotype (n=3). Phenotypes of DLM and of DVM could differ as well (n=4). Thus shi anomalies induced by pupal HP are autonomously expressed in shi tissue, and have no effect on neighboring WT tissues. The patterns of flight muscle phenotype indicate that cell lineages for DLM and DVM are separate in normal development as revealed here by the rela tively late exposure of the shi defect and phenotype with pupal HP. Interestingly, lineage between DVM I and DVM II-III could also be developmentally separate. Supported by NIH-NRSA (MRH) and research funds of OU-COM (WJC).

291.17

EXPRESSION OF A STEROID HORMONE-REGULATED GENE IN THE CNS OF DROSOPHILA MELANOGASTER. L. L. Restifo and K. White*, Department of Biology, Brandeis University, Waltham, MA 02254.

In the nervous systems of both vertebrates and invertebrates, steroid hormones mediate neurogenesis, cell death, and changes in neuronal structure. We are currently investigating steroid hormone-regulated gene expression in the Drosophila CNS during metamorphosis. Others have shown that the steroid molting hormone, ecdysterone, regulates transcription of the X-linked complex genetic locus, the Broad-Complex (BR-C). Genetic evidence indicates that this locus is essential for metamorphosis, both for destruction of larval tissues and for differentiation of the adult epidermis. Furthermore, the BR-C is known to modulate the activity of a number of other ecdysterone-regulated genes at the onset of metamorphosis.

A cDNA clone (provided by G. Guild, U. of Pa.), representing portions of several variably spliced, hormone-inducible transcripts from the BR-C, was used to probe tissue sections of late third instar larvae. Following in situ hybridization, autoradiography reveals transcripts in cells of the larval brain and ventral ganglion. Transcripts are detected in neuronal precursors (neuroblasts) and in the large cells at the cortical-neuropil junction believed to be glia, as well as in mature neurons. In situ hybridization to adult heads indicates that transcription from the BR-C also occurs throughout the mature CNS.

We have begun a neuroanatomical analysis of BR-C mutants to identify aspects of CNS development which are influenced by this locus. In mutants lacking BR-C function entirely, axon bundles of the embryonic CNS appear normal by immunostaining using anti-horseradish peroxidase antibodies. In histologic sections, the larval CNS appears normal at the end of the third instar. However, some mutants possessing partial BR-C function die during metamorphosis and exhibit abnormalies in CNS morphology. In particular, the separation between the subesophageal ganglion and thoracic ganglion occurs only partially, if at all. On the basis of these data, we postulate that BR-C function is not essential for morphogenesis of the embryonic and larval CNS, but is required for proper CNS reorganization during metamorphosis. (Funded by NIH grant NS01259-01 and an MDA postdoctoral fellowship.)

291.18

THYROXINE-INDUCED DEVELOPMENT OF THE OPTIC TECTUM IN LARVAL BULLFROG TADPOLES. N.J. Uray. Dept. of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501

As part of a larger study which explores the effect of thyroxine on brain development during early stages of differentiation and development, the optic tectum of the larval tadpole was examined using routine light microscopic methods. Bullfrog (Rana catesbeiana) tadpoles were raised from eggs and their ages were recorded using the time they swam free of their jelly-coat as a reference point. At various time periods ranging from 1 week to 1 year of age during which time the tadpoles are within the premetamorphic stage, groups of tadpoles were treated with thyroxine (Sigma, L-thyroxine sodium pentahydrate) by immersion for 2-4 weeks at the concentration of 1:100 M. After treatment the tadpoles were anesthetized, fixed and their brains were dissected out. Slides prepared in the three standard planes were stained with H and E and were examined.

Our findings emphasize development during the first half year period, during which time the optic tectum is capable of only partial development following thyroxine treatment. In general, 3 or 4 week treatment results in greater degrees of differentiation than 2 week treatment, and the antero-lateral half of the tectum is better formed than the postero-medial half. The fibrous layers of the tectum are consistently lagging behind the generation of the cellular layers, especially in the posteromedial part of the tectum. Supported by KCOM Warner Fund.

291.19

DEVELOPMENT OF NUCLEUS ISTHMI IN LARVAL STAGES OF BULLFROG TADPOLES. L.C. Towns and N.J. Uray. Dept. of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501

The development of Rana catesbeiana is extremely slow, usually lasting 2-3 years. This study examines the early, partial development of nucleus isthmi in this species.

Rana catesbeiana tadpoles were identified by age in weeks after emerging from eggshells (W) and by the stages of Shumway (S) or Taylor and Kollros (T-K). Ten groups of 20 tadpoles were used; each animal was given three intraperitoneal injections of [3H]-thymidine (10μCi/g per injection) on alternate days. Thus, the injections provided for 6 days of [3H]-thymidine availability. Each group of tadpoles was labeled at developmental periods of either W2+3(S25), W6,W8, W10(T-K I), W12(T-K II), W18, W20, W22(T-K IV) or W26(T-K V). All tadpoles were harvested at age W27(T-K V), and the brains were processed by routine autoradiographic methods. Sections were exposed at 4° C for 6 weeks, developed and lightly stained with H and E.

The autoradiographic data indicate there is some labeling during the entire six-month period. However, the rate of cell division does not appear to be uniform; there are some periods when many cells are labeled while only a few cells are labeled at other times. A spatial pattern of labeling at various ages is also seen.

292.1

AMPHETAMINE SENSITIZATION OF TRANSPLANTED DOPAMINE NEURONS ENHANCES STRESS RESPONSE.
A.M. Snyder-Keller and R.D. Lund. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Three-day old rat pups were given bilateral, intraventricular injections of 6-HDA to destroy dopaminergic nigrostriatal afferents and three days later received a unilateral intrastriatal injection of a cell suspension derived from embryonic ventral mesencephalon (E14). When tested repeatedly during development with both amphetamine (AMPH) and stress, these animals turned in response to AMPH by 15 days post-transplantation and turned to stress at 25 days (Carder, et al., 1987). Here we examined the influence of prior exposure, at 5-day intervals, to AMPH and/or stress on the emergence of stress-induced turning. Animals with prior exposure to AMPH (2 mg/kg, i.p.) and tail pinch, or AMPH alone, developed turning in response to tail pinch by 25 days post-transplantation. None of the animals exposed to tail pinch alone during development turned to stress by this time; however, these same rats did turn to stress after exposure to AMPH. Similarly, animals receiving no stimuli until 30 days post-transplantation rarely turned to stress on the first trial, but did so 5 days after being exposed to AMPH. Thus AMPH appears to have a sensitizing influence on transplanted dopamine neurons: early exposure to AMPH somehow primes the system so that turning to stress can develop by day 25 and later exposure subsequently enhances stress-induced turning. (Supported by USPHS grant NS19608.)

292.2

STRESS-INDUCED RELEASE OF DOPAMINE FROM MESENCEPHALIC TRANSPLANTS IN STRIATUM MONITORED BY MICRODIALYSIS.
R.W. Keller, Jr., A.M. Snyder-Keller, R.D. Lund, and M.J. Zigmond. Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA

Neonatal rats given extensive, bilateral striatal dopamine (DA) depletions and unilateral mesencephalic transplants subsequently turn contralaterally in response to stress and amphetamine (Carder, et al. 1987). We sought to determine whether stress-induced turning was linked to release of dopamine from the transplant. Two months after transplantation, rats were implanted with a chronic loop-style dialysis probe and perfused at 1 ul/min with artificial CSF. Superfusate was collected in 15 min fractions and analyzed by HPLC. Subsequently, the proximity of the probe to the transplant was determined histochemically. Tail pinch (3 sec every 5 min for 30 min) produced contralateral rotation (3-5 turns/min). When probes were positioned adjacent to a transplant, extracellular DA levels rose 15-30 min after the onset of stimulation, peaked within the first post-stimulation fraction, and returned to baseline by 1 hr post stimulation. Delayed increases in DOPAC and HVA also occurred. Elevated levels of DA and its metabolites were observed after administration of amphetamine (2 mg/kg) and during the subsequent period of rotation. Neither neurochemical nor behavioral responses were observed in the absence of transplanted DA neurons. These results suggest that transplants can be influenced by the host tissue environment. (Supported in part by USPHS grant NS19608.)

292.3

STRIATAL ACTIVITY OF ADULT RATS RECEIVING DA DEPLETING LESIONS AND MESENCEPHALIC TRANSLANTS AS NEONATES.
JP Sidney, A Snyder-Keller & TW Berger, Dept. Behavioral Neuroscience, Univ. Pittsburgh, Pgh., PA 15260.

When DA-depleting brain lesions are given to neonatal rats, the spontaneous activity of adult striatal neurons is increased above control levels (Onn et al., Soc. Neurosci. Abstr., 1987). Here we investigated the possibility that transplants of mesencephalic neurons could restore striatal firing rates to control levels. Intraventricular injections of 6-hydroxydopamine (6-HDA) were given at day 3, and at day 6 rat fetal mesencephalic tissue was injected unilaterally into striatum. Animals exhibited tail pinch and amphetamine-induced contralateral turning. At 4 mo, extracellular single unit activity was recorded throughout the transplanted region of the striatum. Striatal cells were identified by spike waveform and orthodromic response to electrical stimulation of cortical afferents. Striatal cells recorded from animals with transplants exhibited lower spontaneous firing rates than animals only injected with 6-HDA on day 3. In addition, neurons often were encountered that did not respond to cortical stimulation and that exhibited spike waveforms and very high spontaneous firing rates atypical of animals not receiving transplants. Some of these putative transplant neurons displayed characteristics similar to those described for DA-containing pars compacta neurons. TH immunocytochemistry revealed transplanted DA cells and substantial innervation of the striatum. (Supported by NS19608 and MH00343).

292.4

FEEDBACK REGULATION OF DOPAMINE METABOLISM IN FETAL SUBSTANTIA NIGRA TRANSPLANTS.
R. Meloni* & K. Gale (SPON: J.A. McConnell). Dept. of Pharmacology, Georgetown Univ. Medical Center, Washington, D.C. 20007.

We previously reported that transplants of fetal substantia nigra respond to blockade of dopamine (DA) receptors with haloperidol (HAL), by increasing DA metabolism within the transplant itself as well as in its terminals in the adjacent regions of the adult host striatum. The present study examined the response of the transplants to the DA receptor agonist apomorphine (APO).

Solid transplants of fetal ventral mesencephalon were placed in cortical cavities overlying the 6-hydroxydopamine denervated (less than 1% DA remaining) striatum. Only those rats in which the transplant resulted in at least 50% reduction in amphetamine-induced rotational behavior, were used for the studies of DA metabolism; this consisted of over 75% of the rats with transplants. At least 3 months after transplantation, rats were given APO (1 mg/kg sc) and sacrificed at 40 min for measurement of DA and its metabolites (DOPAC and HVA). APO treatment induced a significant decrease in the ratio of DOPAC/DA (60% decrease) and HVA/DA (75% decrease) in the transplant terminals in the host striatum; only a small decrease occurred in the transplant itself. This was in contrast to the action of HAL, which caused a somewhat greater change in the transplant itself, as compared with its terminals. These results indicate that feedback mechanisms regulating DA utilization within the graft may be differentiated from those regulating DA utilization in the graft terminals innervating the host striatum.

Supported by a grant from the United Parkinson Foundation and an RSDA (K.G.) MH 00497.

292.5

APPARENT SUPERSENSITIVITY TO AMPHETAMINE OF GRAFTED FOETAL NIGRAL NEURONS IN THE RAT.
L. Rioux, P.J. Bédard, L. Grégoire,* T. Di Paolo, M. Daigle* and C. Rouillard. Lab. Neurobiol., Hôp. Enfant-Jésus and Dép. d'endocrinol. mol., Centre Hosp. de l'Univ. Laval, Québec, Canada.

20 female rats (250 g) were lesioned with 6-OHDA 12 µg in the left substantia nigra. At least one month later they were tested with amphetamine 5 mg/kg s.c. and apomorphine 0.25 mg/kg s.c. A suspension containing approximately 1.5×10^6 cells from the ventral mesencephalon of rat embryos was distributed in three sites in a triangular fashion in the center of the denervated striatum. The amphetamine test was then repeated every month for 6 months. The pattern of circling to amphetamine before the graft was strictly ipsiversive in all animals. From the first month we observed a progressive change and three patterns of evolution could be observed. In eight animals, the total number of ipsiversive turns in 90 minutes actually increased but during the first twenty minutes the animals turned contralaterally to the lesion (and the graft). In seven rats, the total number of turns switched from ipsiversive to contraversive with the animals turning initially towards the intact side and during the second half of the test towards the lesion. Finally four rats progressively switched to turning only towards the intact side. In all cases, maximal contraversive turning occurred during the initial twenty minutes and indeed in the first five minutes. Thus it would appear that especially during the first minutes after amphetamine, the dopaminergic stimulation on the lesioned side is stronger and occurs more rapidly than on the intact side. This could be explained by faster and greater release of dopamine by the grafted neurons, by supersensitivity of the dopaminergic receptors or by the position of the graft in a strategic area for contraversive circling. Apomorphine, 0.25 mg/kg given during the late (4-6 months) period still elicited contraversive circling but the number of turns was reduced. In one grafted rat, microdialysis was performed bilaterally in the striatum. Dopamine was detected before amphetamine only on the intact side. After amphetamine 30 pg/40 µl were detected on the grafted side and this represented one tenth of the level on the intact side. The response to amphetamine appeared on both intact and grafted sides in the first twenty minutes of perfusion. In this rat, tyrosine hydroxylase-positive cells were detected mainly in the dorsal striatum with a few in the central portion. (Supported by MRC of Canada.

292.6

GRAFTING OF GENETICALLY ENGINEERED FIBROBLASTS WHICH PRODUCE L-DOPA IN A RAT MODEL OF PARKINSON'S.
J.A. Wolff, L. Xu, T. Friedmann, M.B. Rosenberg, M.P. Iuvone[3], K. O'Mallery, L. J. Fisher, S. Shimohama[2], and F.H. Gage.[2] (SPON: P.M. Salvaterra). Dept. of Pediatrics (M-009), [2]Dept. of Neurosciences (M-024), UCSD, La Jolla, CA 92093, and [3]Dept. of Pharmacology, Emory University, Atlanta, GA 30322.

Previous studies have shown that rat fibroblasts can be genetically modified in vitro to express an exogenous reporter gene when implanted into the parenchyma of a rat brain. This study was undertaken to determine if this new mode of gene transfer can significantly ameliorate the signs of disease in an animal model of a brain disorder. Rats unilaterally damaged in the substantia nigra serve as an animal model of Parkinson's disease. Fibroblasts genetically engineered to produce L-DOPA should improve the signs of disease in these rats. 208F rat fibroblasts were infected with a retroviral vector carrying the rat gene for tyrosine hydroxylase (TH), which converts tyrosine into L-DOPA. Cell extracts of 208F cells infected with the TH vector had significant levels of TH activity (10.8 pmoles DOPA/min/mg protein) and L-DOPA (1.3 ng/mg protein). These 208F cells expressing the TH gene in vitro were implanted into the caudate of animals with unilateral 6-OHDA injections in the nigrostriatal bundle. In vivo TH expression in these cells transplanted into the caudate was found by immunocytochemical techniques. Studies are in progress to determine whether these cells can reverse the rotational asymmetry induced by unilateral dopamine depletion.

292.7

EXTRACELLULAR CHARACTERIZATION OF HRP-LABELED NEURONS IN DOPAMINE-RICH SUSPENSION GRAFTS TO THE RAT NEOSTRIATUM. L.J. Fisher, S.J. Young*, P.M. Groves, and F.H. Gage. Depts. of Psychiatry and Neuroscience, U.C.S.D., La Jolla, 92093.

We employed extracellular stimulation and recording techniques to characterize physiological properties of neurons in suspension grafts to the striatum. HRP intracellular labelling was combined with tyrosine hydoxylase (TH) immunocytochemistry to identify the location of these cells in relation to catecholamine containing neurons.

Adult rats with a unilateral lesion of the nigrostriatal dopamine pathway were injected with a suspension of fetal mesencephalon into the denervated striatum. Electrophysiological testing was performed after rats no longer displayed amphetamine induced rotational behavior. Recordings were obtained from urethane anesthetized rats using HRP filled microelectrodes. Bipolar stimulating electrodes, placed in striatum surrounding the grafts and in cortical white matter, were used to evoke activity from grafted cells.

Spontaneously active HRP labeled neurons with wide triphasic waveforms and slow regular firing rates, characteristic of pars compacta dopamine neurons, were localized in the grafts. Orthodromic responses were elicited with cortical stimulation in all of these neurons; in one case an antidromic response to striatal stimulation was observed. These cells were found within TH positive neuron clusters with some indication of TH and HRP double labeling. Both regular and deactivating burst firing patterns were observed in unlabeled neurons with characteristic dopamine waveforms. Other labeled neurons, characterized by faster firing rates, were found outside TH positive clusters but within the graft. Grafted neurons were readily distinquished on the basis of waveform and firing pattern from typical striatal neurons labeled outside the graft.

292.8

ADRENAL MEDULLARY AUTOGRAFTS IN NONHUMAN PRIMATES: FINE STRUCTURE OF THE IMPLANT SITE. J.T. Hansen, J.H. Kordower, M.S. Fiandaca, S. Okawara, S.S. Jiao*, and D.M. Gash. Dept. of Neurobiology and Anatomy, and Division of Neurosurgery, Univ. of Rochester Medical Center, Rochester, New York 14642, and *Capital Institute of Medicine, Beijing, China.

In light of the recent use of adrenal medullary autografts for the treatment of human parkinsonism, we have examined the fate of autografted chromaffin cells placed in the caudate nucleus of Cebus monkeys (*Cebus apella*).

Thirty days post-transplantation, the implant site exhibited massive liquefactive degeneration and macrophage infiltration. Fine structure analysis confirmed the presence of extensive macrophage invasion, cellular phagocytosis, and hematogenous cell infiltration. Surviving chromaffin cells, displaying their characteristic cytoplasmic vesicles and a distinct basal lamina, were observed in only two of seven implanted monkeys. In all cases, the chromaffin cells showed overt signs of autolysis and were in close proximity to macrophages. Profiles of portions of degenerating chromaffin cells were seen undergoing phagocytosis, and the interstitium was replete with collagen and cellular debris. Capillaries exhibited a non-fenestrated endothelium and plasma cells commonly were observed throughout the implant site. Overall, the implant site resembled a focal CNS injury with all of the characteristic signs of inflamation and cellular phagocytosis. Chromaffin cell survival was minimal, despite the transplantation of over 200,000 cells into each recipient.

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292.9

ADRENAL MEDULLARY AUTOGRAFTS IN NONHUMAN PRIMATES: REGENERATION AND SPROUTING OF HOST DOPAMINERGIC SYSTEMS. D.M. Gash, J.H. Kordower, M.S. Fiandaca, S.H. Okawara, S.S. Jiao*, M.F.D. Notter, and J.T. Hansen. Dept. of Neurobiology and Anatomy, and Division of Neurosurgery, Univ. of Rochester Medical Center, Rochester, New York 14642, and *Capital Institute of Medicine, Beijing, China.

Twelve Cebus monkeys (*Cebus apella*) have received unilateral intrastriatal adrenal medullary autografts using an open microsurgical approach (n=3), a stereotactic injection technique (n=3), and a stereotactic implantation using a silver tissue carrier (n=6). Upon sacrifice, one month following transplantation, the animals' brains were evaluated via tyrosine hydroxylase immunocytochemistry, electron microscopy, and routine histochemical methods. In both MPTP-treated and normal monkeys, minimal viable adrenal medullary tissue was found in the graft sites one month following the transplant procedure. Macrophages were the primary cells seen in the region of the graft. Despite minimal survival of adrenal medullary cells, we have found dramatic increases in the number of tyrosine hydroxylase immunoreactive fibers in the median forebrain bundle, the nigrostriatal pathway, and the striatum on the grafted side.

There are two major hypotheses for explaining why adrenal medullary grafts may promote functional recovery in human parkinsonism: 1) replacement of lost striatal neurotransmitter (dopamine) by the grafted tissue, or 2) induction of recovery, by the graft, of remaining host catecholaminergic systems. Our data supports the latter theory.

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292.10

ADRENAL MEDULLARY AND PERIPHERAL NERVE GRAFTS AND COGRAFTS IN THE RHESUS HEMIPARKINSONIAN MODEL: ANATOMICAL AND BEHAVIORAL RESULTS. M.S. Fiandaca, J.H. Kordower, J.T. Hansen, M.F.D. Notter, M.D. Kaniucki, J. Snyder, and D.M. Gash. Department of Neurobiology and Anatomy,University of Rochester Medical Center, Rochester, New York, 14642.

The mechanism(s) behind the amelioration of experimental parkinsonism via striatal grafts in primates and humans remains elusive. There is clear evidence, however, that adrenal chromaffin grafts survived poorly and local release of dopamine by implanted chromaffin cells is probably not mediating behavioral improvement. In an effort to delineate the anatomical and behavioral consequences of adrenal medullary and peripheral nerve grafts and cografts, we investigated their effects in the rhesus MPTP hemiparkinsonian model.

Eleven aged rhesus monkeys (Macaca mulatta) were evaluated regarding an upper extremity fine-motor task and rotation in response to intramuscular apomorphine (0.15 mg/kg) before and after unilateral intracarotid MPTP (0.4 mg/kg). One month later, the animals in the experimental groups received stereotaxic striatal grafts ipsilateral to the MPTP infusion. The grafts consisted of either pieces adrenal medulla, pieces of sural nerve, or cografts of both tissues. Control animals received a stereotaxic injection of vehicle only, following adrenalectomy and sural nerve resection. Results will be discussed regarding the behavioral findings (fine-motor task and apomorphine-induced rotation) during the three months following transplantation as well as the anatomical evaluations of the graft sites and surrounding brain regions at the light and electron microscopic levels.

292.11

ADRENAL MEDULLARY AUTOGRAFTS IN NON-HUMAN PRIMATES: EFFECT OF COCULTURE OF CHROMAFFIN CELLS WITH SURAL NERVE EXPLANTS IN VITRO. M.F.D. Notter, M.S. Fiandaca, J.H. Kordower, and D.M. Gash. Dept. of Neurobiology and Anatomy, Univ. of Rochester, Rochester, New York 14642.

In the present study, the importance of non-neural cells obtained from peripheral nerves as a source of trophic factors for medullary cells was investigated. Adult monkey sural nerve obtained from biopsy was placed in explant culture on collagen-coated coverslips. Suspensions of chromaffin cells obtained from the same monkey and enriched by an 18 hour differential plating were seeded onto coverslips with or without explants. Successful explants exhibted outgrowth of fibroblasts and Schwann cells determined by fibronectin or S100 protein staining, respectively. After one week of coculture, chromaffin cells showed tyrosine hydroxylase positive, catecholamine histofluorescent neuritic extensions growing over the surface of the explanted monolayer. Neuritic processes were similar to those induced by treatment of chromaffin cell cultures with NGF (100 ng/ml) alone for the same length of time. Moreover, the presence of chromaffin cells themselves or the conditioned medium from their culture stimulated outgrowth from sural nerve explants reliably and at a faster rate than that seen with cultures of sural nerve alone. These data indicate that factors released from non-neuronal peripheral nerve cultures induce and maintain the neuronal phenotype of chromaffin cells in vitro while, in turn, factors released from chromaffin cells stimulate the growth of these non-neuronal peripheral cells. Furthermore, these data suggest that adrenal medullary cell survival could be improved in neural transplants by cografting with peripheral nerve.

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292.12

COGRAFTS OF ADRENAL MEDULLA WITH PERIPHERAL NERVE IN THE DOPAMINE-DENERVATED RAT STRIATUM. G. Bing, S. Jiao*, M.F.D. Notter, J.T. Hansen and D.M. Gash. Dept. of Neurobiology and Anatomy, University of Rochester, School of Medicine, Rochester, New York 14642, and *Capital Institute of Medicine, Beijing, China.

Adrenal chromaffin tissue from young, 100-125 g, rats was transplanted into the striatum of adult (200-225 g) rat recipients whose substantia nigra were pre-lesioned on the grafted side with 6-OHDA. Functional effects of cografts of adrenal chromaffin cells with sciatic nerve, adrenal chromaffin tissue alone, sciatic nerve alone, optic nerve and culture medium were studied using rotational behavior induced by amphetamine. Immunohistochemistry using antibodies against tyrosine hydroxylase (TH), nerve growth factor receptor (NGFR), glial fibrillary acidic protein (GFAP) and S 100 protein was used to identify and characterize the grafted tissues. A few adrenal medullary cells survived in the group receiving just adrenal chromaffin tissue. However, the number of surviving chromaffin cells was significantly increased by cografting with sciatic nerve. Cografting with sciatic nerve also induced transformation of adrenal chromaffin cells towards a more neuronal phenotype and greatly enhanced nerve fiber outgrowth from the chromaffin cells. Grafts of adrenal medullary tissue had a moderate effect on the rotational behavior, but cografts with sciatic nerve were more effective in decreasing rotational behavior induced by amphetamine. NGFR immunoreactivity was much higher in the striatum of hosts that received grafts of sciatic nerve and cografted recipients. NGFR positive fibers were found both in the grafts and in the host brain adjacent to the grafts. GFAP immunoreactivity was heavier in the transplantation site. S 100 protein positive cells and fibers were found in both cografts and adrenal medulla grafts. The present study demonstrates that cografts of adrenal medullary tissue with peripheral nerve can significantly ameliorate the motor deficit symptoms of experimentally induced unilateral parkinsonism in the rats. Supported by USPHS NS 25778.

292.13

AN EVALUATION OF THE POSSIBLE PROTECTIVE EFFECTS OF NEONATAL STRIATAL TRANSPLANTS ON KAINIC ACID INDUCED LESIONS. D.R. Nash, S.M. Kaplan,* A.B. Norman and P.R. Sanberg. Lab. of Behav. Neurosci., Depts. of Psychiat., Psychol., Neurosurg. and Physiol., Univ. of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

It was recently demonstrated that injection of neonatal striatal grafts into kainic acid (KA) lesioned striatum protected the contralateral striatum from KA lesions (Tullipan et al., Brain Res. 377, 1986, 163-167). In that study, animals with bilateral KA lesions had a 3% survival rate while those receiving transplants had an 85% survival rate. It was postulated that there was a neurohumoral influence provided by the graft in conjunction with the lesioned striatum acting to protect the contralateral striatum from the toxic effects of KA.

Our study examined whether the transplant acting alone may provide protection to the contralateral striatum from KA lesions. Ten male rats received a $1mm^3$ injection of day 3-4 neonatal striatal tissue into one striatum and a 10nMol KA lesion in the contralateral striatum. The control group (n=9) received a sham transplant consisting of 2ul lactated Ringer's solution into one striatum and a 10nMol KA lesion in the other striatum. The transplant did not appear to provide protection from the toxic effects of KA lesions of the striatum. Four of the 10 transplanted animals survived longer than 5 days while 6 of 9 control animals survived longer than 5 days. These results suggest that the transplant (acting without the possible benefit of trophic factors from a lesion) failed to provide protection from KA lesions.

292.14

FETAL STRIATAL TISSUE TRANSPLANTS INTO NORMAL STRIATUM: VOLUME EFFECTS ON BEHAVIOR. S.Y. Lu, M. Giordano, M. Ragozino,* A.B. Norman and P.R. Sanberg. Lab. of Behav. Neurosci., Depts. of Physiol., Psychiat., Psychol. and Neurosurg., Univ. of Cincinnati Coll. of Med., Cincinnati, Ohio 45267.

Fetal striatal transplants into normal striatum produced a decrease in locomotor behavior at three weeks post transplantation followed by a time dependent increase in locomotor activity (1,2). The present study examined the effects of different volumes of transplanted tissue to determine whether the behavioral deficits are volume related.

Male Sprague-Dawley rats (n=26) received three different volumes (1.5 ul, 3 ul and 6 ul) of fetal striatal tissue (E15-17) into both striata. A sham control group received 6 ul of Lactated Ringer's solution. A normal control group consisted of unoperated rats. Nocturnal locomotion was recorded in Digiscan Monitors one week before, and one and three weeks after surgery. Body weight was measured daily.

A volume related weight loss 1-3 days after surgery was found in all transplant groups. The 6, 3 and 1.5 ul groups lost 7%, 3.5% and 1.2% of initial weight respectively. Interestingly, the 6 ul sham group lost 6.3% of the initial weight. The rate of recovery from initial weight loss was similar among groups.

At three weeks post-transplant the experimental and sham transplant groups demonstrated hypoactivity compared to normal controls. Therefore, the initial weight loss and hypoactivity observed following surgery cannot be ascribed to the transplanted tissue. Whether the long-term hyperactivity is dependent on the volume of the transplant remains to be elucidated.

1. Deckel et al., Brain Res. 363:383, 1986.
2. Hagenmeyer and Sanberg, Pharm. Biochem. Behav. 27:583, 1987.

292.15

NIGRA GRAFTS IN NEONATAL BRAIN PROTECT FROM ADULT 6-OHDA LESIONS, BUT NEONATAL 6-OHDA LESIONS DO NOT. D.C. Rogers* and S.B. Dunnett. Dept. of Exptl. Psychology, Univ. of Cambridge, Cambridge CB2 3EB, U.K.

DA-rich grafts in the neonatal brain will protect from the severe ingestive deficits induced by bilateral 6-OHDA lesions in adulthood (Schwarz & Freed, Exp. Brain Res. 65, 1987), whereas similar grafts implanted in mature rats will not. Neonatal 6-OHDA lesions do not induce the severe ingestive deficits that follow comparable adult lesions (Smith et al, J. Pharmacol. Exp. Ther. 185, 1973). Do the grafts therefore disrupt normal dopaminergic development?

Two groups of rats received either i.c.v. saline or 6-OHDA lesions at postnatal day 1 (P1). Half of each group received bilateral embryonic day 15 nigra cell suspension grafts into the neostriatum at P7. At 3-4 months of age all rats received bilateral 6-OHDA lesions of the nigrostriatal bundle. No control rats with adult lesions could spontaneously eat and drink to maintain stable body weight within 12 days. By contrast, 36% of rats with neonatal grafts were able to maintain themselves. 20% of rats with neonatal lesions and 38% with both grafts and lesions could also maintain themselves.

The enhanced survival of the graft group replicates Schwarz & Freed. Although all rats with neonatal lesions were totally unresponsive to haloperidol in either activity or eating tests (indicating that these functions were independent of dopamine receptor activation), surprisingly, 80% of the animals nevertheless showed the full syndrome of ingestive deficits in response to adult lesions.

CATECHOLAMINES III

293.1

NORENEPHRINE DEPLETION IN THE LOCUS COERULEUS FOLLOWING BILATERAL TRANSECTION OF THE VENTRAL NORADRENERGIC BUNDLE. M.A. Cierpial*, L.E. Heyneman* & J.M. Weiss (SPON: G. Marsh). Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

It is well documented that there are adrenergic receptors on the dendrites and/or cell bodies of locus coeruleus (LC) neurons which respond to both epinephrine (EPI) and norepinephrine (NE), and that these receptors play an important role in the regulation of LC firing. A major EPI input to the LC has been identified arising from cells of the C_1 and/or C_2 groups. At present, the exact source of NE innervation to the LC is unknown; however, several studies have shown large depletions of NE in the LC following uncontrollable stress, indicative of a rapid release of this transmitter from nerve terminals. A possible candidate for NE input to the LC is the ascending ventral noradrenergic bundle (VNAB) which originates in brainstem nuclei caudal to the LC and terminates primarily in the hypothalamus. In this experiment, bilateral knife cuts were made through the VNAB caudal to the LC, and NE levels were measured 12-14 days following transection. Bilateral VNAB transections resulted in a 35% decrease in NE content of the LC compared to unoperated and sham operated controls. Hypothalamic NE was decreased by 61% and cortical NE was decreased by 32%, as has been reported with VNAB transection rostral to the LC. Examination of 50 um nissl stained sections revealed no apparent neuropathy in the LC of VNAB lesioned animals. These results suggest that the LC receives an afferent projection from the VNAB in that transections of the bundle caudal to the LC results in NE depletion in this nucleus.

293.2

NEUROPEPTIDE-Y LEVELS IN THE LOCUS COERULEUS AFTER UNCONTROLLABLE SHOCK. T.M. Brown*, M.A. Cierpial*, P.E. Simson, P.D. Butler & J.M. Weiss (Spon: L. Demski). Dept. Psychiatry, Duke Univ. Medical Center, Durham, NC 27710.

Neuropeptide-Y (NPY) is a 36-amino-acid peptide co-localized with noradrenaline in noradrenergic cells of the locus coeruleus (LC). It is known that the LC is highly activated by stress. To investigate the effect of stress on NPY in the LC, male Sprague-Dawley rats were exposed to stressful conditions. In the first experiment, one group received randomly-spaced, uncontrollable electric shocks for 19 hours while a control group consisted of unstressed animals removed directly from their home-cage. The shock condition significantly elevated NPY in the LC (107.4 $\pm$ 8.6 ng/mg tissue) relative to the control animals (39.2 $\pm$ 8.6 ng/mg tissue). In a second experiment, effects of a briefer shock exposure were tested. One group received randomly-spaced, uncontrollable electric shocks for 3 hrs; a second group was placed in the shock apparatuses for 3 hrs but received no shocks; and a third control group consisted of unstressed animals removed directly from their home-cage. Exposure to the shock apparatus for 3 hrs significantly elevated NPY in the LC (78.0 $\pm$ 10.2 ng/mg tissue) relative to the controls (39.0 $\pm$ 12.2 ng/mg tissue). In animals exposed to 3 hrs of shock, however, the NPY concentration was not elevated in the LC (39.6 $\pm$ 9.2 ng/mg tissue). These results suggest that mild acute stress (i.e., 3 hrs of confinement in the shock apparatus) elevates the NPY concentration in the LC whereas more severe acute stress (3 hrs of shock) causes sufficiently high release of the peptide that an elevation is not seen. However, if the severe stress is long-lasting (i.e., 19 hrs), then mechanisms increasing the availability of the peptide compensate for the increased release and an elevation in NPY is again seen.

293.3

QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF α1- AND β-ADRENERGIC RECEPTORS AND ELECTROPHYSIOLOGICAL ANALYSIS OF CONNECTIVITY IN INTRAOCULAR RAT LOCUS COERULEUS-HIPPOCAMPUS CO-GRAFTS. M. Mynlieff, P. Curella*, N.R. Zahniser, and T.V. Dunwiddie. Univ. Colo. Hlth. Sci. Ctr. and VAMC, Denver, CO.

Previous studies have shown the feasibility of "building" an isolated circuit between the locus coeruleus (LC) and hippocampus in oculo. The present study investigated the role of noradrenergic innervation in regulating α1- and β-adrenergic receptor development in the hippocampal portion of LC/hippocampus co-grafts and relate this to the development of functional connections between the co-grafts. LC from embryonic Sprague-Dawley rats was transplanted to the anterior eye chamber of sympathetically denervated 6-8 week old rats, followed 4 weeks later by co-transplantation of fetal hippocampal tissue. Two month and 6 month old co-grafts, single hippocampal grafts grown in normal hosts, and single hippocampal grafts grown in sympathetically denervated hosts were analyzed.

Quantitative autoradiographic analysis of saturation curves showed no significant difference in receptor numbers for either the α1-receptor (31-65 fmol/mg protein) or the β-receptor (14-19 fmol/mg) in the single grafts, 2 month co-grafts, or 6 month co-grafts. HPLC analysis in these grafts also showed approximately equal amounts of norepinephrine (NE) in all three types. The hippocampal grafts grown in the absence of NE input from the iris or LC exhibited higher numbers of α1-(109 fmol/mg) and β-receptors (41 fmol/mg). Intracellular electrophysiological studies showed that stimulation of the LC portion of 2 month old co-grafts almost always resulted in a β-adrenergic response in the hippocampus while in 6 month old co-grafts 1/3 showed α-adrenergic responses, 1/3 showed β-adrenergic responses, and the remainder showed a combination of α and β responses. Electrophysiological studies have shown that the denervated grafts are not more sensitive to NE. Immunohistochemical analysis of co-grafts showed that at 6 months the NE fibers have grown farther into the hippocampus and are denser than at 2 months. These results suggest that the number of adrenergic receptors in the hippocampus are regulated by NE content but the physiological response to NE is not necessarily predicted by the receptor number. Grant Support: USPHS AG04418, DA 02702 and VA Medical Research Service.

293.4

REGULATION OF BRAIN Na,K-ATPase BY INTRAVENTRICULAR NORADRENERGIC RECEPTOR LIGANDS. Alan C. Swann and Jeffrey D. Steketee. Department of Psychiatry, University of Texas Medical School, Houston, Texas 77225.

Previous results have suggested that indirect noradrenergic stimulation increased brain Na,K-ATPase. We investigated the role of specific noradrenergic receptors using intraventricular infusions of receptor ligands and measuring ouabain binding in cerebral cortex and hippocampus. In the initial experiments, norepinephrine (NE; 1, 2, and 5 μg/h), phenylephrine (PH; 1, 2.5, 5, and 7.5), isoproterenol (ISP; 2.5, 5, 7.5, and 10), clonidine (CL; 1 and 5) were infused into the lateral cerebral ventricles with osmotic minipumps for one week. In addition, forskolin (FSK; 3, 6, and 12) was infused in order to examine the effect of direct stimulation of adenylate cyclase. Then, the optimal dose was infused into rats with 6-hydroxydopamine lesions of the dorsal noradrenergic bundle (>90% NE depletion). NE, ISP and FSK increased ouabain binding, PH had a biphasic effect with maximal stimulation at 2.5 μg/h, while CL reduced ouabain binding. All ligands except CL increased binding in DB lesioned rats. These results show that NE receptor stimulation, partially through cyclic AMP, increases ouabain binding in vivo.

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293.5

NORADRENERGIC STIMULATION OF CYCLIC AMP SECRETION BY RAT EMBRYONIC CEREBRAL CORTEX IN DISSOCIATED CELL CULTURE. P. A. Rosenberg and M. A. Dichter. Depts. of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115; Graduate Hospital and University of Pennsylvania, Philadelphia, PA 19146.

Beta adrenergic receptors have been demonstrated in cortical astrocytes but not neurons in primary culture. Secretion of cyclic AMP in response to beta adrenergic stimulation has been described in many cells of vertebrate origin. Cyclic AMP secreted by astrocytes stimulated by NE or other agents might affect the function of nearby neurons either directly or by way of metabolites.

Isoproterenol (ISO) at 10 uM stimulated intracellular cyclic AMP ($cAMP_{in}$) accumulation in mature (4-6 weeks in vitro) mixed neuron and glial cultures, with a peak occurring at approximately 10 minutes. ISO also stimulated extracellular cyclic AMP ($cAMP_{ex}$)accumulation, with a peak occurring at 40-60 minutes. The quantity of $cAMP_{ex}$ assayed in the medium at peak was comparable to $cAMP_{in}$ at peak (180 nmol cAMP/mg protein). We have also been able to demonstrate extracellular phosphodiesterase as well as nucleotidase activity in these cultures resulting in the degradation of $cAMP_{ex}$ to AMP and adenosine. Whether cAMP secretion affects neurons and if so by what mechanism remains to be determined. This work was supported by a Robert Morison Fellowship from the Grass Foundation, NS 00-993, CH MR Core HD 06276, and the Milton Fund.

293.6

EXPRESSION OF TYROSINE HYDROXYLASE mRNA AND MORPHOMETRIC CHARACTERISTICS OF DOPAMINERGIC NEURONS IN TISSUE CULTURE. DK Sundberg*, BA Bennett, P Skrincosky*, F Baldino Jr, and M Morris*, Dept. of Physiol. and Pharmacol., Bowman Gray School of Medicine, Wake Forest University, Winston Salem, N.C. 27103 and Cephalon Corp, West Chester, PA.

Studies in our laboratory have focused on the use of primary cultures of specific brain regions. Results show that hypothalamic arcuate cultures develop dendritic processes, stain immunocytochemically for tyrosine hydroxylase (TH) and synthesize dopamine. The objective of these studies was to characterize the cultures using morphometric analysis and to determine whether the TH positive cultures express mRNA for this enzyme.

Hypothalami from 3-5 day old rats were dissociated enzymatically. The cultured cells were fixed and stained for TH or neuron specific enolase after 3,6 or 9 days of culture. Morphometric indices included cell size, density and length of processes. TH-positive neurons represent 6-8% of the total number of surviving neurons. They are somewhat smaller than the mean neuronal population and send out significantly longer projections ($p<.05$) than the mean population. TH mRNA was measured by dot blot in 6 day cultures. In addition to staining for TH, extracted mRNA hybridized to a 30 mer DNA probe complimentary to TH mRNA. (Supported by NIH grants NS 24723 and NS 22492).

293.7

SUCCESSFUL CRYOPRESERVATION OF DOPAMINE PRODUCING HUMAN SUBSTANTIA NIGRA CELLS. R. J. Robbins, I. Torres-Aleman*, C. Leranth, C. Bradberry, A. Deutch, D. E. Redmond, Jr., R. Roth, and F. Naftolin*. Sections of Neuroendocrinology and Neuropharmacology, Dept. of Medicine, OB/GYN, and Pharmacology, Yale Medical School, New Haven, CT 06510

Recent advances in transplantation biology have made possible the concept of implanting human dopamine producing cells into the brains of patients with Parkinson's Disease (PD). Survival of fetal substantia nigra (SN) cells in the striatum of adult rats and adult monkeys has been reported. In order to allow time to screen potential donor cells for a variety of characteristics including infectious agents, and to allow careful neurosurgical preparation of the recipients we have attempted cryopreservation of freshly isolated human dopamine cells. Cells from 9 to 12 week old human fetal cadavers were collected within one hour of death, transferred to cryoprotective solutions (DMSO or propanediol) and frozen according to several gradual freezing paradigms. Cells were stored at -196C. Viability of thawed cells was above 95% when thawed within two weeks of freezing but only 65% when thawed after three months. Monolayer cultures of these cells had tyrosine hydroxylase (+) neurons with long processes and HPLC analysis revealed authentic dopamine in the cells and medium. We conclude that human SN cells can survive short term cryopreservation and express specific adult dopaminergic characteristics.

293.8

MESOLIMBIC DOPAMINE AUTORECEPTORS CONTROLLING TYROSINE HYDROXYLASE ACTIVITY : EVIDENCE FOR THE INVOLVEMENT OF CYCLIC AMP AS A SECOND MESSENGER. P. Onali and M.C. Olianas. Dept. Neurosciences, University of Cagliari, Italy.

The cyclic AMP-dependent stimulation of synaptosomal tyrosine hydroxylase (TH) by forskolin (FSK),an activator of adenylate cyclase,has been used as a model to test whether dopamine (DA) autoreceptors of nucleus accumbens inhibit TH activity by a mechanism involving cyclic AMP. Homogenate of rat nucleus accumbens was preincubated with the test agents and the enzyme activity was assayed in extracts prepared by centrifugation and sonication of the tissue. FSK maximally stimulated TH by 2.5-3 fold with an EC50 of~1 μM. Quinpirole,a selective D2 DA agonist,inhibited basal TH activity by 25-30% and reduced the stimulation of the enzyme elicited by FSK (0.5-10 μM) by 35-50%. The IC 50 value of quinpirole was~ 25 nM. The quinpirole inhibition was antagonized by the selective D2 DA blocker l-sulpiride (2 μM). On the other hand,quinpirole failed to inhibit the stimulation of TH elicited by dibutyryl cyclic AMP (2 mM), which acts independently of adenylate cyclase. These results suggest that mesolimbic DA autoreceptors can regulate the cyclic AMP-induced activation of TH presumably by exerting an inhibitory input on a presynaptic adenylate cyclase system.

293.9

CENTRAL REGULATION OF ADRENAL TYROSINE HYDROXYLASE; INTERACTION BETWEEN DOPAMINE AND GABA SYSTEMS. S. Regunathan*, R. Gabor* and T.L. Sourkes. Douglas Hosp. Res. Cent. & Dept. of Psychiatry, McGill University, Montreal H3A 1A1.

The nigrostriatal dopamine (DA) system participates in the regulation of adrenal tyrosine hydroxylase activity (ATHA). To determine whether activation or inhibition of this system is responsible for the induction of adrenal TH, the role of presynaptic DA receptors was investigated. Apomorphine (0.2 mg/kg), (+)3PPP (10 mg/kg) and BHT920 (up to 3 mg/kg) caused significant increases of ATHA. These drugs bind to presynaptic DA receptors and thereby inhibit the release of DA. As a central GABA system is believed to exert inhibition over DA release, GABA agonists were tested for their effects on ATHA. Muscimol (3 mg/kg), GOBA (0.5 mg/kg) and HA966 (150 mg/kg) produced significant induction of adrenal TH which was not blocked by DA postsynaptic receptor antagonists such as sulpiride or SCH 23390. It is concluded that the inhibition of the nigrostriatal DA system is responsible for the induction of adrenal TH. This is achieved by activation of the striatonigral GABAergic feedback system or by presynaptic regulation of DA release through autoreceptors in the striatum. (Research supported by the MRC, Canada)

293.10

PROTEIN KINASE C PHOSPHORYLATES THREE PUTATIVE SITES ON TYROSINE HYDROXYLASE IN INTACT BOVINE ADRENAL CHROMAFFIN CELLS. J.P. Johnston, K. Hummer-Lickteig and J.C. Waymire. Dept. Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77225.

Tyrosine hydroxylase (TH) is phosphorylated *in vitro* by several protein kinases. Alberts *et al.* (PNAS 81:7731, 1984) have reported that purified TH is phosphorylated by PK-C on a site that is identical to that phosphorylated by PK-A. However, Tachikawa *et al.* (J Neurochem 48:1366, 1987) have reported that TH is phosphorylated *in situ* in PC-12 cells on distinct sites by forskolin and phorbol 12-myristate-13-acetate. We have investigated the role of PK-C phosphorylation of TH in bovine adrenal chromaffin cells where it appears there are seven putative phosphorylation sites (Waymire *et al.*, Neurosci Abstr 13, 1987). RP-HPLC analysis of TH tryptic peptides revealed that stimulation of intact chromaffin cells with 1 μM phorbol 12,13-dibutyrate produced an increased incorporation of ^{32}P into 3 of the 7 peptides. This response was dependent on both concentration and time (EC_{50} = 316 nM, $t_{\frac{1}{2}}$ = 4 min). One of the peptides (peptide 6) is also a substrate for protein kinase A both *in vitro* and *in situ*. Two other peptides (peptides 4 and 7) have not been shown to be substrates for any protein kinase *in vitro*, and correspond to those peptides which are phosphorylated with a slow time course in response to ACh stimulation of intact chromaffin cells. Supported by USPH NS 11061.

293.11

MAPPING THE ALTERATION OF THE COFACTOR DOMAIN OF TYROSINE HYDROXYLASE DUE TO cAMP DEPENDENT PHOSPHORYLATION. S.W. Bailey*, S.B. Dillard* and J.E. Ayling. (SPON: R.B. Moore). Dept. Pharmacology, Univ. South Alabama, Mobile, AL 36688.

The dihydroxypropyl side chain of R-tetrahydrobiopterin (R-BH_4) is not essential for catalytic activity of tyrosine hydroxylase (TH), the first committed step in catecholamine biosynthesis. That it enables several important regulatory properties is well established. Despite major changes in the kinetics of TH with assay pH, no study on the role of cofactor structure on specificity and function under physiological conditions has yet been reported. More than a dozen 6-mono- and 6,6-disubstituted tetrahydropterins have been synthesized and kinetics with bovine TH with respect to cofactor and tyrosine determined. The decrease in K_m for cofactor upon cAMP dependent phosphorylation that has been previously found with R-BH_4 and 6-methyl-tetrahydropterin is seen with all the analogs, but ranges from 40 to 2000 fold. With both forms of TH side chain hydrophobicity plays an important but limited role in binding in a manner similar to other carbohydrate-protein interactions. Phosphorylation induces a major rearrangement of cofactor specificity. For example, K_m for R-BH_4 shifts from a lowest to a moderate ranking. Several analogs possess significantly higher affinity for either form. This along with the unusual kinetics of the disubstituted analogs, and molecular mechanical calculations, suggest a model for the effects of phosphorylation different from those previously proposed. (Supported by GM-30368.)

293.12

INDUCTION OF TYROSINE HYDROXYLASE BY NICOTINIC AGONISTS IN BOVINE ADRENAL CHROMAFFIN CELLS IS ACCOMPANIED BY A MARKED, DISPROPORTIONATE INCREASE IN CATECHOLAMINE BIOSYNTHESIS CAPACITY. J.C. Waymire and G.L. Craviso. Dept. Neurobiol. & Anat., Univ. Tex. Med. Sch., Houston TX 77225.

Long-term treatment of chromaffin cells with the nicotinic agonist dimethylphenylpiperazinium (DMPP), increases the synthesis of tyrosine hydroxylase (TH). This study examined whether catecholamine (CA) synthesis rates in DMPP-induced cells are affected by the increase in TH levels. When chromaffin cells were treated with 1 μM DMPP for 3 days, TH activity, measured *in vitro*, increased 1.5- to 2-fold over that of untreated cells, whereas CA synthesis rates, measured *in situ*, increased in excess of 3.5-fold. The more pronounced stimulation of synthesis relative to the amount of increased TH was not due to TH activation or enhanced dopa decarboxylase activity. Elevated CA synthesis, unlike TH induction, was not completely blocked by protein synthesis inhibitors. Incubation of DMPP-induced cells with 100 μM acetylcholine for 10 min further increased CA synthesis (2- to 3-fold), indicating that the cells retained their ability to respond to subsequent short-term stimulation. Our results demonstrate that prolonged nicotinic receptor stimulation greatly augments CA synthesis capacity (at least 6-fold over control cells), and that induction of TH alone does not appear to account for the magnitude of the changes in synthesis rate. Supported by USPH NS11061.

293.13

INDUCTION OF TYROSINE HYDROXYLASE BY NICOTINIC AGONISTS IN BOVINE ADRENAL CHROMAFFIN CELLS IS CA^{2+}-DEPENDENT. G.L. Craviso and J.C. Waymire. Dept. Neurobiology and Anatomy, Univ. Texas Medical School, Houston, TX 77225.

Acute regulation of adrenal chromaffin cell tyrosine hydroxylase (TH) by acetylcholine is dependent upon extracellular Ca^{2+}. The present study examined whether Ca^{2+} also plays a role in the cholinergic-receptor mediated induction of TH. A 3-day exposure of chromaffin cells to varying concentrations of carbachol or dimethylphenylpiperazinium (DMPP) increased TH activity in a dose dependent manner (EC_{50} carbachol = 20 μM; EC_{50} DMPP = 0.25 μM) to a maximum of 158 ± 10%, and 146 ± 10% of control, respectively; muscarine had no significant effect at any concentration tested. Induction of TH by 56 mM K^+ (255 ± 6%) was similar in many respects to that produced by prolonged nicotinic receptor stimulation; induction was maximal at 3 days, blocked by protein synthesis inhibitors, and the increases in TH activity corresponded to the increases observed for TH immunoreactive protein. Increased TH synthesis also required Ca^{2+}. Induction of TH by DMPP increased with increasing extracellular Ca^{2+} up to 2.5 mM (121 ± 6% at 50 μM Ca^{2+}; 148 ± 4% at 0.5 mM Ca^{2+}; 184 ± 2% at 2.5 mM Ca^{2+}), and induction by K^+ was maximal at 0.5 mM Ca^{2+}; TH levels in non-induced cells were not affected by Ca^{2+}. These data strongly suggest that a Ca^{2+}-dependent mechanism is important in the long term regulation of TH. Supported by USPH NS 11061.

293.14

TYROSINE HYDROXYLATION IN INTACT CHROMAFFIN CELLS IN RESPONSE TO TETRAHYDROBIOPTERIN AND ANALOGS. J.E. Ayling and J.C. Waymire. Dept. Pharmacology, Univ. South Alabama, Mobile, AL 36688 and Dept. Neurobiology and Anatomy, Univ. Texas Med. Sch., Houston, TX 77225.

The activity of tyrosine hydroxylase (TH) is dependent on several factors, among which are its state of phosphorylation and the availability of its cofactor, tetrahydrobiopterin (BH_4). The current studies were undertaken to ascertain whether kinetic parameters for BH_4 and BH_4 analogs determined for TH in vitro (see abstract S.W. Bailey, S.B. Dillard and J.E. Ayling) parallel those in the intact cell. If so, these compounds may be useful probes of the state of phosphorylation in vivo.

Primary suspension cultures of bovine chromaffin cells were assayed for tyrosine hydroxylation in the absence or presence of varying concentrations of an added cofactor. Activity was determined by measuring $^{14}CO_2$ released from ^{14}C-1-tyrosine. HPLC analysis of dopa accumulated in the presence of a dopa decarboxylase inhibitor, m-hydroxybenzylhydrazine, gave similar results. All reactions were in pH 7.15 buffer and at 37°C, as with the isolated enzyme, and rates were linear for at least 18 minutes. Apparent Michaelis constants and maximum velocities for BH_4 and a series of BH_4 analogs with resting intact cells correlate with those determined with the isolated non-phosphorylated enzyme in vitro. This is consistent with findings that TH in these cells in the resting state is minimally phosphorylated. (Supported by NIH GM-30368 and NS-11061).

293.15

STIMULATION OF TYROSINE HYDROXYLASE (TH) ACTIVITY BY AN ANTISERUM AGAINST A PEPTIDE CORRESPONDING TO A SEGMENT OF TH. K.Y Lee[1*], J.Y. Lew[1], D. Tang[1*], D. H. Schlesinger[2*], A.Y. Deutch[3] and M. Goldstein[1] (SPON: R. Margolis). N.Y. Univ. Med. Ctr., New York, NY 10016, Dept. of Psychiatry[1], Dept. of Cell Biol. and Med.[2] and Yale Univ., Dept. Psychiatry, New Haven, CT 06510[3]

We have synthesized a peptide corresponding to position 34-47 in TH (TH-16) which is a substrate for phosphorylation by protein Kinase A, protein Kinase C and Ca^{+2}/calmodulin protein Kinase II (M. Goldstein et al., to be published). Antibodies to TH-16 were raised (anti-TH-16) and their effects on TH activity were compared with those raised against rat TH (anti-TH). The addition of anti-TH inhibits, while the addition of anti-TH-16 stimulates striatal TH activity. The stimulation elicited by anti-TH-16 is dose-dependent and the maximal stimulation was approx. 80%. To determine whether stimulation of TH activity by anti-TH-16 mimics the stimulation elicited by phosphorylation of TH we have determined the pH dependency and the effect on K_M for the cofactor $DMPH_4$. The stimulation is not pH dependent and the K_M for $DMPH_4$ was not significantly changed. Thus the enzyme stimulated by anti-TH-16 does not have the same kinetic properties as the phosphorylated TH. Immunohistochemical studies show that anti-TH-16 recognizes catecholaminergic neurons in the rat brain, and some immunoreactive sites are similar to those identified with anti-TH. Supported by grants from NIMH (MH 02717-30) and the Leon Lowenstein Foundation.

293.16

EFFECT OF TETRAHYDROBIOPTERIN (BH_4) ON RAT CIRCLING BEHAVIOR. W.E. Stroo* and J.E. Ayling. (SPON: P.R. Dyken). Dept. Pharmacology, Univ. South Alabama, Mobile, AL 36688.

BH_4, the natural cofactor for the aromatic amino acid hydroxylases, is clinically useful in treating the neurological symptoms of BH_4 deficiency and may have application in other neurological disorders associated with decreased levels of biogenic amines. However, the study of BH_4 and synthetic analogs (see abstract J.E. Ayling et al.) has been limited by the availability of an animal model to evaluate the CNS effectiveness of these compounds. Circling behavior of unilaterally lesioned rats (8 µg 6-OH-dopamine into substantia nigra) was monitored as an indirect index of dopamine (DA) release. BH_4 alone had no effect on circling behavior. However, BH_4 (0.1 mmole/Kg IP) after 0.5 mg/Kg amphetamine IP increased the 360° turns/2 hours from 173 ± 57 to 353 ± 70 (n=8). BH_4 was effective even when given up to 2 hours prior to amphetamine. Centrally effective inhibitors of DA synthesis (α-methyl tyrosine; m-hydroxybenzylhydrazine) at doses which did not significantly affect amphetamine induced behavior, blocked the effect of BH_4. A peripheral inhibitor (carbidopa) did not alter the response to BH_4. These data demonstrate that peripheral administration of BH_4 has behavioral effects consistent with enhanced synthesis of DA in the CNS. The unilaterally lesioned rat may be a useful animal model to evaluate synthetic analogs of BH_4. (Supported by GM-30368.)

293.17

THE EFFECTS OF PHYSIOLOGICAL STRESSORS ON TYROSINE HYDROXYLASE ACTIVITY IN CENTRAL AND PERIPHERAL CATECHOLAMINERGIC SYSTEMS. L.K. Nisenbaum, E.M. Stricker, and M.J. Zigmond. Dept. of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

Norepinephrine turnover is elevated in the central and peripheral nervous systems during physiological stress. Changes in tyrosine hydroxylase (TH) activity have been implicated in this response. We have examined the effects of stress on TH activity in locus coeruleus (LC) of rat. TH activity in LC was increased by 26% when shaved rats were exposed to chronic cold (5°C) for 3 days and by 38% after 7 days. In cerebellum, TH was unchanged after 3 days of cold stress, but increased by 34% after 7 days, and remained elevated for at least 21 days. Comparable changes in TH activity were obtained with chronic insulin-induced hypoglycemia (PZI, 4-8 U/day). These responses to the two stressors are considerably lower than those we observe in the adrenal gland (e.g. +95% after 5 days of exposure to cold). This difference does not reflect a lower capacity for TH induction in brain, because reserpine (5 mg/kg) increased TH activity by 151% in LC, an amount comparable to the change in adrenal. Instead, the difference may reflect different demands placed on LC and adrenal during exposure to these stressors, different turnover rates of catecholamines in the two structures, and/or the requirement of LC to transport TH from the cell body to diffuse terminal projections. (Supported by USPHS Grants MH29670 and NS19608.)

293.18

EFFECTS OF REPEATED COCAINE OR AMPHETAMINE ON DOPAMINE SYNTHESIS MODULATING AUTORECEPTORS (ARs). M.J. Keegan*, J.B. Stahl*, and M.P. Galloway. (SPON: F.J. White) Wayne State Univ. Sch. Med.& Lafayette Clinic, Detroit, MI 48207

Although the ability of cocaine (COC) or amphetamine (AMPH) to increase synaptic levels of dopamine (DA) probably contributes to their acute psychoactive properties, less is known concerning their effects on nerve terminal ARs after repeated exposure. We have previously shown that COC, but not procaine, decreases both DA synthesis in vivo and K+-stimulated synthesis in vitro in a sulpiride sensitive fashion. To determine DA-AR responsivity after repeated COC or AMPH, we have measured DOPA synthesis both in vivo and in vitro after DA agonist challenge. In striatal slices obtained from rats treated with COC (20 mg/kg, bid, x16d), the dose response to 7-OH-DPAT was shifted to the right. However, this apparent decrease in AR responsivity was not accompanied by substantial alterations in regional levels of parent monoamines or metabolites, measured either 24 hrs or 5 wks after cessation of treatment (as above, x7d). After AMPH pretreatment in vivo, AMPH induced DA release in vitro was enhanced, however there was little change in the ability of quinpirole (QUIN) to inhibit synthesis in striatal slices. This dissociation between the effect of QUIN on DA synthesis (AR sensitivity) and AMPH induced release is consistent with the observation that QUIN (10uM) did not attenuate AMPH (3uM) induced release. Support: USPHS DA-04120, MH-41227, Mich. Dept. Mental Health.

293.19

THE CATECHOLAMINE TRANSPORTER IN CHROMAFFIN VESICLES IS INHIBITED BY 5-METHOXYTRYPTAMINE BUT NOT BY MELATONIN, A NON-PROTONATABLE ANALOGUE. Patrick M. Kelley, David A. Turner*, and David Nius*. Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

The catecholamine transporter of adrenal medullary chromaffin vesicles concentrates biogenic amines some 3-4 orders of magnitude above the cytosolic concentration. Transport occurs via an exchange of catecholamines for protons but the mechanism for this exchange is controversial. Much of the controversy centers around which form of the amine (protonated or unprotonated) binds to and is transported by the transporter. We have used two derivatives of 5-hydroxytryptamine (5-HT), 5-methoxytryptamine and melatonin, to help elucidate the transport mechanism. 5-Methoxytryptamine, which is protonatable, is a good competitive inhibitor of 5-HT uptake (K_i about 5µM). By contrast, melatonin, which is N-acetylated and has an unprotonatable nitrogen, is a relatively poor inhibitor. Melatonin does inhibit catecholamine transport at very high concentrations (above 500µM) but the mechanism does not seem to involve inhibition of the transporter. Instead, melatonin abolishes the ATP-dependent membrane potential which drives amine transport. This indicates that melatonin interacts with the transporter very poorly if at all. Steric considerations are not likely to account for the poor binding of melatonin, since N-ethyl 5-methoxytryptamine is a good competitive inhibitor and isoproterenol is a good transportable substrate. This argues against the neutral, unprotonated form of the amine being the substrate for the transporter. This work was supported by NIH grant GM33849.

294.1

DOPAMINERGIC MESOCORTICAL-MESOLIMBIC INTERACTIONS MEASURED BY IN VIVO INTRACEREBRAL DIALYSIS. C.A.Leslie[1] and J.P.Bennett[2]. Departments of Behavioral Medicine and Psychiatry, [1,2] Neurology,[2] and Pharmacology,[2] University of Virginia School of Medicine, Charlottesville, VA 22908.

The "positive" symptoms of schizophrenia such as hallucinations, delusions and thought disorder may reflect "hyperactive" dopamine (DA) neurotransmission in subcortical mesolimbic sites. There are as well, "negative" symptoms in schizophrenia; flat affect, social withdrawal and avolition, and cognitive "deficit" symptoms which are more referable to higher cortical functions subserved by terminal fields of the mesocortical DA pathways. Many recent studies using a variety of techniques point to anatomical and functional abnormalities in the mesocortical DA system in schizophrenia. Biochemical and behavioral experiments suggest that mesocortical DA activity exerts modulatory influence over mesolimbic DA activity. A more thorough understanding of the neurochemistry of these feedback pathways, including the functional roles of D1 and D2 receptor subtypes, may enable a better understanding of the pathophysiology of schizophrenia and lead to more effective neuropharmacological interventions.

We are examining in the rat the effects of acute microinjection of selective D1 and D2 DA receptor agonists and antagonists into the medial frontal cortex (MFC) on DA synaptic transmission in the nucleus accumbens as measured by in vivo intracerebral dialysis. Preliminary studies have shown a rapid rise in DOPAC and HVA in the nucleus accumbens when the specific D1 agonist SK&F 38393 (500 pmol) is microinjected into the MFC. Biochemical studies suggest that this is mediated by augmentation of DA release in the nucleus accumbens. There is no change in mesolimbic DA synthesis estimated by acute DOPA decarboxylase inhibition.

294.2

DIALYSIS MEASUREMENTS OF DOPAMINE IN NUCLEUS ACCUMBENS AND VENTRAL TEGMENTAL AREA: EFFECT OF COCAINE.
C.W. Bradberry and R.H. Roth. Depts. of Pharmacology and Psychiatry, Yale Univ., School of Medicine, New Haven, CT.

Cocaine has previously been reported to increase dopamine (DA) levels in dialysis perfusates in striatum and nucleus accumbens, consistent with its ability to block DA reuptake. Cocaine has also been shown to inhibit the firing rate of meso-accumbens (m.-a.) DA neurons. One postulated mechanism by which cocaine might inhibit the firing rate of m.-a. DA neurons is by increasing the availability of DA to inhibitory dendritic autoreceptors. We report here that cocaine (1 mg/kg i.v.) increases extracellular DA levels in the ventral tegmental area as well as the nucleus accumbens in the chloral hydrate anesthetized rat. In the first twenty minute collection following cocaine administration, DA increases to 275%, and 175% of basal levels in nucleus accumbens and ventral tegmental area respectively. These data are consistent with a cocaine-induced enhancement of DA availability for somatodendritic regulation of DA neurons, thus providing a mechanism whereby cocaine may exert an inhibitory influence on m.-a. DA neuronal firing rate.

Supported in part by NIH Grant DA05119.

294.3

MICRODIALYSIS AND TISSUE LEVEL STUDIES OF PRESYNAPTIC DOPAMINE FUNCTION IN CHRONICALLY COCAINE-TREATED RATS.
P. Glue*, A. Mele*, C.C. Chiueh, D.J. Nutt*, and A. Pert. (SPON: L. Erinoff). NIAAA and NIMH, Bethesda, MD 20892.

Repeated administration of the psychostimulants cocaine (coc) and d-amphetamine (d-amp) cause behavioural sensitisation in rats, seen as increasing degrees of locomotor behaviour and stereotypy. Because these drugs act by increasing release and blocking reuptake of dopamine (DA), these may be the neurochemical mechanisms underlying this phenomenon. Although d-amp pretreatment causes increased d-amp-induced DA release, the effects of coc pretreatment on aspects of presynaptic DA function are unclear.

We investigated this by giving rats i.p. coc 30 mg/kg or saline daily for 1 week. Two indices of presynaptic function were studied; DA and metabolite levels in the striatum, and DA and metabolite responses to 1 mM coc infused via the microdialysis probe in the striatum and n.accumbens.

Striatal tissue levels of DA and metabolites were identical in the two groups, and ratios of metabolites to DA were also similar. In the microdialysis study, DA and metabolite baselines and responses were again identical in both groups. This suggests that, in contrast to d-amp, coc pretreatment does not alter presynaptic DA function. In addition, it would appear that in behavioural sensitisation, coc-induced changes in DA function are likely to be postsynaptic.

294.4

HIPPOCAMPAL NE AND DOPAC RELEASE FOLLOWING DAMAGE TO THE DORSAL BUNDLE: DIALYSIS STUDIES.
E.D. Abercrombie and M.J. Zigmond. Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Microdialysis was used to examine the capacity for transmitter release of NE neurons spared after partial lesion of dorsal bundle (DB) afferents to the hippocampus. Hippocampal NE content was reduced by 30% to >90% by administration of 6-HDA (4 - 10 ug in 2 ul) into the DB. Two weeks later, extracellular levels of hippocampal NE and DOPAC were determined under basal conditions and in response to tail shock and elevated K^+ (60mM) in the perfusate.

% DEPLETION	pg NE/25 ul	pg DOPAC/25 ul	n
Sham	16 ± 3	26 ± 6	3
≤50%	18 ± 2	13 ± 3	3
70%	9 ± 3	4 ± 1	3
80%	5 ± 1	9 ± 3	3
>90%	2 ± 1	3 ± 1	5

Basal NE level did not decline significantly unless NE depletion exceeded 50%; however, basal DOPAC level declined linearly with lesion size. Stress and elevated [K^+] increased NE level in sham animals by 65% and 179%, respectively, and by 61% and 166%, respectively, in lesion animals with ≤50% NE remaining. DOPAC level was increased only modestly by these stimuli in sham animals but was increased dramatically in lesioned animals with ≤50% NE. Thus, compensatory processes exist by which a system of neurons can function normally despite the loss of up to half the population.

294.5

COMPARATIVE EFFECTS OF D-AMPHETAMINE AND COCAINE ON DOPAMINE RELEASE IN STRIATUM AND MEDIAL PREFRONTAL CORTEX: BILATERAL *IN VIVO* MICRODIALYSIS IN NORMAL RATS.
J.L. Baird*, J.N. Carlson and S.D. Glick. Department of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208.

Using the technique of *in vivo* brain microdialysis, we have monitored the bilateral release of dopamine and its metabolites (DOPAC and HVA) in the striatum and medial prefrontal cortex (PFC) of both anesthetized and awake female Long-Evans rats. Both d-amphetamine sulfafte (1.25 mg/kg, i.p.) and cocaine hydrochloride (20 mg/kg, i.p.) increased dopamine and decreased DOPAC and HVA in striatum; these changes were usually greater in one side of the brain than in the other and were more pronounced after d-amphetamine than after cocaine. In some rats we utilized an automated system for recording rotation (circling) concomitantly with collection of dialysis samples. For both d-amphetamine and cocaine, there appeared to be within-animal correlations between behavioral (i.e., rotation) and neurochemical (i.e., striatal dopamine) asymmetries during the time-course of drug effects. Preliminary data indicate that both drugs also increase dopamine in PFC but that, in contrast to striatum, the effect of cocaine is greater than that of d-amphetamine. Further studies are in progress to characterize dose-response relationships of these effects (Supported by NIDA grant DA03817 to S.D.G.).

294.6

EFFECT OF COCAINE AND AMPHETAMINE ON EXTRACELLULAR DOPAMINE LEVELS IN THE RAT PREFRONTAL CORTEX: A COMPARISON TO THEIR EFFECT IN THE NUCLEUS ACCUMBENS.
B. Moghaddam and B.S. Bunney, (Spons: R.N. Adams), Depts. Pharmacol. & Psychiat., Yale Univ. Sch. Med., New Haven, CT.

Several lines of evidence suggest that cocaine and amphetamine may elicit their psychotropic actions primarily by altering dopamine (DA) levels in the synapse. Of the major anatomical projections of the DA neurons, the nucleus accumbens (NAc) and prefrontal cortex (PFC) have been implicated most in reinforcing properties of cocaine and amphetamine. To test the involvement of NAc and PFC DA in cocaine and amphetamine reward, we have investigated the effect of systemic administration of these drugs on the extracellular levels of DA in the PFC and NAc using in vivo perfusion dialysis. These experiments were performed in chloral hydrate anesthetized rats and extracellular levels of DA were monitored simultaneously in PFC and NAc. Amphetamine (1 mg/kg i.v.) increased the extracellular DA levels in the NAc by 200-300% and in the PFC by 100-200%. Cocaine (1 mg/kg i.v.) increased DA levels in the NAc by 200-300% whereas very small or no effects were observed in the PFC (0-10%). Higher doses of cocaine (3-4 mg/kg i.v.) were fatal and lower doses (0.5 mg/kg i.v.) had no effect on the levels of DA in the PFC. These results indicate that systemic administration of cocaine has a more profound effect on the extracellular levels of DA in the NAc than in the PFC.

294.7

RELEASE OF DOPAMINE FROM STRIATUM OF FREELY MOVING RATS AFTER SINGLE AND REPEATED HALOPERIDOL. H. Tilson, M. K. Stachowiak, W. Zhang*, and J. S. Hong (SPON: R. King). Lab. Molec. Integ. Neurosci., NIEHS, Research Triangle Park, NC 27709.

The effects of acute or repeated administration of haloperidol on release of dopamine (DA) was studied in rats using a microdialysis procedure. 24 hrs after unilateral striatal implantation, rats were perfused with artificial CSF for 4 hrs; haloperidol (1 mg/kg, i.p.) was given and perfusate collected for 4 more hrs. Haloperidol produced a time-dependent increase in DA, peaking 1 hr after dosing, while HVA levels were still elevated 4 hrs later. Increase in DA but not HVA release was blocked by chloral hydrate anesthesia (300 mg/kg, i.p.). In another experiment, rats received 21 daily injections of haloperidol (1 mg/kg, i.p.) and were perfused 24 hrs after last injection. Repeated injections elevated basal DA release but not HVA. Acute administration of haloperidol to rats repeatedly treated with this drug failed to increase DA release, while HVA release increased. These observations suggest that repeated exposure to haloperidol causes a compensatory increase in extracellular DA release. The association of the inability of haloperidol to acutely increase DA release in those animals with the development of behavioral tolerance warrants further study.

294.8

REGIONAL DIFFERENCES IN THE EFFECT OF TYPICAL AND ATYPICAL NEUROLEPTICS ON TERMINAL RELEASE OF DOPAMINE: IN VIVO MICRODIALYSIS STUDIES. B.S. Bunney and B. Moghaddam. Depts. of Pharmacol. & Psychiat., Yale Univ. Sch. Med., New Haven, CT.

In vivo microdialysis has been used to study the acute effect of antipsychotic drugs on the release of dopamine (DA) from the nucleus accumbens (NAc), striatum, and prefrontal cortex (PFC). As previously shown by other investigators, we found that low doses of l-sulpiride (10-20 mg/kg i.v.) and haloperidol (0.05-0.1 mg/kg i.v.) cause a marked increase in striatal DA extracellular levels. However, we found that these low doses did not effect the release of DA from NAc and PFC. Higher doses of sulpiride (150-200 mg/kg i.v.) were required to effectively increase the extracellular levels of DAin the PFC. The effect of various doses of haloperidol on the release of DA from PFC and NAc is currently under investigation. The atypical neuroleptic clozapine, at low doses (5-10 mg/kg i.v.), had a larger effect on the release of DA from NAc than striatum. Our preliminary results indicate that clozapine (10 mg/kg) also increases the extracellular DA levels in the PFC. These data suggest that atypical neuroleptics are more effective than typical neuroleptics in inducing release of DA from the terminals of A10 dopaminergic neurons.

294.9

ENHANCEMENT OF IN VIVO DOPAMINE RELEASE FROM THE RAT STRIATUM BY 6R-L-ERYTHRO-TETRAHYDROBIOPTERIN AS STUDIED BY BRAIN DIALYSIS. K.Koshimura*, S.Miwa*, K.Lee*, M.Fujiwara* and Y.Watanabe*,¶ (SPON:K.Imamura). Dept. of Pharmacol., Kyoto Univ. Sch. of Med., Kyoto 606 and ¶Dept. of Neurosci., Osaka Bioscience Institute, Suita, Osaka 565, Japan.

Intracerebroventricular injection of 6R-L-erythro-tetrahydrobiopterin (BH_4), a cofactor for tyrosine hydroxylase, increases catecholamine (CA) synthesis (Miwa, S. et al., Arch.Biochem.Biophys., 239:234, 1985). In the present study, to clarify effects of BH_4 on CA release, we examined effects of BH_4 on in vivo dopamine (DA) release from the striatum of male Wistar rats using brain dialysis. DA in perfusates was assayed using HPLC with electrochemical detection after purification with alumina batch method. BH_4 applied in the perfusion fluid (0.1 mM - 5 mM) dose-dependently increased DA concentration in perfusates (2-fold increase for 0.1 mM BH_4, 5-fold for 0.5 mM, 10-fold for 1 mM and 60-fold for 5 mM), whereas biopterin had little effect. The increase was abolished by tetrodotoxin (10 μM in perfusion fluid) but was persistent after inhibition of tyrosine hydroxylase by α-methyl-p-tyrosine or inhibition of DA uptake by nomifensine. These results show that BH_4 enhances DA release in the brain and that this enhancement is not a result of an increase in DA synthesis or inhibition of DA uptake but a result of direct effects of BH_4 on the mechanism of DA release.

294.10

DIFFERENCES IN THE EFFECTIVE SURFACE AREA OF MICRODIALYSIS PROBES IN VIVO VERSUS IN VITRO. G.M. Alexander, J.R. Grothusen* and R.J. Schwartzman. Dept. of Neurology, Jefferson Medical College, 1025 Walnut St., Phila, PA 19107

The relative efficiency of microdialysis probes in vivo differs from that determined in vivo. The relative efficiencies were determined both in vitro and in vivo using tritiated water (THO). THO freely distributes throughout the fluid spaces of an experimental animal and, at equilibrium, the brain extracellular concentration of THO is the same as the plasma concentration. Microdialysis probes were inserted into the right caudoputamen of anesthetized rats. The rats were injected with THO and after one hour microdialysis samples were collected. The in vitro relative efficiency for THO was computed as the ratio of the THO concentration in the dialysate to that of the solution the probe was immersed in. The in vivo relative efficiency was computed as the ratio of the THO concentration in the brain dialysate to that measured in the plasma. The ratio of the in vivo to in vitro relative efficiencies is both flow and temperature dependent. This study demonstrates how the effective surface area can be computed at any probe flow rate and how it can be used as a correction factor.

294.11

TEMPERATURE EFFECTS ON THE CALIBRATION OF MICRODIALYSIS PROBES T. J. Parry* and J. G. McElligott (SPON: R. Murray) Temple University School of Medicine, Dept. of Pharmacology, Philadelphia, PA 19140.

Temperature, an often ignored variable, is important for *in vitro* calibration of microdialysis probes since thermodynamic principles dictate that the diffusion coefficient and percent recovery of analyte vary with temperature. This study illustrates the influence of temperature on percent recovery and stability of catecholamines and indolamines during microdialysis probe calibration. Standards containing norepinephrine (NE), dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindolacetic acid (5-HIAA), dopamine (DA), and serotonin (5-HT) were prepared (1 to 0.1 μM) with a deaerated Krebs-bicarbonate buffer with glutathione and cysteine as antioxidants in open test tubes. Microdialysis probes (membrane size; 250 μm OD X 3mm length, total probe dead volume ≈7 μl), constructed in our laboratory, were immersed in the above standards and incubated at 21.5, 39, and 50°C for ten minutes. The dialysate (10 μl), collected at a flow rate of 1 μl/min, was placed in 5μl of 0.1M $HClO_4$ containing an internal standard (dihydroxybenzylamine DHBA) and was analyzed immediately by HPLC-EC. Percent recovery was calculated from temperature matched standards. The percent recovery of 5-HT and 5-HIAA rose in a non-linear fashion when compared to standards at 21.5 and 50°C (for 5-HT from 10.1% to 18% and for 5-HIAA from 15.8%, to 25%). The percent recovery of catecholamines also rose until a temperature of 39°C but then decreased at 50°C. We have subsequently found that the catecholamines in standard solutions even with antioxidants break down over the ten minute incubation time at elevated temperatures (20 to 50°C) while the indolamines remain stable. Results show that it is essential to calibrate microdialysis probes *in vitro* at brain temperature and to compare them with standards at the same temperature in order to minimize an underestimation of neurotransmitter and metabolite concentrations.
(Supported by a grant from NIH S07-RR05417)

294.12

PHARMACOLOGICAL MODIFICATIONS OF CATECHOLAMINERGIC METABOLISM MEASURED BY IN VIVO VOLTAMMETRY AND MICRODIALYSIS IN THE ROSTRAL VENTROLATERAL MEDULLA OBLONGATA OF ANAESTHETIZED RATS. L.Lambás-Señas*, JY.Gillon*, JP.Bouilloux*, M.Seccia*, L.Quintin and B.Renaud. Neuropharmacologie, Univ. Claude Bernard and CNRS, Lyon, France.

Two recent in vivo techniques are available to measure central catecholaminergic (CA) metabolism: voltammetry and microdialysis. We used these two techniques to monitor the CA metabolism of the C1 adrenergic neurons located in the rostral ventrolateral medulla oblongata (RVL). In vivo voltammetry allowed the recording, every 2 min, of a catechol oxidation peak (CA.OC) (+55mV) in the C1 group. Microdialysis allowed the measurement (by HPLC-ED) of 3,4-dihydroxyphenylacetic acid (DOPAC) released in the RVL during 10 min periods. Pharmacological manipulations showed that: i) the tyrosine-hydroxylase inhibition(∝-MPT, 250 mg/kg,i.p.) and the MAO inhibition (pargyline, 75mg/kg, i.p.) suppressed both the CA.OC and the extracellular DOPAC, ii) the dopamine-β-hydroxylase inhibition (FLA 63, 30mg/kg, i.p.) doubled both the height of the CA.OC and the extracellular DOPAC. The changes in CA.OC (voltammetry) and extracellular DOPAC (microdialysis) exhibited very similar time courses in the 3 experiments. These results confirm that, as previously suggested, DOPAC is the catechol responsible for the in vivo oxidation current observed in the C1 group. Additional validation experiments are in progress to determine if the combination of in vivo voltammetry and microdialysis provide a useful approach of the CA metabolism of the C1 adrenergic neurons.

294.13

DIFFERENTIATION OF DOPAMINE OVERFLOW AND UPTAKE IN VIVO. L.J. May*, W.G. Kuhr and R.M. Wightman. Dept. of Chemistry, Indiana University, Bloomington, IN 47405.

Fast-scan cyclic voltammetry with Nafion-coated,carbon-fiber microelectrodes was used to measure the stimulated overflow of dopamine (DA) in vivo. DA concentrations in the extracellular fluid of the rat caudate nucleus were sampled in less than 10 ms at 100-ms intervals. Overflow of DA was induced by electrical stimulation of the medial forebrain bundle with 120-pulse trains of 300-uA biphasic pulses. Stimulated overflow was measured as a function of stimulus frequency both before and after administration of various pharmacological agents. The maximum DA observed at frequencies between 9 and 60 Hz was correlated with the maximum DA predicted by a simple uptake/overflow model. Observed overflow was assumed to be a function of DA overflow per stimulus pulse and the kinetics of cellular DA uptake. Correlation of experiment with simulation was obtained at 95% confidence for an increase in Km after administration of 20 mg/kg nomifensine or 25 mg/kg bupropion, whereas an equivalent increase in DA overflow per pulse did not. Results obtained following administration of 1-DOPA methyl ester were best modeled by an increase in DA overflow per pulse. Effects of GBR 12909 best fit an increase in DA available for overflow.

Supported by NSF BNS 86-06354.

294.14

ASCORBATE INTERFERES WITH DOPAMINE DETECTION AT NAFION-COATED ELECTRODES. D.J. Wiedemann, A. Basse-Tomusk, R.M. Wightman & G.V. Rebec, Dept. Chem. & Psychol., Indiana University, Bloomington, IN 47405. (SPON: R.S. Gurd)

Attempts to measure the low extracellular concentrations of dopamine(DA, 20 nM) using in vivo voltammetry have been hindered by the high levels of ascorbate (AA, 300-400 uM) present in DA terminal areas. The advent of nafion, a cation exchange polymer which can be used to coat electrodes, has greatly improved the ability to selectively detect DA under certain conditions (Gerhardt et al., Brain Res.,290: 390,1984).

In this study, the voltammetric response of nafion-coated microdisk electrodes to varying concentrations of DA and AA was measured (scan rate 812 mV/s or 100 mV/s). While many of these electrodes were able to detect extremely low concentrations of DA (20-50 nM), no electrode tested (n=20) was capable of rejecting 100 uM AA regardless of film thickness. In fact, current produced by 100 uM AA was of similar magnitude to that produced by 250 nM DA. Several of these electrodes were lowered into the striatum of urethane-anesthetized rats and the response to 2.5 mg/kg d-amphetamine monitored. In some cases, a peak resembling DA appeared during the first 10 min of the response but this was always subsequently overwhelmed by a peak resembling AA. Thus, these limitations must be considered when using this technique.

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294.15

INVERSE RELATIONSHIP BETWEEN ASCORBATE AND DIHYDROXYPHENYLACETIC ACID IN THE STRIATUM OF THE RAT. A. Basse-Tomusk & G.V. Rebec, Dept. Psychol., Indiana University., Bloomington, IN 47405

Accumulating evidence suggests that ascorbate (AA) modulates dopaminergic transmission in the striatum (Gardiner et al., Brain Res., 344:181, 1985). In this study in vivo voltammetry was used to study the regional distribution of AA as it relates to the distribution of the dopamine metabolite, dihydroxyphenylacetic acid (DOPAC). In each rat (n=20), an electrochemically modified carbon-fiber electrode was lowered through one of four regions which were selected to sample the entire extent of the striatum. During each dive, voltammetric scans were made at 1 mm increments to estimate extracellular DOPAC and AA concentrations at each dorsoventral level.

Within each striatal region, there was a large amount of variability in AA levels (200-500 µM) between animals. However, within individual animals, a consistent pattern emerged in all areas. AA levels were highest at the most dorsal and most ventral extent of the striatum. The opposite was true for DOPAC. Because DOPAC may serve as an index of dopamine turnover, our data suggest that AA levels are inversely related to dopamine turnover in the striatum.

Supported by DA 02451 and BNS 87-11240.

294.16

SELF-STIMULATION OF THE VENTRAL TEGMENTUM AND CONCURRENT RELEASE OF DOPAMINE MEASURED BY IN VIVO ELECTROCHEMISTRY. **C.D. Blaha[1,2], A.G. Phillips[1], and H.C. Fibiger[2], Depts. of Psychology[1] and Psychiatry[2], University of British Columbia, Vancouver, BC, Canada, V6T 1Y7.**

Ex vivo studies of dopamine (DA) turnover and metabolism support the dopaminergic hypothesis of brain-stimulation reward in the VTA. We examined this hypothesis by monitoring extracellular DA concentrations during intracranial self-stimulation (ICS) using in vivo chronoamperometry (10-60 sec sampling rate). Male rats were implanted with a bipolar stimulating electrode in the VTA and a stearate-modified graphite paste recording electrode in the ipsilateral N. accumbens.

Initiation of ICS was accompanied by an immediate increase in the electrochemical DA signal. The DA signal remained elevated over the course of a 10 min period of ICS using a fixed current intensity (60Hz, 0.2s, 15-25uA test range) and returned to baseline levels within 5 min during extinction; a positive correlation was seen between changes in DA release and ICS response rates. Rate-intensity experiments also revealed a corresponding increase in both the ICS rates and DA release levels with each successive increase in current intensity (10-40uA, 5uA step/10 min). These data provide the first demonstration of DA release in the N. accumbens during ICS of DA cells in the VTA. Accordingly, these findings are consistent with a role for DA in brain-stimulation reward.

294.17

IN VIVO ELECTROCHEMICAL DETECTION OF DOPAMINE WITHIN THE NUCLEUS ACCUMBENS OF RATS SELF-ADMINISTERING COCAINE. J.M. Finlay, H.C. Fibiger, C.D. Blaha and A.G. Phillips. Div. of Neurol. Sci., Depts. of Psychiatry and Psychology, Univ. of British Columbia, Vancouver, BC, V6T 1W5.

Intravenous self-administration (SA) of cocaine is thought to be mediated by an increase in extracellular dopamine (DA) concentrations within the terminal regions of mesolimbic DA neurons. Lesions and pharmacological manipulations of mesolimbic DA neurons have been used to provide indirect support for this hypothesis. In the present experiment, in vivo electrochemistry was used to directly monitor extracellular DA concentrations within the nucleus accumbens of rats self-administering cocaine.

During daily 3-hr sessions rats were allowed to bar press for intravenous infusions of cocaine. When responding had stabilized, a stearate-modified carbon paste electrode was implanted within the anteromedial nucleus accumbens. Each rat was then given access to cocaine and DA concentrations were determined at 60 s intervals using chronoamperometry. Intravenous SA of cocaine was associated with a significant increase in extracellular DA concentrations within the nucleus accumbens.

The ability to monitor changes in extracellular DA concentrations associated with drug SA should permit the further characterization of mesolimbic DA neurons in reinforcement processes.

294.18

DOPAMINE DIFFUSION IN TURTLE BRAIN MEASURED WITH HIGH-SPEED VOLTAMMETRY. M.E. Rice and C. Nicholson. Physiology & Biophysics, NYU Med. Center, New York, NY 10016.

Dopamine (DA), besides acting as a classical neurotransmitter at discrete synapses, may also act as a neurohumoral agent on neurons distant from a release site. To estimate the range of DA influence, parameters affecting DA diffusion, including tortuosity (λ) and extracellular volume fraction (α) of the tissue, uptake and interactions with the extracellular matrix, must be determined. Previous work suggests that these factors may vary between brain regions, such that diffusion is less efficient in regions with high DA uptake like rat striatum (Rice et al., Neurosci., 15:891, 1985) compared to cortex (Dayton et al., J. Electroanal. Chem., 146:189, 1985). Using 8µm carbon fiber electrodes with fast-scan (300 V/s) cyclic voltammetry, we examined iontophoretically introduced DA diffusion in isolated regions of turtle (Pseudemys scripta and Chrysemys picta) brain, including cerebellum, olfactory bulb and frontal cortex. Curve-fitting based on simplex optimization of α, λ and concentration dependent uptake (k') revealed that DA diffusion was largely governed by the same parameters as the standard extracellular marker, tetramethylammonium (Nicholson & Phillips, J. Physiol. 321:225, 1981). These data imply that DA may diffuse relatively freely in turtle brain and hence may exert its influence over considerable distances. Supported by NS-13742.

296

WORKSHOP: CODING VISUAL SIGNALS AT THE PHOTORECEPTOR SYNAPSE. P. Sterling, UPenn and D. Copenhagen, UCSF (Co-chairmen); M. Wilson, UC Davis; A. Kaneko, Natl. Inst. Physiol., Okazaki; A. Stuart, UNC.

The photoreceptor axon terminal integrates neural signals from many sources: currents from the inner segment and adjacent photoreceptors, and feedback chemical signals from second order neurons. This complexity most likely relates to the problem of encoding an optical image whose intensity can vary over many log units using membrane potentials that can vary by only tens of millivolts. Wilson describes how electrically coupled photoreceptors filter voltage-gated photocurrents and suggests that feature extraction begins at the first synapse (salamander). Copenhagen treats the kinetics and gain of synaptic transfer from photoreceptors to second order neurons and explains the different issues in transmitting small and large signals (turtle, toad). Kaneko reports that the presumed photoreceptor transmitter (glutamate) itself excites cone terminals and suggests a possible role for this chemical signal in rod-cone interactions (fish). Stuart compares problems faced by an invertebrate visual system with those of the vertebrate and summarizes the physiological basis for their solution (barnacle). Sterling describes cone circuits to the ganglion cell and computations to suggest that the ganglion cell's contrast sensitivity originates in the cone axon terminal (cat).

297

SYMPOSIUM. SPECIFICITY IN THE CONTROL OF REGIONAL SYMPATHETIC OUTFLOW: PHYSIOLOGICAL, NEUROCHEMICAL AND ANATOMICAL APPROACHES. L.C. Weaver, Robarts Research Institute and Univ. of Western Ontario (Chairperson); R.M. McAllen*, Univ. of Melbourne; S.M. Barman, Michigan State Univ.; T.L. Krukoff, Univ. of Alberta; E.M. McLachlan*, Univ. of New South Wales.

The sympathetic nervous system provides discrete control of cardiovascular function by means which remain unknown. What characterizes a vasomotor sympathetic neuron? How can target organ specificity of central sympathetic neurons be determined? Do anatomical or neurochemical distinctions provide clues about the specificity of neuron function? Dr. McAllen will describe responses to stimulation of groups of brainstem neurons which demonstrate differential control of hindlimb skin and skeletal muscle vessels. Dr. Barman will discuss relationships between firing of brainstem neurons and different sympathetic nerves and selective medullo-spinal projection patterns. Dr. Weaver will provide evidence that visceral postganglionic neurons receive differential tonic and reflex inputs. Dr. Krukoff will correlate information about neurochemicals contained within sympathetic preganglionic neurons and terminal near them with possible functions of these neurons. Dr. McLachlan will demonstrate that characteristics of channels within postganglionic cell membranes provide these neurons with physiological properties that may distinguish them functionally.

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY IV

298.1

THE FATE OF THE SUBPLATE, A TRANSIENT STRUCTURE IN THE DEVELOPING NEOCORTEX. J.J.M. Chun and C.J. Shatz, Dept. of Neurobiology, Stanford Univ. Sch. of Medicine, Stanford, CA 94305.

During the development of the neocortex, the region of the future white matter directly beneath the cortical plate (CP) contains a subdivision called the subplate (SP). The SP includes a transient population of early-generated cells that are also immunoreactive (ir) for the neuron-specific marker MAP2, as well as for several neuropeptides (Chun et al.,1987, Nature 325, 617-620). Here, immunolabeling is used to examine the fate of this special population. The SP, as defined by MAP2-ir, increases in width relative to the CP between E46 and E56 and then decreases most rapidly during the first 4 postnatal weeks. Between P0 and P401, the density of MAP2-ir cells within the white matter of the lateral gyrus decreases by 5.5X. There is also a decrease in the density of peptide-immunoreactive cells within the SP during the same period: somatostatin (SRIF)- 5X; NPY-23X. SRIF-ir cells comprise a constant fraction of all MAP2-ir cells throughout postnatal life (22 to 23%), whereas the fraction of NPY-ir cells decreases between P0 and P401 (18 to 4%). These changes in cell density occur within a region of the white matter that increases in area by only 2X. Thus, the disappearance of the SP cannot be due to simple dilution. Cell death must also contribute significantly. (NIH Grants EY02858 to C.J.S. and GM07365 to J.J.M.C.)

298.2

RELATIONSHIP OF GENICULOCORTICAL AFFERENTS TO DEVELOPING NEURONS IN THE FERRET VISUAL CORTEX. Jean D. Peduzzi. Dept. of Physiol. Optics, School of Optometry, University of Alabama at Birmingham, Birmingham, AL 35294.

Previous studies (Jackson, Peduzzi & Hickey, 1984; Jackson & Hickey, 1986) described the position of neurons of a given birthdate in the developing and adult ferret visual cortex. In this study, the relationship of geniculocortical (GC) fibers (identified by a TMB reaction following embryonic or postnatal intraocular injections of WGA-HRP) to particular cortical neurons (identified by autoradiography after ^{3}H-thymidine injections) was examined. The GC afferents do not reach the area of the presumptive visual cortex until postnatal day 3 (P3). The pattern of GC fibers can be divided into 4 primary stages. The first stage is a "waiting period" (from P3-P7) where GC afferents are waiting or expanding in the intermediate zone but have not reached the cortical plate. The second stage is a "widely dispersed stage" (from P10-P15) where GC fibers are found throughout the primitive cortex. The widespread distribution of fibers affords the possibility of interaction with virtually all visual cortical neurons during this stage. The third stage is a "right place-wrong amount stage" (from P20-P36) where GC fibers are present in the normal geniculo-recipient layers but the projection to layer VI is much greater than that found in the adult. The fourth stage is the adult pattern where the major geniculate projection is to layer IV and minor projections to layers I and VI. (EY01338 and EY03039)

298.3

PRENATAL DEVELOPMENT OF AXONAL PROJECTIONS FROM THE CAT'S VISUAL CORTEX. S.K. McConnell and C.J. Shatz. Dept. of Neurobiology, Stanford Med. School, Stanford, CA 94305.

In the adult cat, neurons in the visual cortex project with remarkable specificity to cortical and subcortical targets. We have examined the development of these axonal projections, using either [3H]-leucine (injected into the visual cortex of living fetal or neonatal kittens) or the fluorescent tracer dil (placed in fixed prenatal brains).

The first cortical axons reach the thalamus by E39. Labeled axons have arrived in the vicinity of all cortical and subcortical targets by E52, at which time some axons (e.g., those to the superior colliculus and claustrum) have invaded their targets, whereas other sets of axons--most strikingly, those headed for the lateral geniculate nucleus and contralateral cortical areas--accumulate in regions just outside the target itself. Cortical axons invade the LGN at around E59, while both callosal axons and ipsilateral corticocortical axons continue to wait beneath the cortical plate well into postnatal life. A transient aberrant projection was observed prenatally, when some axons from visual cortex are misrouted from the corpus callosum into the fornix.

Cortical axogenesis in the cat thus shows three characteristic features: directed outgrowth to normal targets, early "waiting periods" in which axons accumulate outside the target, and early "exuberant" axonal projections that are eliminated in later life. (Supported by NIH EY02858 and EY06028.)

298.4

LOCALIZATION OF CYTOCHROME OXIDASE IN MACAQUE STRIATE CORTEX DURING PRENATAL DEVELOPMENT. M. Wong-Riley, T. Trusk, and D. Hoppe*. Dept. Anatomy & Cellular Biology, Med. Col. of WI, Milwaukee, WI 53226.

The mature pattern of cytochrome oxidase (C.O.)-rich laminae and supragranular puffs of macaque striate cortex is present with slight modifications at birth (Kennedy et al., '85). We sought to determine if C.O. is expressed in early prenatal development and whether the pattern changes over time. Twelve fetal macaque monkeys at 4 age groups were examined: (1) Embryonic day (E) 70, when layers V and IVC neurons had attained their final positions; (2) E101-103, when the last striate neurons had been generated; (3) E135, when most geniculocortical fibers had invaded the cortex (Rakic, '74;'76); and (4) E158-160, 1 week before birth. We found that at E70, the major site of high C.O. activity was in upper II, where small, spindle-shaped cells with darkly reactive mitochondria were aggregated. The outer half of I was moderately reactive, while the rest of the cortical plate was only lightly reactive. At E101, upper II retained its dark staining, and a moderately dark band appeared in VI and upper half of V. C.O. activity in the cell dense IV was quite low. By E135, however, IVA, IVCα and the lower border of IVCβ (IVCβ_{dark}) and VI were distinctly reactive, while the rest of IVCβ, II/III, and I were only lightly reactive. IVB and V were unusual in that IVB was wider at this stage, and both of these lightly reactive layers had aggregates of C.O. reaction product along their midportions. No distinct puffs were found at this age. Finally, between E158 and E162, the newborn pattern of C.O. was achieved with dense staining in supragranular puffs, IVA, IVCα, IVCβ_{dark}, and VI. Most neurons within puffs contained reactive mitochondria at this age. Since puffs are present at E143 (Horton, '84), they must have developed sometime between E135 and E143, approximately 3.5 weeks before birth. [Supported by NIH EY05439 to MWR. Fetal brains were gifts of Dr. S.A. Sholl (NIH HD18865 & RR00167)]

298.5

SENSITIVE EXTRACELLULAR RECORDINGS OF SPONTANEOUSLY ACTIVE PYRAMIDAL AND NONPYRAMIDAL NEURONS IN EMBRYONIC VISUAL CORTEX. M.G. Blanton and A.R. Kriegstein. Dept. of Neurology, Stanford University, Stanford, CA 94305-5300.

An understanding of normal and disrupted neuronal development requires information on the physiological behavior of individual developing neurons. Using a non-invasive extracellular patch clamp technique (Forda et al, Brn Res 249: 371, 1982), we obtained sensitive, high resolution unit recordings from slabs of embryonic turtle visual cortex, chosen for its simple cytoarchitecture and resistance to hypoxia. Our capacity to visualize the tissue allowed selection of specific neuronal types, permitting anatomical-physiological correlations. During early cortical plate formation, identified nonpyramidal cells fired periodic repetitive bursts of rapid action potentials (APs), while pyramidal units fired single, relatively slow APs. With development, AP duration shortened and firing patterns of the cell types became more distinct. Evoked activity patterns suggested that nonpyramidal GABA-mediated inhibition of pyramidal cells was functional early, when synapses were still sparse. Presumed synaptic currents, graded and often associated with APs, became more frequent during development. Similar spontaneous APs and synaptic currents were recorded in early developing rat neocortex, indicating that distinct patterns of nonpyramidal and pyramidal spontaneous electrical activity may be a general feature of early cortical development in higher vertebrates.

298.6

EFFECTS OF VISUAL DEPRIVATION ON SYNAPTIC ACTIVITY IN THE RAT VISUAL CORTICAL SLICE. R. L. Berry and T. J. Teyler. Dept. of Neurobiology, NE Ohio Univ. College of Medicine, Rootstown, Ohio 44272.

Visual deprivation effects, widely studied in intact cat cortex, have not been studied with in vitro slice techniques. The present study investigates the effects of monocular (MD) and binocular deprivation (BD) on the electrophysiology of rat primary visual cortical slices.

Long Evans hooded rats were given monocular or binocular lid suture (or no treatment) before natural eye opening and reared to 3-6 mos. Animals were then sacrificed and cortical slices were obtained and maintained in slice chambers where white matter (geniculocortical fibers) underlying cortical area OCl was stimulated and cortical field potential profiles were obtained. Integration of current sinks obtained by current source density analysis provided laminar estimates of synaptic activity. Slices from cortex opposite the sutured eye in MD rats demonstrated a marked reduction in multisynaptic (5-40 msec post stimulus) layer II-IV synaptic activity when compared with slices from the experienced cortex and from BD and normally reared rats. BD slices exhibited near normal patterns of responses. These results indicate that in rat, as in cat, MD is much more potent than BD in reducing responsivity of cortical neurons--a surprising finding considering that in cats this difference is attributed to binocular competition (in MD and not BD) not likely to play a major role in the much less binocular rat. This research was supported by ONR Grant #86K0664.

298.7

NMDA-EVOKED CALCIUM UPTAKE BY SLICES OF KITTEN VISUAL CORTEX MAINTAINED *IN VITRO*. J.E. Sherin*, D. Feldman* and M.F. Bear, Center for Neural Science, Brown University, Providence, RI 02912.

We have developed a neurochemical assay of NMDA receptor effectiveness in the visual cortex of kittens. Slices of visual cortex are prepared and maintained *in vitro*, and uptake of ^{45}Ca is monitored as different concentrations of NMDA are applied. We find that Ca uptake is saturated by a 2 minute exposure to 50 μM NMDA; the maximum value attained is ~5.4 nmoles Ca/ mg protein which is 145% of basal uptake (3.7 nmol/mg). NMDA-stimulated uptake is significantly reduced by the selective NMDA receptor antagonist AP5 in concentrations ≥ 50 μM. In contrast, Ca uptake evoked by 62.5 mM K (144% of control) is unaffected by 100 μM AP5.

Both norepinephrine (NE) and NMDA receptors are thought to contribute to the mechanisms underlying experience-dependent plasticity in the visual cortex during the critical period. We therefore examined the effects of NE on NMDA-evoked Ca uptake. Slices were pretreated for 5 min. with different concentrations of NE before NMDA and ^{45}Ca were added. 50 μM NE (in 0.5 mM ascorbate) doubled the Ca uptake evoked by 25 μM NMDA, from 113±2 % to 128±2 % of basal levels. These data support the hypothesis that NE exerts its effects on synapse modification, at least in part, by modulating the effectiveness of visual cortical NMDA receptors.

Rearing kittens in the dark from birth leaves the visual cortex largely unresponsive to retinal stimulation, but also highly modifiable by subsequent visual experience. To assess the functional state of NMDA receptors in striate cortex under these conditions, we assayed NMDA-stimulated Ca uptake in tissue taken from P31-40 dark-reared (DR) kittens. We found that Ca uptake evoked by 50 μM NMDA in slices from DR kittens was only ~1/3 that of control (116±4 % vs. 145±7 % of basal). These data suggest that the increased modifiability of DR cortex is not explained simply by an increase in the sensitivity or number of NMDA receptors. (Supported by ONR contract N00014-81-K0136 and NIH grant NS06929)

298.8

PHARMACOLOGICAL MODULATION OF LONG-TERM POTENTIATION IN SLICES OF VISUAL CORTEX. Barry W. Connors and Mark F. Bear, Section of Neurobiology and Center for Neural Science, Brown University, Providence, RI 02912.

Neocortex displays a remarkable degree of experience-dependent plasticity. Accumulated evidence suggests that some types of modification require the presence of noradrenergic or cholinergic inputs. In addition, cortical NMDA receptors and the general level of GABA-mediated inhibition have been implicated. A crucial issue concerns the mechanisms by which these variables interact to orchestrate synaptic modifications in visual cortex.

Slices of area 17 were prepared from kittens aged 32 to 56 days of age, or from adult rats, and maintained *in vitro*. Bipolar stimulating electrodes were placed at the white matter-layer VI border and field potentials were recorded in layer III. Baseline responses to stimulation in the range of 15 to 50 μA were collected every 30 sec. for ≥10 min., followed by conditioning stimulation applied at twice the test intensity. A wide range of stimulation parameters were tried, ranging from 2 Hz for 10 min. to repeated bursts of 50 Hz stimulation. In general, conditioning stimulation of any type was ineffective in eliciting potentiation unless a small amount of the $GABA_A$ receptor antagonist bicuculline methiodide (BMI) was added to the bathing solution. When BMI was present in concentrations ranging from 0.3 to 1 μM, conditioning stimulation of 4 Hz for 30-60 sec. was most effective in producing a lasting (>30 min.) potentiation (LTP). LTP always followed a period of post-tetanic depression that lasted several minutes. The induction of LTP was most probable when facilitation and afterdischarge occurred during the tetanus. Addition of 10 μM norepinephrine (NE; in 20μM ascorbate) to the bath had no effect on LTP induction in the absence of BMI. However, NE did reliably lower the threshold concentration of BMI required for conditioning stimulation to elicit potentiation. In two experiments, slices treated with BMI and NE were conditioned in the presence of 50-100 μM AP5, a selective NMDA receptor antagonist. No facilitation or afterdischarge occured during the tetanus and no LTP was induced. However, LTP could be induced after the AP5 was washed out.

Taken together, these results suggest that long-term, activity-dependent synaptic potentiation in neocortex requires activation of NMDA receptors that are relatively ineffective unless network inhibition is slightly suppressed. NE facilitates these modifications by reducing the requirement for disinhibition. (Supported by the Klingenstein Fund, and NS25983 and NS06929 from the NIH)

298.9

THE LOCATION AND FUNCTION OF NMDA RECEPTORS IN CAT AND KITTEN VISUAL CORTEX. K. Fox, H. Sato and N. W. Daw. Dept. Cell Biol., Wash. Univ. Med. Sch., St. Louis MO 63110. We studied the effects of NMDA antagonists in cat and kitten area 17 to assess the hypothesis that NMDA receptors play a role in cortical plasticity. Carbon fiber micro-electrodes were used to record from neurons and apply D-APV. The standard drug trial of 10nA for three minutes was ineffective on kainate responses but abolished equipotent NMDA induced excitation. Sensitivity to APV was correlated with cortical layer but not with receptive field type, stimulus evoked firing rate, or sequence within penetration, and with age only in layer IV. Layers II and III of 28-35 day kittens and adults were more sensitive to APV than were deeper layers: visual responses were reduced to 10-45% of control at all ages. Layer IV cells were insensitive to APV in adults but in kittens 57% showed small but significant reductions in response (55-70% of control). Preliminary results suggest layer IV may be more sensitive in 21 day kittens. Spontaneous activity was reduced more than visual response in both kittens and cats. Cells which showed no response to 10nA of APV were unaffected by higher doses. The developmental time course of NMDA receptor efficacy in layer IV more closely follows that of geniculo-cortical axon segregation than the critical period. The persistence of NMDA receptors in layers II and III of adult may indicate a role in the transmission of sensory input to these layers rather than plasticity.

298.10

POSTNATAL DEVELOPMENT OF GABAERGIC SYNAPSES IN THE VISUAL CORTEX (AREA 17) OF NORMAL AND DEPRIVED CATS. R.E. Kalil and A.N. Lies*. Dept. of Ophthalmology and Neurosciences Training Program. Univ. of WI, Madison, WI 53706.

GABAergic inhibitory mechanisms are thought to play an important role in molding the response selectivity of cortical neurons. With the light microscope, GABA+ cells are evident in area 17 of the newborn kitten, but their maturation, as gauged by cell body growth, is not complete until at least 8 weeks postnatal. At present there is little information available concerning the morphological development of GABA+ synapses in visual cortex. We have therefore combined electron microscopy with immunocytochemistry to study this problem directly.

Antibodies raised against GABA conjugated to bovine serum albumin were used to stain cortical neurons in normal cats that ranged in age from newborn to adult. Also studied were cats that had been reared from birth to adulthood with binocular eyelid suture. GABA+ cells and processes are present in the cortex of normal animals at all ages studied. In cats younger than 4 weeks, presynaptic GABA+ terminals are not prominent, although stained cell somas and dendrites often are postsynaptic. During the second postnatal month, GABA+ terminals increase in size and frequency. By 8 weeks, they are qualitatively similar to those in adult cats. Long-term deprivation appears to have no major effect, suggesting that normal visual experience is not essential for the development of GABA+ synapses in area 17.

298.11

Postnatal development of Substance P-ir structures in the cat visual cortex:neurons with ascending axons become less and less detectable. P.Wahle*,C.Sanides*,K.Albus, MPI fuer Biophysik. Chemie/110, Am Fassberg, 3400 Goettingen, FRG.

In 30 cats aged postnatal day (P) 0 to adulthood SP-ir was demonstrated using the PAP-method.SP-ir cells are first detected at P2 in early stages of morphological differentiation in layer V. During the following 2 weeks more and more neurons become SP-ir and differentiate. As a consequence SP-ir axons and terminals increase in density,and the staining intensity increases reaching a peak from P 12-20. Neurons now are completely labeled. The somata sit in layers V/VI, they are bitufted to multipolar. The axon arises from the upper pole,even from secondary dendrites.Initially issued recurrent collaterals form a dense terminal plexus in layer V, further collaterals ascend as a bundle to superficial layers , where a second terminal plexus forms in II/III. Fibers reach layer I. From P 15 onwards cells become lighter and lighter labeled and decrease in number. Until the end of the third month the innervation pattern has disappeared; only occasionally could a faintly labeled soma be detected in the 12 month old cat. The concurrent analysis of Somatostatin-ir structures, however, reveals the presence of a cell type that displays the same morphological variability, same axonal projection and postnatal chronology of appearance. It can be easily detected in the young and adult cat cortex.This suggests that a persisting cell type that uses SOM/GABA as transmitter expresses high amounts of SP during the first three postnatal month.

298.12

DEVELOPMENT OF ASTROCYTES IN THE CAT VISUAL CORTEX. C.M. Müller*, A.K. Engel* and W. Singer (SPON: European Neuroscience Association). Max-Planck-Institut für Hirnforschung, Frankfurt/M., F.R.G.

Neurones of the cat visual cortex acquire their response selectivity under the influence of visual experience. This process starts at three weeks of age, lasts for several weeks and coincides with an activity-dependent elimination of synaptic connections. Since astrocytes have been shown to participate in synapse elimination, we studied their development in the cat visual cortex by immunolabeling the astrocyte specific proteins GFAP and S-100. The distribution of immunopositive cells was similar with both markers. At all ages (1 week to adult) heavily labeled cells were present in the white matter. Developmental changes in the cortex followed the characteristic inside-out pattern. Initially (from 7-21 days postnatally) labeled cells were confined to infragranular layers and only increased in number. At 4 weeks they occured throughout all layers. Staining intensity in gray matter was weak at these developmental stages. Between the fifth and seventh week the adult pattern with heavily labeled cells in all layers was attained. Preliminary studies in dark reared animals revealed a reduced density and staining intensity, especially in supragranular layers. As dark rearing delayes also the decay of cortical susceptibility to experiential modifications there may be an inverse relation between the maturity of astrocytes and cortical malleability.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY III

299.1

CHRONIC QUINOLINIC ACID LESIONS IN RATS CLOSELY MIMIC HUNTINGTON'S DISEASE. M. Flint Beal, Neil W. Kowall, Kenton J. Swartz, Robert J. Ferrante and Joseph B. Martin. Neurology Service, Mass. General Hospital, Boston, MA.

Our previous work has shown a relative sparing of NADPH-diaphorase neurons with preserved concentrations of somatostatin and neuropeptide Y following acute quinolinic acid (1 week) striatal lesions. These results are similar to those in Huntington's disease (HD) although in this illness there are 2-3 fold increases in striatal somatostatin and neuropeptide Y concentrations. In the present study we studied the effects of lesions at 1 year. Lesions were made by injecting 240 nmol of quinolinic acid in 1 ul into the anterior striatum. Neurochemical studies showed a significant decrease of GABA of 38% and substance P of 56% while somatostatin was significantly increased by 41% and neuropeptide Y by 74%. Histologic evaluation of the lesions was carried out using NADPH-diaphorase staining combined with enkephalin staining. The histologic staining showed a marked shrinkage of the striatum with reabsorbance of the acute lesion core. Neuronal counts showed marked approximately 3 fold significant increases in the numbers of NADPH-diaphorase neurons in regions in which there were significant 60% reductions in the numbers of enkephalin neurons. Chronic quinolinic acid lesions therefore closely resemble the neurochemical and histologic features of HD, since they result in significant increases in both somatostatin and neuropeptide Y concentrations and marked increases in the density of NADPH-diaphorase neurons.

299.2

GLUCOSE-DEPRIVATION CORTICAL NEURONAL INJURY IS STRONGLY INFLUENCED BY THE AVAILABILITY OF EXTRACELLULAR AMINO ACIDS. H. Monyer, M.P Goldberg, and D.W. Choi. Stanford Univ. Med. Sch., Stanford, CA 94305.

We developed a murine cortical cell culture model of glucose-deprivation neuronal injury (GDNI). In our initial experiments (Brain Res. 446:144), cultures were exposed for 3 - 6 h to Eagle's minimal essential medium (MEM) without vitamins or glucose, prior to the addition of glucose. By the next day, substantial amounts of neuronal injury developed, that could be attenuated by the addition of the NMDA antagonist APV. Here we explored the dependence of GDNI on the availability of the essential amino acids (AAs) normally present in MEM.

If all AAs were omitted from the glucose-deprivation medium, GDNI was usually somewhat reduced compared with that found in the standard MEM condition. Adding back AAs but not glutamine (Glut), the synthetic precursor for transmitter glutamate, resulted in GDNI which was both more extensive, and less sensitive to APV, than that found in the standard MEM condition. We conclude that: 1) GDNI is enhanced by the presence of extracellular essential AAs (not including Glut); and 2) in the presence of these AAs, Glut has a dual effect on GDNI, increasing a component mediated by NMDA receptors (possibly by serving as a substrate for glutamate synthesis in presynaptic terminals), but also decreasing another component not mediated by NMDA receptors.

299.3

DELAYED ANTAGONISM OF NMDA RECEPTOR-MEDIATED CORTICAL NEURONAL INJURY: AN IN VITRO MODEL. D.M. Hartley and D.W. Choi. Stanford Univ. Med. Sch., Stanford, CA 94305.

Present experiments explored the neuroprotective efficacy of manipulations performed "late" after excitotoxin-induced injury. Murine cortical cell cultures exposed to 500 μM NMDA for 3 min developed over the next day substantial neuronal cell loss. When added at t = 0 - 30 min after NMDA exposure, concentration-dependent neuroprotection was produced by the competitive NMDA antagonist D-APV (EC_{50} 10 μM) and the non-competitive NMDA antagonists MK-801 (EC_{50} 0.3 μM) and dextrorphan (EC_{50} 3 μM). Maximal benefit (at t = 0) was similar for all three drugs (50 - 80% reduction in neuronal damage) and somewhat less than the essentially complete protection afforded by these drugs if added during the toxic exposure. In contrast, little benefit was produced by late addition of 1 mM secobarbital, 100 μM phenytoin, 10 μM nifedipine, 3 μM TTX, or non-NMDA antagonists (1 mM GAMS, 1 mM GDEE, or 10 μM FG 9065). Rescue benefit could also be demonstrated by removing Ca but not Na from the medium for 30 min at t = 0; interestingly, if both Ca and Na were omitted for this period, neuronal injury could be essentially blocked. The ability of NMDA antagonists to rescue cortical neurons "doomed to die" by previous toxic exposure may be a basis for the protective efficacy of these drugs administered subsequent to ischemic insults in vivo.

299.4

CORTICAL NEURONAL INJURY IN VITRO FOLLOWING COMBINED GLUCOSE AND OXYGEN DEPRIVATION: IONIC DEPENDENCE AND DELAYED PROTECTION BY NMDA ANTAGONISTS. M.P. Goldberg, H. Monyer and D.W. Choi. Stanford Univ. Med. Sch., Stanford, CA 94305.

We have previously described NMDA receptor-mediated neuronal injury in cortical cell cultures deprived of either oxygen or glucose for 4 - 8 h. Combined removal of both oxygen and glucose resulted in more rapid neuronal injury, such that a 30 - 45 min exposure was sufficient to produce marked neuronal swelling, followed over 24 hr by widespread neuronal degeneration. Both acute swelling and delayed death were blocked by addition of the NMDA antagonist dextrorphan (DX, EC_{50} 3 μM) during exposure (D-APV, CPP, and MK-801 were also effective). DX reduced neuronal loss by 50 - 75% even when administered following completion of the deprivation insult.

Substitution of choline for Na in the exposure solution blocked acute swelling but not late neuronal cell loss. In this Na-free solution, removal of Ca substantially reduced, and excess Ca (5.4 mM) enhanced, subsequent injury; DX however still blocked injury. Removal of Ca in normal Na yielded destructive neuronal swelling, which could also be blocked by DX.

Neuronal injury in this in vitro model of ischemia may be due to an NMDA receptor-mediated influx of Ca and Na, in part occuring in a delayed fashion subsequent to the actual insult.

299.5

NON-NMDA RECEPTOR-MEDIATED NEUROTOXICITY IN CORTICAL CULTURES. J. Koh, D.M. Hartley, D.W. Choi, Stanford Univ. Med. Sch., Stanford, CA 94305.

The neurotoxicity of non-NMDA receptor agonists was quantitatively investigated in dissociated murine cortical cell cultures. 5 min exposure to 1 mM kainate produced acute neuronal swelling, but little in the way of neuronal loss by the next day. 5 min exposure to 1 mM AMPA produced neither acute neuronal swelling nor much late neuronal loss. With 24 h exposures, both kainate (EC_{50} 15 μM) and AMPA (EC_{50} 3 μM) produced concentration-dependent widespread neuronal loss; AMPA but not kainate toxicity was sensitive to attenuation by GAMS, whereas both AMPA and kainate toxicity were sensitive to attenuation by kynurenate (Kyn). Thus the non-NMDA agonists kainate and AMPA, which probably act on separate receptors, differ from NMDA agonists in requiring lengthy exposure to destroy most cortical neurons. Somewhat puzzling then is the ability of quisqualate (Quis) (30 μM - 1 mM) to produce widespread late neuronal loss with 5 min exposure. Since this toxicity is resistant to glutamate antagonists (APV, GAMS, GDEE, Kyn) added acutely during agonist exposure, but can be attenuated by Kyn (but not APV) added "late" to the medium after agonist washout, we speculate that Quis even upon brief application, may be taken up into neurons and released gradually, thus achieving in fact lengthy exposure (see Harris et al., Brain Res. 418:361).

299.6

BIPHASIC EFFECTS OF PURINES ON QUINOLINIC ACID NEUROTOXICITY. T.W. Stone, J.H. Connick* and C. Chumbley* (SPON: European Neuroscience Association) Departments of Physiology and Anatomy, St George's Hospital Medical School, University of London, London SW17 0RE.

The ability of the endogenous neurotoxin quinolinic acid to produce neuronal damage may involve presynaptic factors. We have recently shown that quinolinate can induce a release of glutamate and aspartate in vivo and we reasoned that compounds which suppress transmitter release might protect against quinolinate toxicity.

Male rats were anaesthetised with pentobarbitone and stereotaxic injections made into the dorsal hippocampus. Quinolinate was used at doses of 100 nmoles for a limited lesion, and 120 nmoles for an extensive lesion. Animals were killed 5 days later and the brain taken for histology. The extent of hippocampal damage was assessed blind on a 1-10 scale. The co-injection of R-PIA (100ng) reduced the amount to damage produced by quinolinate. Similarly the intraperitoneal administration of R-PIA (0.01mg/Kg) also afforded significant protection when injected at the same time as the intrahippocampal quinolinate. Higher doses of systemic R-PIA caused an enhancement of the toxic effects of quinolinate probably due to hypotension inducing a degree of hippocampal hypoxia.

Support by Action Research.

299.7

RELATIVE RESISTANCE TO QUINOLINIC ACID TOXICITY OF NEURONS CONTAINING NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE DIAPHORASE (NADPH-d) IN CULTURES OF RAT CORTICOSTRIATAL SYSTEM. W.O. Whetsell, Jr., and B. Christie-Pope*. Neuropathology, Vanderbilt Univ. Sch. Med., Nashville, TN 37232

Mature (21 days *in vitro*) organotypic cultures of rat corticostriatal system (Whetsell & Schwarcz, J. Neural Trans. Suppl 19, 53, 1983) were used to investigate sensitivity of subpopulations of neurons to toxicity of the endogenous excitotoxin, quinolinic acid (QUIN). Specifically, the question of sensitivity of NADPH-d-containing neurons to QUIN toxicity was examined in this system. Corticostriatal (CXCA) cultures were exposed to a range of QUIN concentrations (10^{-3}M, 10^{-4}M, or 10^{-6}M) for different periods of time and compared by light microscopy to control (non-QUIN-treated CXCA cultures) after histochemical staining for NADPH-d activity (Vincent et al.,Comp. Neurol. 217:252, 1983). CXCA cultures exposed to QUIN 10^{-3}M showed generalized degeneration in the living state after 24 hours; NADPH-d positive neurons were either not present or were rarely found in these cultures. Identical sibling cultures incubated in QUIN 10^{-4}M showed vacuolization and destruction of many regions by 24 hours, but in those regions, the population of NADPH-d positive neurons was indistinguishable from control cultures. Other sibling CXCA cultures chronically exposed to QUIN 10^{-6}M for four-to-five weeks showed scattered focal degeneration similar to that reported previously (Whetsell & Schwarcz, Soc. Neurosci. Abstr., 1987), but NADPH-d-containing neurons were well-preserved. Results indicate that NADPH-d-bearing neurons appear to be relatively resistent to QUIN neurotoxicity at moderate to low concentrations of QUIN but susceptible to higher concentrations of QUIN in this paradigm. (Supported by USPHS grant NS-20509.)

299.8

ADENOSINE ANALOGUES PROTECT AGAINST KAINATE-INDUCED TOXICITY IN RAT STRIATUM AND HIPPOCAMPUS. B. Arvin*, L.F. Neville* and P.J. Roberts Dept. of Physiology and Pharmacology, University of Southampton, Southampton, U.K.

Kainate when administered intracerebrally or systemically in rats, produces well documented axon-sparing lesions, by a mechanism at least partially dependent on the presence of glutamate.

In this study, we have examined the effects of a number of adenosine analogues in relation to the possibility of modifying kainate-induced striatal or hippocampal damage. Co-injection of 2-chloroadenosine (3-25 nmol) with kainate (2.2 nmol intracerebrally) resulted in a dose-dependent protection in both rat striatum and hippocampus. The selective A1 agonist, CPA (1-5 mg/kg i.p.) prevented loss of hippocampal CA1 neurons following systemic kainate (8-12 mg/ kg i.p.). In contrast, the A1 antagonist CPT (5 mg/kg) enhanced kainate toxicity in the hippocampus.

These findings may lead to a better understanding of the mechanisms underlying excitotoxin cell loss and also suggests a potential anti-excitotoxic strategy directed at pre- rather than postsynaptic targets.

299.9

ROLE OF CALPAIN ACTIVATION IN THE MECHANISM OF EXCITATORY AMINO ACID NEUROTOXICITY. R. Siman, J.C. Noszek*, and C. Kegerise*, Neuroscience Group, The DuPont Company, Wilmington, DE. 19898.

Sustained stimulation of receptors for excitatory amino acids (EAAs) leads to the death of receptive neurons, and to activation of the calcium-dependent protease calpain I (Siman and Noszek, *Neuron*, in press). Here, we have investigated the relationship between the protease activation and neurotoxicity. Calpain activation was detected and quantified on immunoblots as increases in the levels of major proteolytic fragments of the calpain substrate spectrin. Intraventricular administration of NMDA (80 ug) or kainate (0.2-1 ug) produces hippocampal damage and calpain I-induced spectrin degradation. The NMDA antagonist CPP (2 ug) selectively blocks both NMDA-induced neurotoxicity and calpain activation. Only those doses of EAAs sufficient to cause neuronal damage also markedly activate calpain I. The calpain activation occurs over the course of the first day, temporally coincident with the onset of hippocampal neurodegeneration monitored by silver impregnation histochemistry. Colchicine lesions of hippocampus are not accompanied by calpain activation, indicating that protease stimulation does not occur simply as a secondary response to neuronal destruction. These results indicate that calpain activation can serve as an intracellular mediator of EAA action, and further support the hypothesis that calcium influx and calpain I activation are obligatory events in the initiation of EAA neurotoxicity.

299.10

MK-801 PRETREATMENT ENHANCES NMDA MEDIATED BRAIN INJURY AND ALTERS NMDA RECEPTOR AND PCP RECEPTOR BINDING CHARACTERISTICS IN PERINATAL RATS. M.V. Johnston, F.S. Silverstein and J.W. McDonald. MSTP and Neuroscience Programs and Departments of Pediatrics and Neurology, University of Michigan, Ann Arbor, MI. 48104.

MK-801, a non-competitive NMDA receptor antagonist, effectively reduces NMDA mediated brain injury in 7 day old rats when administered (i.p., 1 mg/kg) after an intrastriatal injection (25 nmol NMDA/0.5 ul; McDonald et al., 1988). NMDA mediated brain injury is markedly enhanced in 7 day old rats (*Brain Res.*, In press) which allows quantitation of injury by comparison of hemisphere weight disparities in pups that received unilateral injections. In this model, MK-801 (1 mg/kg, i.p.) antagonized NMDA mediated injury when administered 2, 6, and 12 hours before intrastriatal injection of NMDA. However, when MK-801 was given 24 hours prior to the NMDA injection, NMDA mediated injury was enhanced relative to NMDA injected controls as assessed by hemisphere weight disparities [% Damage (injected vs contralateral hemisphere) in controls (n=11) was 31.8±0.9 vs MK-801 pre-treated (n=6) 39.4±3.7, $p<0.05$ independent t-test].

To examine the mechanisms for this effect, in vitro quantitative autoradiography was carried out in 7 day old rats treated with MK-801 (1 mg/kg, i.p.) 24 hours before sacrifice. NMDA sensitive [3H]glutamate binding was enhanced relative to saline treated controls (130-140% of control) while [3H]TCP binding to the PCP receptor was reduced (60-80% of control): NMDA binding in CA_3 region of hippocampus (fmol/mg protein, mean±SEM), MK-801 treated (n=3) 1,092.9±81.0 vs control (n=3) 894.3±95.2, $p<0.01$; TCP binding in CA_3, MK-801 treated (n=3) 71.8±6.6 vs control (n=3) 114.9±6.3, $p<0.01$. The data suggest that pretreatment with non-competitive NMDA antagonists in vivo increases the ratio between NMDA receptors and PCP receptors on NMDA channels. This change may be associated with enhanced sensitivity to NMDA toxicity.

299.11

GANGLIOSIDES AND NMDA-SENSITIVE GLUTAMATE RECEPTOR ANTAGONISTS PREVENT GLUTAMATE NEUROTOXICITY VIA DIFFERENT MECHANISMS. H. Manev*, M. Favaron*, H. Alho, M. Bertolino*, A. Guidotti and E. Costa. FGIN, Georgetown Univ., Medical School, Washington, D.C. 20007.

In primary culture of rat cerebellar and cortical neurons excitatory amino acids regulate translocation of protein kinase C (PKC) and induce neuronal cell death. PKC translocation was assessed within 15 min by measuring ^{3}H-phorbol ester binding, while neuronal viability was measured 24 h later by staining with fluorescein diacetate/propidium iodide. The two noncompetitive allosteric antagonists of NMDA sensitive glutamate receptors (phencyclidine and MK-801) counteracted more effectively neuronal death and PKC translocation induced by glutamate than those induced by kainate. Pretreatment with gangliosides (GT1b, GD1b and GM1, 50 μM or lower) for two h followed by washing similarly prevented the action of the two excitatory amino acid agonists. Asialo ganglioside was inactive. In contrast to PCP and MK-801, the doses of gangliosides that counteracted glutamate-and kainate-induced neuronal death failed to change the agonist stimulation of ionotropic and metabolotropic responses. Probably, gangliosides protect from neuronal death by limiting the translocation of PKC and of other Ca^{2+} regulated enzymes. This action is unrelated to a modification of excitatory amino acid receptor mediated responses.

299.12

CORRELATION BETWEEN GANGLIOSIDE CONTENT AND PROTECTION OF GLUTAMATE-INDUCED NEUROTOXICITY IN CEREBELLAR NEURONAL CULTURE TREATED WITH GT_{1b}.
B. Ferret*, H. Manev*, A. Guidotti and E. Costa., (SPON: F. Vaccarino) FGIN, Georgetown Univ., Washington, D.C. 20007.

Cerebellar granule cells (8 days in vitro) have the following relative abundance of ganglioside: 0.24 GM3; 0.05 GM2; 0.22 GM1; 0.57 GD3; 1.8 GD1a; 0.39 GX; 0.84 GD1b; 0.93 GT1b O-Acetyl; 2.9 GT1b; 0.37 GQ- (μg neuraminic acid/mg protein). GX is a ganglioside tentatively identified as lactone of GT1b. In primary culture of rat neuronal cells, 2 hrs pretreatment with 10-50 μM GT1b, or GD1b or GM1 prevents the glutamate-induced neurotoxicity. Incubation of cells with GT1b (10-50 μM) for 2 hours induces a dose-dependent increase in cell content of GT1b (up to 5 fold) and a slight increase in GD1a and GM1. The increased GT1b content and the protection against glutamate-induced neurotoxicity appear to be related. The protective effect of gangliosides (10-50μM) lasts for 24-48 hours after their removal from the medium. Following incorporation of GT1b in the cell membranes, GT1b content declines at a rate of about 6% per hour. We conclude that the long lasting protective effect of gangliosides on glutamate-induced neurotoxicity is related to the presence of an increase in the sphingoglycolipid membrane content.

PROCESS OUTGROWTH, GROWTH CONES, AND GUIDANCE MECHANISMS V

300.1

VARIOUS THY-1 ANTIBODIES DIFFERENTIALLY ENHANCE OUTGROWTH BY RODENT RETINAL GANGLION CELLS. Stuart A. Lipton, Leonard A. Levin* and Colin J. Barnstable. Dept. of Neurol., Harvard Med. Sch., Boston, MA; Lab. of Neurobio., Rockefeller Univ., NYC 10021.

When immobilized, the Thy-1 monoclonal antibodies (MAbs) 2G12 and OX7 have been shown to promote the outgrowth of postnatal rat retinal ganglion cells in culture [Leifer, Lipton, Barnstable & Masland, *Science* 1984;224:303]. OX7 reacts with the allelic site at amino acid #89 while 2G12, although binding elsewhere, can compete for this site. In the present study we tested various Thy-1 MAbs that react with mouse including three developed by Tim Springer [*PNAS* 1981;78:4535]. We found that these MAbs bind differentially to various Thy-1 positive tissues, for example, to spleen and neurons but not thymocytes from the same animal. This finding suggests the possibility of binding to carbohydrate moieties, which vary among tissues, rather than solely to the amino acid residues, which are invariant. In contrast, other Thy-1 MAbs bind to all three tissues. Interestingly, despite approximately equal concentration and equal affinity for substrate, the MAbs with disparate binding patterns differentially affect process outgrowth by retinal ganglion cells. These findings confer an element of selectivity to the effect of Thy-1 MAbs. Explanations may include a conformational change or active site on Thy-1 that is affected by specific Thy-1 MAbs.

300.2

MONOCLONAL ANTIBODIES TO G4, A NEURAL CELL SURFACE GLYCOPROTEIN, INTERFERE WITH NEURITE EXTENSION ON AN AXONAL SUBSTRATE AND ON A G4 SUBSTRATE. S. Chang, F.G. Rathjen*, and J.A. Raper. Max-Planck-Institut für Entwicklungsbiologie, Spemannstr. 35, D-7400 Tübingen, FRG.

Embryonic neurons have been observed to elongate upon the surfaces of other axons in vivo. Thus axons are a natural substrate for neurite outgrowth, and could be an important source of information for growth cone pathfinding. In order to identify the molecules which promote neurite extension on axons, we have developed an assay which measures chick sympathetic neurite extension on chick sympathetic axons.

Polyclonal antibodies to G4, a recently identified cell surface glycoprotein, interfere with neurite extension in this assay. We have now tested eight monoclonal antibodies and all were effective in reducing the lengths of neurites growing on axons. The monoclonal antibodies reduced the average neurite length to about 60-70% of control values, which is the same result obtained with polyclonal antibodies to G4.

In addition, purified G4 is a potent promoter of neurite outgrowth from both central and peripheral neurons. This finding is in agreement with the recent report that 8D9, another glycoprotein related to L1, promotes neurite outgrowth as well (Lagenauer & Lemmon, Proc. Natl. Acad. Sci. USA 84:7753, 1987). Monoclonal antibodies to G4, when incubated on substratum bound G4 and washed off, can inhibit completely the neurite promoting activity of G4.

Our results identify G4 as one of the molecules on axonal surfaces which promote neurite outgrowth.

300.3

THE 8D9 ANTIGEN, A MEMBER OF THE NILE/L1/NgCAM CLASS OF CELL ADHESION MOLECULES, IS INVOLVED IN AXON OUTGROWTH ON GLIA. J. Drazba* and V. Lemmon (SPON: M. Ontell). Dept. of Neurobiology, Anatomy and Cell Science and Ctr. for Neuroscience, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

A novel assay for studying axon growth on glia has been developed. Confluent monolayers of chick retina Muller cells are prepared by culturing dissociated E13 retinae. Neurons are removed by treating the cultures with an anti-ganglioside MAb (8A2) and complement. Subsequently, strips of retina attached to nitrocellulose (after W. Halfter et al., Dev. Biol., 95:56, 1983) are placed on the Muller cells. Axons from the retinal ganglion cells grow out perpendicularly from the strips. The rate of outgrowth is rapid, up to 2 mm/24 hours, but varies depending on the substrate (matrigel>laminin>glia> collagen>polylysine). MAb JG22, which inhibits integrin function, did not inhibit axon outgrowth on glia; neither did polyclonal Fab' fragments to NCAM. In contrast, Fab' fragments to the 8D9 antigen inhibited neurite outgrowth by about 50%. These results strongly suggest that the 8D9 antigen is one of the molecules that mediates axon growth along glia. (Supported by NIH EY05285 and MOD 1-979)

300.4

ANTIBODIES TO L1 INHIBIT RETINAL NEURITE GROWTH ON SCHWANN CELLS. D.K. Simon*, N. Kleitman, M. Schachner+, R.P. Bunge. Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110; +Dept. Neurobiol., Univ. Heidelberg, FRG.

Embryonic rat retinal explants extend long neurites on Schwann cell surfaces in vitro. We have explored this interaction using antibodies to the cell adhesion molecule L1. Anti-L1 blocked outgrowth on Schwann cell surfaces in a dose dependent manner which was specific to neurite growth on Schwann cells. Hemi-retinas from day 15-16 rat embryos were plated on Schwann cell monolayers cultured on ammoniated type I collagen (AC). AC alone did not support retinal neurite outgrowth. Affinity purified polyclonal antibodies to L1 (400 μg/ml) or Fab fragments (50-200 μg/ml) were present in the culture medium from the time explants were plated onto the monolayers. Outgrowth was quantified by measuring the area covered by neurites. The intact polyclonal anti-L1 inhibited neurite outgrowth by 95% relative to control cultures. Fab fragments blocked growth in a dose dependent manner: 24% at 50 μg/ml, 85% at 100 μg/ml, and 90% at 200 μg/ml. At the 50 μg/ml dose, neurites were defasciculated. Anti-L1 Fab fragments did not appear to affect outgrowth on an air-dried collagen substratum, suggesting that L1 specifically affected the neurite-Schwann cell interaction. Other antibodies to Schwann cell or neurite surfaces tested (anti-laminin, anti-Thy-1, 217C) did not affect outgrowth in this system and anti-L1 appeared not to inhibit retinal outgrowth on astrocytes (Ard et al., this volume). Our results suggest that L1 is a mediator of retinal neurite outgrowth on Schwann cells and may prove important in supporting regeneration of these CNS neurons into a peripheral nerve environment. (Supported by NIH NS09923, NS08069.)

300.5

ISOLATED SCHWANN CELLS SUPPORT NEURITE REGENERATION FROM EXPLANTS OF ADULT RAT RETINA. J.M. Hopkins and R.P. Bunge. Dept. Anat., Washington U. Sch. Med., St. Louis, MO 63110.

The ability of sciatic nerve grafts to support regeneration of retinal ganglion cell axons in the adult rat raises the question of whether Schwann cells or extracellular matrix is primarily responsible for this phenomenon. We have used explants of adult rat retina, stimulated to regeneration by intraorbital axotomy, to explore this question in vitro. Primary cultures of isolated Schwann cells were prepared from embryonic day 15 rat dorsal root ganglion cultures which were denervated prior to addition of retinal explants (orphaned Schwann cells), or from postnatal day 1 rat sciatic nerves (sciatic Schwann cells). Ammoniated rat tail collagen served as a primary substratum for Schwann cells. Retinal explants were prepared from adult rats 7 days after crush of the optic nerve. Neurite growth from these explants was assessed by neurofilament immunofluorescence after 3 days in vitro. Air dried or ammoniated collagen alone did not support neurite growth. Explants seeded onto a bed of sciatic Schwann cells extended long neurites onto the cells but did not cross bare collagen between the cells. Orphaned Schwann cells supported growth of retinal neurites for distances up to 2 mm, in a pattern that closely paralleled the linear organization of the Schwann cells in these cultures. Since the Schwann cells prepared for these experiments do not produce basal lamina we concluded that the Schwann cell surface is a sufficient substrate for the growth of axons regenerating from adult rat retina. We are presently using this model to explore the molecular basis for the ability of Schwann cells to support regeneration of adult mammalian retinal axons. (NIH EY06073, NS09923.)

300.6

RETINAL NEURITE GROWTH ON ASTROCYTES AND OLIGODENDROCYTES IN CULTURE. M.D. Ard+, P. Wood, M. Schachner++, and R.P. Bunge. Dept. Anatomy, Washington Univ. Sch. Med., St. Louis, MO 63110; +M.D.A. currently at Dept. Anat., Univ. Mississippi Med. Ctr., Jackson, MS 39216; ++Dept. Neurobiol., Univ. Heidelberg, FRG.

In an effort to model conditions for CNS regeneration in vitro, we have cultured neonatal rat cortical astrocytes (method of McCarthy & de Vellis, 1980, J. Cell Biol. 85:890) as a substratum for neurite growth from embryonic day 15 rat retinal explants. Retinal neurites grew well (to an average length of 2 mm in 4 days) on the surfaces of astrocytes in various conditions, independent of the amount of extracellular matrix present in these experiments. By electron microscopy neurites were seen to grow in channels formed between layered astrocyte processes, often in contact with astrocyte surfaces. Antibody to the cell surface molecule L1 was added to cultures in preliminary experiments. Retinal neurite growth on astrocytes was not inhibited by anti-L1 at a concentration (100 μg/ml) which blocked retinal neurite growth on Schwann cell surfaces (Simon et al., this volume).

Recent reports (e.g., Caroni & Schwab, 1988, Neuron 1:85) have implicated mature oligodendrocytes in obstruction of axonal regeneration. To test whether oligodendrocytes could inhibit neurite growth on an otherwise permissive substratum, we added cultured oligodendrocytes derived from adult rat spinal cord and partially purified using fluorodeoxyuridine (Wood & Bunge, 1986, J. Neurol. Sci. 74:153) to our astrocyte preparations. After allowing one day for the mixed glial cells to attach, retinal explants were added. Neurite growth on oligodendrocytes plus astrocytes was not inhibited compared to neurite growth on astrocytes alone. (NIH NS09923; National Multiple Sclerosis Society.)

300.7

Identification of radial glial growth cones. JP.Misson*,P. Evrard*, JF. Gadisseux*,VS Caviness Jr.(Spon. M. Edwards)E.K. Shriver Center, Developmental neurobiology, Waltham MA 02254.

Radial glia are transiently present in the cerebral wall during neocortical histogenesis and are believed to serve as guides for neuronal migration. Radial glial cells have been demonstrated to be mitotically active and presumably are progenitors for new cells and radial fibers (Misson et al. Dev.Br.Res.1988,38, 183). The density of RGF appears to remain constant as the volume of the cerebral wall increases,implying that radial glial cells increase in number (Gadisseux et al. Neurosc. Abstr. 1987, 1117). The present analysis focuses on the shape transformation of the daughter cells, in particular on the manner in which newly formed radial processes are redeployed and incorporated into the transcerebral system of fascicles. RG are stained with RC2 (Misson et al. Neurosc. Abstr. 1987,1345) and for glycogen. Selectively stained growth cone at the outgrowing tips of newly formed RGF are found almost exclusively within the radial glial fascicles. The growth cones are of two forms: cylindric form or flattened form. The cylindric form,which is similar in shape to axonal growth cones within developing axonal fascicles are found within the glial fascicles. The flattened form which are similar to growth cones at the tips of terminal arborizations of axons are observed on RGF which are deflected singly from the glial fasicles suggesting a different mechanism of growth. These observations are consistent with the view that the radial glial cell population is dynamically increasing. At least some of the newly formed radial glial daughter cells are elaborating new growth-cone-tipped processes.The new processes become incorporated into the glial fiber system by fasciculation within the pre-existing fascicles. Their deployment behavior, and growth cone configuration are similar to those of axons. Supported by the Charles A King Trust and a NIH grant 12005.

300.8

RADIAL GLIA: A CELLULAR SOURCE FOR FIBRONECTIN DURING EARLY CORTICAL DEVELOPMENT A.L. Pearlman, J. Cohen* and W. Puckett*. Depts. of Cell Biology and Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110

Fibronectin (FN) is transiently present in the developing cerebral cortex of the mouse (Stewart and Pearlman, 1987) and cat (Chun and Shatz, 1988) in a distribution that suggests an important role in cortical development. To determine the relationship between FN and the cellular elements of developing cortex, we double-labeled unfixed cryostat sections of murine cerebral cortex from embryonic day 11 (E11) through E13 with a polyclonal antibody to FN (provided by J. McDonald) and with the RC1 monoclonal antibody (provided by M. Yamamoto), which labels radial glia but not neurons. We found that FN is distributed along most of the length of the radial glia at early stages of cortical formation (E11-12). Slightly later (E12-13) the laminar distribution of FN (op. cit.) is reflected by discontinuous labeling along radial glial processes. To determine which cells of developing cortex produce FN, we cultured dissociated cortical cells from E13 embryos and 48 hours later treated them with monensin to block the export of FN. We found immunolabeling for FN within RC1+ cells with the morphology of radial glia, but not within RC1- neurons. Thus our evidence indicates that radial glia produce FN and distribute it along their processes in the zones involved in early axonal elongation and cortical plate formation. (Supported by NIH grant EY621).

300.9

A POSSIBLE ROLE OF GLIA IN THE DECUSSATION OF HINDBRAIN AUDITORY FIBERS IN THE DEVELOPING FERRET. J.K.Brunso-Bechtold and C.K.Henkel, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

At birth, the ferret lateral and medical superior olivary nuclei are extremely immature ultrastructurally as well as in their dendritic development. Somatic synapses which typify these nuclei in the adult are not common until 2 weeks of development and the mature dendritic morphology is not seen until the second postnatal month. The immature ferret hindbrain is also characterized by the presence of a midline glial structure which is similar to that described in the rat by Van Hartesveldt, et al. (1986). In the ferret, however, this structure reacts intensely with antibodies to GFAP as well as to S-100. It is comprised of large caliber, dense fibers and extends from the dorsal to the ventral surfaces of the hindbrain. The structure is evident at birth and is no longer present by the end of the first postnatal month. Despite the presence of what would seem to be a formidable barrier to growing axons, the midline trapezoid body is quite large at birth. In an effort to determine the manner in which developing axons might negotiate the midline glial barrier, we studied the midline in the developing ferret using immunocytochemical and electron microscopic techniques. Our results suggest that very fine caliber glial processes, some of which may be extensions of what had been radial glia, may be playing a role in guiding growing axons through the glial midline barrier present early in development. (Supported by NIH Grant NS23092-03.)

300.10

MECHANISM OF ACTION ON THE GROWTH CONE OF *APLYSIA* HEMOLYMPH IN PROMOTING NEURITE OUTGROWTH. D.J. Goldberg. Dept. of Pharmacology, Columbia U., New York, NY 10032.

Little is understood of how the binding to neurons of factors on the substratum promotes neurite outgrowth. I have used high-resolution video microscopy to examine how *Aplysia* hemolymph, which contains such a factor(s) (as yet unpurified), acts on the growth cone. Growth normally occurs by the extension of flat, organelle-free veils of membrane, followed by the engorgement of some of the veils with organelles and cytoplasm moving in from the central region of the growth cone and then the conversion of the engorged veils into cylindrical neurite. I find that a primary action of hemolymph is to promote the step of veil engorgement. In the absence of hemolymph, dihydrocytochalasin B, which weakens and depolymerizes networks of actin filaments, produces the same effect. This demonstrates that the actin filament network of the veil *resists* an intrinsic forward thrust of organelles and cytoplasm (presumably slow axonal transport). It may be that an important mode of action in promoting neurite growth for the factor(s) in hemolymph, and perhaps other substratum-bound factors, is weakening of the actin filament network of newly formed veils. Protein phosphorylation could be the route by which this is accomplished, in that stimulation of protein kinase A or C also causes veil engorgement, though inhibition of protein kinase C, at least, does not reduce the effect of hemolymph on the veil.

300.11

GLYCOPEPTIDES ON THE GROWTH CONE SURFACE: SPECIFIC INTERACTIONS WITH TARGET CELLS IN APLYSIA. R.T. Ambron, H. Den*, I. Protic*, C. Gabel*. Dept. Anatomy and Cell Biology, Columbia University, N.Y., NY 10032.

Neurons of *Aplysia* growing in vitro form specific chemical synapses on their follower cells, implying that macromolecules at the growth cones of these neurons recognize their target. To identify these constituents, we isolated growth cones from RUQ cells and identified several classes of glycopeptides, including one (GPwga) that is enriched and exposed on the surface. Studies now indicate that a GPwga is present at the growth cones of many neurons. To see if GPwga interacts with target cells, we isolated GPwga from the neurons of 180 abdominal ganglia. GPwga and ovalbumin glycopeptides (GPov), were each coupled to fluorescent beads and the beads were added in vitro to target cells known to be innervated by many neurons from the ganglion. We found that: 1) Beads containing GPwga, but not GPov, bound to muscle cells isolated from the circulatory system or the heart; 2) Binding depended on the glycopeptide since GPwga in the bath diminished binding of GPwga-beads; 3) GPrt, another class of glycopeptide at growth cones, did not bind to the cells. When added to neurons as targets, GPwga-beads bound to certain neurons; others not only failed to bind GPwga, but excluded the beads from the area around the cell. To see if the neurons release proteins, cells were exposed to ^{35}S-met for 24h. Autoradiography showed that each neuron is surrounded by labeled proteins bound to the substrate.

These results indicate that among the glycopeptides present at the growth cone, GPwga is involved in some aspect of neuron-target interaction and that this interaction may be modulated by material released from the neuron.

300.12

SELECTIVE SYNAPSE FORMATION IN VITRO BY NEURON L10 OF APLYSIA IS ASSOCIATED WITH SPECIFIC PATTERNS OF REGENERATION. D. Hawver* and S. Schacher (SPON: J.E. Goldman), Ctr. Neurobiol. & Behav., Columbia CPS & NYS Psych. Inst., New York, NY 10032

In cell culture, L10 forms specific connections with appropriate abdominal ganglion neurons. To explore the mechanisms underlying this specificity, we have combined phase contrast and fluorescent light microscopy to examine the pattern of neurite outgrowth by L10 after 4-5 days of co-culture with either target or non-target cells alone, or with both cell types simultaneously.

Action potentials in L10 evoke a dual-action (fast excitatory-slow inhibitory) response in RB cells. Injection of the fluoresscent dye 5(6)-carboxyfluorescein into L10 reveals many labeled processes extending along and in close apposition to the axons and processes of the RB cells (N=5). In contrast, when co-cultured with non-target right upper quadrant (RUQ) cells (N=3), the neurites of L10 generally form loops and only intersect the processes of the RUQ cells. These differences in the pattern of growth are also observed when L10 is co-cultured with both RB and RUQ cells (N=4). In addition, nearly all the neurites of L10 extend along RB cells when the processes of both the RB and the RUQ cells are in close proximity.

These results are consistent with the idea that synapse specificity may involve the activation of cellular mechanisms for both target selection and avoidance. We plan to examine the nature of the signals from the postsynaptic cells and how they influence neurite outgrowth from L10.

HUMAN BEHAVIORAL NEUROBIOLOGY II

301.1

SEX DIFFERENCES IN THE ANATOMY OF THE CORPUS CALLOSUM AND RELATION TO FUNCTIONAL ASYMMETRY AND COGNITION. S. F. Witelson. Department of Psychiatry, McMaster Univ., Hamilton, Canada. L8N 3Z5.

At least 15 studies have been reported in which gross anatomy of the human corpus callosum in midsagittal section was studied for possible sex differences. Almost all found that males either had or tended to have a larger callosal area and a larger splenium. Relative to brain size, females still did not have larger callosa. Only two studies reported opposite results: splenial breadth was larger in females in both, and splenial area tended to be greater in one. I now report a series of sex differences observed in callosal anatomy in a sample of 56 brain specimens when different subregions of the callosum and factors of hand preference and age were considered. In contrast to males, callosal size was not associated with hand preference in females. The genu was found to be significantly larger in males, but females had a larger relative posterior trunk region than males for consistent-right-handers. Callosal size decreased with age in men, but not in women. These results suggest that the biological rules governing hand preference and functional asymmetry may be different in the sexes; that the neurobiological basis of aging may be different in women than men, and that the sex difference in callosal proportional morphology may be related to the sex differences in brain lateralization and cognition. (Supported; Grant NS 18954)

301.2

SEX DIFFERENCES IN CORTICAL ACTIVATION PATTERNS DURING AN ORTHOGRAPHIC ANALYSIS TASK USING rCBF METHODOLOGY. C.E. Naylor and F.B. Wood*. Bowman Gray School of Medicine, Winston-Salem, NC 27103.

The present study examines rCBF findings on 52 normal readers, 30 females and 22 males, during an orthographic analysis task requiring subjects to identify words that were four letters in length. There were no sex differences in behavioral performance. The more accurate a subject's performance, the greater was the relative activation in Wernicke's area compared to activation in the left angular gyrus region.

A unique relationship between the probe over the left temporal region and several other probes (holding all other probes constant) was found, yet the pattern differed depending on sex. For females, significant unique partial correlations were found between the probe over the left temporal region and numerous intra- and interhemispheric sites. For males, there was a signifiaant positive correlation between Wernicke's area on the left and the homologous region in the right hemisphere.

These results suggest that Wernicke's area serves to "drive" a greater number of other cortical regions in females compared to males. In other words, there may be more language "driving" in females due to more extensive unique linkages both within and between the hemispheres.

301.3

NEURAL CORRELATES OF HUMAN ATTENTION TO TOUCH AND FLUTTER J.V. Pardo, P.T. Fox, and M.E. Raichle. Depts. of Psychiatry, Neurology and Neurological Surgery, and Radiation Sciences, Washington University School of Medicine, and the McDonnell Center for Studies of Higher Brain Function, St. Louis, Mo. 63110

The regional cerebral blood flow responses to touch and flutter were measured using PET in normal right handed subjects performing somatosensory tasks requiring attention. Three task conditions were compared to the control state (eyes closed, rest): count pauses in a volley (3-5 Hz) of suprathreshold touches applied using a von Frey hair to the pad of either the left (A; nine subjects) or the right great toe (B; 10 subjects), or monitor the right great toe for changes in flutter (35 Hz) amplitude (C; 14 subjects). In contrast to the predominantly Rolandic responses observed when potent vibrotactile stimuli (130 Hz, 2mm) are applied to subjects in a passive (no task) state, these tasks recruited a major response in the contralateral supramarginal gyrus (Brodmann 40). In fact, monitoring for changes in flutter did not activate Rolandic toe cortex. Response location was asymmetric; the left response was about 1 cm anterior to the right. Additional studies indicated that neither the somatosensory stimulus without attention, nor the attentional task without a stimulus, activated the supramarginal gyrus.

301.4

SYNTACTIC AND SEMANTIC CONTRIBUTIONS TO SENTENCE COMPREHENSION IN ALZHEIMER'S DISEASE (AD). J. Sherman*, J. Schweickert*, J. Growdon, S. Corkin. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

A sentence-picture matching task was presented to 12 AD patients and 8 age-matched healthy control subjects (HCS). The subjects heard sentences that varied in syntactic complexity, semantic constraint, and plausibility, and were asked to choose which of two pictures matched each sentences' meaning. The incorrect picture violated either a lexical or syntactic property of the sentence. HCS performed almost perfectly on the task, whereas 10 of the 12 AD patients performed below the normal range. The 10 impaired patients were worse on sentences paired with pictures that violated syntactic properties rather than lexical properties. Further, syntactic complexity hindered their sentence interpretation irrespective of semantic constraint and plausibility. Sentence interpretation was also affected by semantic constraint and plausibility: For all sentence types, patients performed better on constrained than unconstrained sentences, and better on plausible than implausible sentences. The results indicate that AD patients attempted to apply independent syntactic and semantic analyses in their sentence interpretation, but were often unsuccessful at both. The findings suggest that syntactic and semantic processing are compromised in AD patients' sentence comprehension and contrast with the general characterization of the language function in AD as showing preserved syntactic knowledge.

301.5

ACTIVATION OF THE TEMPORAL POLES IN NORMAL AND PATHOLOGICAL FORMS OF HUMAN ANXIETY, EM Reiman, ME Raichle, E Robins, MJ Fusselman, PT Fox, MA Mintun, and JL Price Washington Univ. Schl. of Med., St. Louis, MO 63110

Positron emission tomographic (PET) measurements of regional blood flow were used to investigate the neuroanatomical correlates of anticipatory anxiety. Eight normal volunteers were studied with and without the anticipation of a painful electric shock. In anticipation of the shock, the subjects had significant increases in subjective and physiological measurements of anxiety; they also had significant increases in blood flow in the region of the right and left temporal poles.

In a separate study, PET was used to investigate the neuroanatomical correlates of experimentally induced anxiety attacks in patients with panic disorder. Patients with panic disorder and normal control subjects were studied before and during the infusion of sodium lactate. The production of lactate-induced panic was associated with blood flow increases in the same bilateral regions of temporopolar cortex; bilateral regions corresponding to the lateral putamen, claustrum or insular cortex; bilateral regions corresponding most closely to the superior colliculus; and a region corresponding most closely to the left side of the anterior cerebellar vermis. Lactate was not associated with significant blood flow changes in non-panicking patients or controls.

These studies demonstrate activation of the temporal poles in normal and pathological forms of human anxiety.

301.6

PET SCAN CHANGES IN CORTICAL (3-N-[11-C] METHYL) SPIPERONE BINDING FROM DEPRESSED TO NON-DEPRESSED STATES IN POST-STROKE DEPRESSION. R.M. Parikh,* R.G. Robinson, H.S. Mayberg, D.F. Wong,*, T.R. Price,*, E.P. Brousolle,* J.M. Links,* R.F. Dannals,* H.N. Wagner,* (SPON: A. Justice) Johns Hopkins Univ. Sch. Med. Baltimore, Md 21205.

In a previous PET study, we reported that spiperone (NMSP) binding in the temporal cortex of stroke patients with left hemisphere lesions was highly correlated with severity of depression (r=.92). This case study reports the effect of treatment of depression on NMSP binding in the temporal cortex in one patient. The patient, a 60 year old right-handed black female had sudden onset of right hemiparesis. CT and MRI scans demonstrated a lacunar infarct of the left caudate nucleus. At one week post stroke, she was found to have a major depression (Hamilton score 22) and after undergoing an NMSP PET scan, was started on a double-blind treatment study using nortriptyline and placebo. Her depression improved dramatically on placebo over the 6 week study (Hamilton score became 0). Her NMSP binding ratio in the ispsilateral to contralateral frontal and temporal cortex changed from .91 and .92 respectively before treatment to 1.20 and 1.17 respectively after treatment. This increased NMSP (primarily 5HT2 receptor binding) over the course of treatment could not be attributed to a drug effect and is to our knowledge, the first known case of a patient with post-stroke depression showing a commensurate change in in vivo chemistry.

301.7

CSF MONOAMINE METABOLITE MEASURES IN POST-STROKE DEPRESSION R.G. Robinson, R.M. Parikh,* R.A. Rodriguez,* G.M. Greene* and T.R. Price*. Johns Hopkins Univ. Sch. Med. and Univ. of Maryland Sch. Med. Baltimore, Md 21205.

Acute stroke patients (n=21) and non-stroke neurologic controls (n=9) were examined for cerebro-spinal fluid (CSF) concentrations of monoamine metabolites MHPG, 5-HIAA and HVA. The CSF collection was controlled for lumbar gradient, time of day, diet, exercise and time since stroke. Measurements of CSF MHPG, 5-HIAA and HVA were carried out using a high pressure liquid chromatography (HPLC) assay. CSF MHPG levels were significantly lower in patients with left compared with right hemisphere lesions (19.0 ng/ml± 7.3 vs 28.2 ng/ml±8.2, t=-2.5, df=17, p<.02) or control patients (27.0 ng/ml±5.8, t=-2.5, df=15, p<.05). There were no significant differences in MHPG between right lesion and control groups or among right, left and control groups in 5-HIAA or HVA levels.

Mean Hamilton Depression Scores were significantly higher in the left compared to control group (10.5±8.9 vs 2.4±5.9, t=-2.1, df=13, p<.05) and for the entire study group, CSF MHPG levels were inversely correlated to Hamilton scores (r=-.61, df=23, p<.01). These findings are consistent with our previous hypothesis that noradrenergic dysfunction may play a role in post-stroke depression.

301.8

MAPPING TWO VISUAL PATHWAYS IN MAN WITH REGIONAL CEREBRAL BLOOD FLOW (rCBF) AS MEASURED BY POSITRON EMISSION TOMOGRAPHY (PET) AND $H_2{}^{15}O$. J.V. Haxby*, C.L. Grady, B. Horwitz, M.B. Schapiro, R.E. Carson*, L.G. Ungerleider, M. Mishkin, P. Herscovitch, R.P. Friedland, S.I. Rapoport. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

The presence and neuroanatomical location of separate visual pathways for processing spatial location and object identity were investigated by measuring rCBF with PET in 4 healthy young men during visual processing and sensorimotor control tasks. Visual processing was induced with two-choice, match-to-sample visual discrimination tasks. The object identity task involved face recognition. The spatial location task involved perceiving the location of a dot in a square with the sample and choice stimuli rotated relative to each other. PET rCBF measurements were obtained using bolus injections of $H_2{}^{15}O$ and the weighted integration method. Difference images contrasting each visual processing task to the sensorimotor control task were obtained and smoothed, the latter resulting in an in-plane resolution of 9 mm.

Each subject demonstrated a double dissociation between regions activated by the two visual tasks. Areas consistently activated during the spatial but not the face recognition task were in the superior parietal lobule, bilaterally. Areas consistently activated more by the face recognition than by the spatial vision task were in the region of the occipitotemporal junction, bilaterally, just inferior to the angular gyrus. These results demonstrate two distinct visual processing pathways at work in humans. These human neural systems are similar to visual pathways in nonhuman primates, though there seem to be cross-species differences in their anatomical location.

301.9

LATERALITY PATTERNS IN TWO COGNITIVE ACTIVATION TASKS USING THE rCBF METHODOLOGY. F.B. Wood* and C.E. Naylor (SPON: I.Brown). Bowman Gray School of Medicine, Winston-Salem, NC 27103.

Regional cerebral blood flow was measured during an orthographic analysis task and a recognition memory task. The subjects were 52 normal readers, 30 females and 22 males, with no history of physical or emotional illness.

The difference between the activation at homologous probes was calculated. These difference scores were then subjected to a principal components analysis. Two major factors emerged. The first involved a reciprocal pattern of activation between frontal and posterior regions. Greater activation in the frontal regions of one hemisphere was associated with a pattern of greater activation in posterior regions of the opposite hemisphere around the angular gyrus and a relative reduction of activation in the ipsilateral posterior region. A second factor involved a similar reciprocal relationship between central regions and more inferior temporal flow. Increased flow in central regions of one hemisphere was related to a relative increase in flow in the inferior temporal region of the opposite hemisphere.

The relative weighting of these two factors was found to vary depending on the task. The anterior-posterior factor was found to be the principal component in the memory task, while the second factor involving laterality patterns in central regions accounted for most of the variance in the orthographic analysis task.

301.10

SOURCE LOCALIZATION OF COMPONENTS OF THE VISUAL-EVOKED NEUROMAGNETIC RESPONSE. C. J. Aine, J. S. George*, M. T. Oakley, P. A. Medvick*, and E. R. Flynn. Neuromagnetism Laboratory, MS M882, Los Alamos National Laboratory, Los Alamos, NM 87545.

Neural sources were determined from neuromagnetic field distributions recorded in humans for each temporal component of an evoked response elicited in a visual attention task. The dipole-like current sources of the magnetic components were then located on anatomical images of the subjects' brains produced by Magnetic Resonance Imaging. The results demonstrated a strong correlation between magnetic waveform morphology and simultaneously recorded electrical responses. Computer modeling experiments were performed to aid in determining whether the time-series of field patterns reflected a single source with a location and orientation that changed with time or two independent sources. In another case, the field distribution was fit by a linear combination of two discrete dipole sources. The two dipoles were modeled by first fitting one dipole and then fitting a second dipole to the residual field distribution; the predicted field patterns were then summed and compared to the empirical field distribution. Some electrical/neuromagnetic "components" apparently reflect focal activation of discrete cortical loci; however, other "components" may reflect extremes of a spatial-temporal path of cortical activation. These results demonstrate the value of combining several complementary methodologies for mapping brain function onto structure.

301.11

"HORIZON OF SIMULTANEITY" FOR AUDITORY AND VISUAL STIMULI - AND HOW THE BRAIN MAY GET RID OF IT: E. Pöppel, K. Schill, N. von Steinbüchel. Dept. of Medical Psychology, Goethestr. 31, 8000 München 2, FRG

Reaction time (RT) to auditory stimuli is shorter than to visual stimuli (130 ms to auditory vs. 160 ms to visual stimuli under optimal conditions). This difference is mainly due to principally different transduction mechanisms in the two sensory systems. A transduction time of at least 30 ms even under high contrast conditions has to be assumed for the visual system, whereas in the auditory system it is well below 1 ms. This temporal difference implies that an object which is defined in visual and auditory space will be available centrally with a considerable temporal difference. We have studied whether the difference in transduction is compensated by the time that sound travels through space. Auditory stimuli were systematically removed from subjects and it was tested whether RT to these stimuli increased accordingly. This was the case and it was found that at approximately 10 m distance RT to visual and auditory stimuli was about equal. Thus, at 10 m we observe a "horizon of simultaneity" for these two sensory channels. These facts pose a particular problem for sensory integration: How is object identity possible if there is a temporal delay in central availability? One solution could be that one channel is disregarded altogether. We would like to suggest, however, that the brain solves this temporal problem by a mechanism that allows to disregard non-simultaneity within a certain range. This could be done by using neuronal oscillators that define system states. If these are relaxation oscillators that are entrained by either sensory system, the other system could be linked to defined system states. If a system state means that within one period of an oscillation no before-after relationship is defined, temporal differences within the two sensory systems would be disregarded. Experimental evidence for oscillatory processes and order threshold with 30-40 Hz support experimentally this hypothesis. Supported by DFG PO 121/13-1

301.12

TOPOGRAPHIC BRAIN MEASURES OF HUMAN PAIN AND PAIN RESPONSIVITY. A.C.N. Chen, S.F. Dworkin,* and J. Gehrig.* Lab. of Brain/Pain Research SC-63, Univ. of Washington, Seattle, WA 98195.

We have been analyzing ongoing EEG activities based on Fast-Fourier Transform under naturalistic pain states. In this research, we have a) quantified topographic brain activities by computer analysis of the ongoing EEG cortical spectrum in relation to a longer lasting, more diffuse type of tonic pain, the cold-pressor test (CPT); and b) assessed neurophysiological correlates of individual differences in pain responsivity which we have observed consistently.

EEG was recorded bilaterally at F3, F4, T3, T4, P3, P4, and O1, O2 with linked-ears reference for 42 normal healthy male subjects (mean=24.7years, s.d.=5.8). Psychological and physiological data were gathered in three experimental stages: Baseline, Noxious Stimulation (Pain), and Post Test. EEG sampling rate and filter setting were at 250 Hz and 0.5-70 Hz, respectively. Pain was assessed by endurance time to withdrawal from the 1±0.3°C icewater, and by subjective ratings of pain intensity and aversiveness.

A dichotomy of pain responsivity was observed: A pain-tolerant group (PT) tolerated the required 3 min of cold pressor, while the pain-sensitive group (PS) tolerated a mean of only 57 sec ($t=16.7$, $p<10^{-9}$). The PS (n=13) group reported significantly higher pain ratings than the PT (n=29) subjects. State anxiety was heightened equally for both PS and PT subjects under the CPT. For all subjects, pronounced topographic changes in specific band densities were demonstrated for both hemispheres during the CPT pain condition. Greater changes occurred in the delta band power density at all topographic loci. Neither theta nor alpha activities changed under CPT pain state, specifically. However, the higher frequency beta-1 and beta-2 power densities increased significantly under CPT pain, especially at the temporal loci. Comparing PS and PT groups, both showed no discernible differences at Baseline in EEG findings. During the CPT, PS exhibited clearly greater reactivity in the delta band than PT. In contrast, both groups exhibited an equal degree of heightened power density for the high frequency beta bands. These results indicate that even though both delta and beta band power densities increased under the pain state for PS and PT groups, differences in the delta band, only, distinguished between these two groups.

The topographic activation of delta activity in the anterior-posterior loci for both hemispheres may be interpreted as global activation of the protocritic sense/feelings of pain and discomfort. By contrast, the enhancement of beta activity may be interpreted as heightened vigilance related to scanning mechanisms that govern both perceptual and cognitive functions.

MESSENGER RNA REGULATION II

302.1

CHANGES OF TYROSINE HYDROXYLASE mRNA IN DOPAMINERGIC NEURONS OF RAT SUBSTANTIA NIGRA DURING RETROGRADE DEGENERATION. *T. Shirao, M.J. Evinger, L. Iacoviti and D.J. Reis.* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Tyrosine hydroxylase (TH) is the initial and rate-limiting enzyme necessary for catecholamine biosynthesis in dopaminergic neurons. In this study we analyzed changes of TH mRNA in dopaminergic neurons of rat substantia nigra (SN) during retrograde degeneration. Axons of the unilateral nigrostriatal pathway were transected by a small knife cut placed stereotaxically at the level of posterior lateral hypothalamus. At various times after placement of lesions, total RNA was extracted from SN tissue using the guanidine monothiocyanate-lithium chloride method. Total RNA content was determined spectrophotometrically. TH mRNA levels were quantitated by Northern blot and slot blot analyses using TH cRNA (380 ntd.) (T.H. Joh) as a probe. Bovine adrenal medulla RNA served as a standard. Some brains were processed for *in situ* hybridization analysis. On Northern blots a single mRNA band of 1900 bases was detected in both ipsilateral and contralateral SN during retrograde degeneration. In the ipsilateral SN, the TH mRNA level was not changed at postoperative days 2 and 3. However, by postoperative day 14, the TH mRNA level was reduced to less than 10% of unoperated control. No change in TH mRNA level was detected in the contralateral SN. Our previous immunohistochemical studies have demonstrated a cell loss of dopaminergic neurons maximal at 12 days following transection of the nigrostriatal tract with survival of only 30% of dopaminergic neurons in the SN pars compacta (Saji and Reis, 1985). In our studies TH activity in the ipsilateral SN was 40% of control at postoperative day 14. Therefore, TH activity in those surviving neurons appeared unchanged. *In situ* hybridization analyses with ^{35}S-labelled TH cRNA showed a reduced number of dopaminergic neurons in the ipsilateral SN at postoperative day 14. Furthermore, reduced grain density over those surviving neurons was observed in the ipsilateral SN compared with the contralateral SN. Therefore, we conclude that TH mRNA levels within the surviving dopaminergic neurons in the ipsilateral SN are decreased, although the levels of TH activity within those neurons remain similar to control.

302.2

NEURAL MODULATION OF PHENYLETHANOLAMINE N-METHYL-TRANSFERASE mRNA IN BOVINE CHROMAFFIN CELLS. *M.J. Evinger, P. Ernsberger, B.N. Reynolds*, D.J. Reis.* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Phenylethanolamine N-methyltransferase (PNMT), the enzyme catalyzing the synthesis of epinephrine from norepinephrine, is regulated in the adrenal medulla by hormonal and neural stimuli. Previously, we demonstrated a 5-8 fold increase in PNMT mRNA levels in response to depolarization by potassium (50 mM). In this study, we sought to resolve whether neurotransmitters which bind to defined adrenal medullary cell receptors are able to influence expression of the PNMT gene. Primary cultures of dispersed bovine chromaffin cells were established following collagenase digestion of adrenal medullary tissue. After 48 hr, cells were incubated 18-24 hr in fresh media containing agents known to influence PNMT enzymatic activity or catecholamine release from chromaffin cells. Total RNAs were prepared from harvested cells disrupted by treatment with 0.5% NP-40, then fractionated on denaturing agarose gels for Northern blot analyses by hybridization with PNMT (T.Joh) and 18 S rRNA (R. Guntaka) cDNAs. The cholinergic agonists nicotine (10 μM) and carbachol (200 μM) elicited increases in the steady state levels of PNMT mRNA relative to untreated control cultures. The stimulatory effect of carbachol could be partially blocked by incubation with the nicotinic antagonist hexamethonium (0.5 mM), thereby implicating nicotinic receptors in the response. Reversal of carbachol stimulation by the muscarinic antagonist atropine (1 μM) was considerably smaller in magnitude although radioligand binding assays using ^{3}H-quinuclidinyl benzylate reveal that both M1 and M2 receptor subtypes are present on chromaffin cells in moderate abundance (B_{max} = 58 ± 9 fmol/mg protein; or 7600 ± 1200 sites/cell) and exhibit very high affinity (K_d = 300 ± 73 pM). The ability of other neurotransmitters to regulate PNMT gene expresssion is suggested by the increase in PNMT mRNA using histamine at concentrations up to 10 μM. We therefore conclude that, in addition to hormonal regulation, the PNMT gene is responsive to neural stimuli and can be modulated by specific neurotransmitters.

302.3

SHORT- AND LONG-TERM EFFECTS OF ANGIOTENSIN ON CATECHOLAMINE SYNTHESIZING ENZYMES IN CULTURED BOVINE ADRENAL MEDULLARY CELLS (BAMC). M.K. Stachowiak, A. Poisner*, H.K. Jiang*, P. Hudson*, V. Owyang* and J.S. Hong. LMIN, NIEHS, Res. Triangle Pk., NC 27709.

Renin-angiotensin and adrenal medullary (AM) catecholamines are two major hormonal systems controlling circulation. The presence of renin in the adrenal cortex suggests paracrine functions of angiotensin in AM. In this report, we have examined effects of angiotensin on cultured AM cells. Exposure of BAMC for 10-30 min to 200 nM sar^{1}-angiotensin II (ANG) produced transient increase in the affinity of tyrosine hydroxylase (TH) to BH_4 cofactor. No change in the activity of PNMT was found at 30 min; however, 48 hrs exposure produced a 2-fold increase and also elevated total met-enkephalin content. 24 hrs incubation with ANG produced increases in TH, PNMT, and proenkephalin (pEK) mRNA levels (ED_{50} 1.2 nM), which were additive with the increases produced by forskolin or veratridine. Induction of mRNAs by ANG was inhibited by nifedipine, calmidazolium and TPA indicating involvement of calcium channels, calmodulin and protein kinase C. Thus, the adrenal medulla appears to be a locus of the interactions between the renin-angiotensin and sympathoadrenal systems. In addition to modulating secretion of catecholamines and enkephalins, angiotensin could be involved in the short-term regulation of TH activity and the expression of TH, PNMT and pEK genes.

302.4

DETECTION OF TRYPTOPHAN HYDROXYLASE mRNA IN RAT CNS. T.L. Green*, P.D. Walker, L. Ni*, G.M. Jonakait and R.P. Hart (SPON: H.H. Feder). Dept. of Biol. Sci., Rutgers University, Newark, NJ 07102.

We have devised an assay for tryptophan hydroxylase (TPH) mRNA that is quantitative and detects tissue-specific messages. This assay utilizes a radiolabeled 69 nt synthetic TPH oligonucleotide primer hybridized with mRNA in a reverse transcriptase-catalyzed extension reaction. The size of the cDNA product, measured by gel electrophoresis, indicates the distance between the priming site and the 5' end of the mRNA. The amount of cDNA product is linear with respect to RNA added to the reaction.

The primer extension assay reveals two species of TPH mRNA in pineal and one in the CNS. The larger, pineal-specific species yields a cDNA size (738 nt) by primer extension that would be predicted from the sequence of the rabbit pineal cDNA that has been cloned[1]. A smaller TPH mRNA (producing a 550 nt extended cDNA) was found in pineal and in midbrain and medullary raphe nuclei, but not in the cerebellum or the superior cervical ganglia (SCG). The absence of signal in a catecholaminergic region (SCG) argues against detection of the highly homologous tyrosine hydroxylase mRNA. In order to demonstrate this specificity further, rat serotonergic neurons were lesioned at birth with 5,7-dihydroxytryptamine following protection of catecholaminergic neurons with desmethylimipramine. Lesioned adult rats had depleted TPH mRNA in brainstem nuclei, but little mRNA depletion in the pineal. (Supported by NS 23687. GMJ and RPH are Johnson & Johnson Discovery Research Fellows.)

[1] Grenett, H. et al. (1987) Proc. Natl. Acad. Sci. USA 84:5530.

302.5

REGULATION OF TRYPTOPHAN HYDROXYLASE mRNA IN RAT CNS FOLLOWING pCPA ADMINISTRATION. R.P. Hart, T.L. Green*, P.D. Walker, L. Ni*, S. Schotland*, and G.M. Jonakait. Dept. of Biol. Sci., Rutgers University, Newark, NJ 07102.

Inhibition of tryptophan hydroxylase (TPH), the rate-limiting enzyme in 5-HT biosynthesis, occurs following administration of *p*-chlorophenylalanine (*p*CPA). In order to determine effects of enzyme inhibition on subsequent biosynthesis of TPH, we assayed TPH mRNA levels following chronic *p*CPA administration.

Adult rats were injected with 300 mg/kg s.c. *p*CPA prior to implantation of Alzet minipumps containing sufficient *p*CPA to deliver 100 mg/kg/day for 14 days. At various times after implantation, dorsal raphe nuclei were assayed for TPH enzyme activity. TPH mRNA from medullary raphe was assayed using a radiolabeled 69 nt synthetic TPH oligonucleotide primer that hybridized with total RNA in a reverse transcriptase-catalyzed primer extension reaction[1].

During the first week of *p*CPA infusion, TPH enzyme activity was 77% inhibited. By contrast, TPH mRNA levels increased relative to control rats receiving sham surgery. TPH activity remained inhibited to the same extent 2 and 3 weeks after implantation. TPH mRNA levels, however, approached control levels at 2 weeks and dropped below control at 3 weeks.

These results are consistent with an end-product regulation of TPH biosynthesis via control of specific mRNA levels. (Supported by NS 23687. GMJ and RPH are Johnson & Johnson Discovery Research Fellows.)

[1]Green, T.L. et al. (1988) Soc. Neurosci. Abstr. (supra vide).

302.6

Transneuronal alterations of nigral GAD mRNA containing neurons following long term striatal lesions. G.M. Pasinetti, D.G. Morgan, S.A. Johnson, M.M. Meyers* and C.E. Finch, Gerontology Ctr. and Dept of Biological Sciences USC, Los Angeles, CA 90089

Neostriatal injection of ibotenic acid (IBO) elicits transneuronal degeneration of neurons in s. nigra (SN) reticulata (Saji and Reis, Science 1987). We extended these findings in adult Fisher 344 male rats, unilaterally injected (4 µg IBO in 1 µl) in three different striatal sites. GAD mRNA containing neurons (GABAergic neurons) were identified by in situ hybridization; tyrosine hydroxylase (TH) immunopositive neurons (dopaminergic neurons) were identified using a primary antibody to TH (Eugene Tech). In situ hybridization and immunocytochemistry were performed on 10 µm paraffin sections, GAD (Sal I/Eco RI, 1.3 Kb) cDNA fragment (A.Tobin), was recloned into Bluscribe M13 vector to produce single strand cRNAs. Three months after striatal IBO lesions there was a 50% loss of GABAergic neurons throughout the rostral-caudal extent of the striatum. The striatal cross-sectional area on the lesioned side was a 30% less than the contralateral side. SN reticulata cross-sectional area, ipsilateral to the IBO lesions showed 30% reduction. None of these alterations were evident in the vehicle injected group. The SN pars compacta dopaminergic neurons on the lesioned side showed small apparent cell loss (10-15%), compared to the contralateral side; similar neuron loss was observed in the vehicle injected group. GABAergic neurons in SN reticulata ipsilateral to the lesioned side showed a 40% loss in the central and ventromedial portions, with no apparent loss in the dorsolateral portion. GABAergic neurons were unchanged in the vehicle injected group. Experiments are in progress to determine the long term effect of striatal excitatory lesions on TH mRNA prevalence in the remaining SN dopaminergic neurons. (Supported by John D. and Katherine T. MacArthur Foundation Research Program on Succesfull Aging)

302.7

CARBOXYPEPTIDASE E EXPRESSION IN RAT BRAIN, STUDIED WITH IN SITU HYBRIDIZATION. C.A. Ross, L. Fricker and S.H. Snyder (SPON: G. Pearlson). Johns Hopkins University School of Medicine, Department of Neurosci., Baltimore, MD 21205.

Carboxypeptidase E (CPE, also called enkephalin convertase) is believed to participate in the processing of enkephalins, as well as possibly other neuropeptides. In order to map its expression in brain, we synthesized and labelled three antisense and two sense orientation 45 base oligodeoxynucleotides, and performed in situ hybridization. All three antisense probes yielded similar patterns of label, while sense strand probe labelling was negligible. Antisense labelling was highest in amygdala, paraventricular hypothalamus, cerebral cortex, and nucleus of the tractus solitarius--areas known to contain enkephalins. Label was also dense in hippocampus and cerebellar granule cell layer--areas not known to contain enkephalins. Label was present at lower levels in many other areas of brain. We conclude that CPE is expressed in widespread regions of the brain, and is probably involved in processing of a number of neuropeptides.

302.8

MULTIPLE PATHWAYS MEDIATE c-fos INDUCTION BY NGF IN PC12. R. C. Armstrong* and S. Halegoua* (SPON: A. Carlson). Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook N.Y. 11794

In PC12 cells, NGF stimulates transcription of the proto-oncogene c-fos. We have investigated the molecular mechanisms underlying this transcriptional activation. In PC12 cells c-fos mRNA is induced by cAMP activated A-kinase, phorbol ester (PDBU) activated C-kinase, or increased intracellular Ca^{++}. Since each of these pathways is thought to mediate NGF action, we questioned whether any of these pathways mediate c-fos induction by NGF. A reinduction strategy was employed that takes advantage of the transient nature of c-fos induction. After c-fos is induced by PDBU, it cannot be reinduced by either PdBu or Ca^{++} for at least four hours after the primary event. However reinduction can occur by dbcAMP or NGF (~50% of non-preinduced level) after a primary induction by PDBU. Similarly, if c-fos is first induced by Ca^{++}, reinduction by Ca^{++} or PDBU is blocked, however, dbcAMP and NGF can reinduce c-fos (~50% of non-preinduced level). With both dbcAMP and NGF, an initial induction of c-fos blocks reinduction by the same agent. In contrast, when cells are pretreated with dbcAMP, a rechallenge with PDBU or NGF results in a level of c-fos mRNA which is similar to that observed in non-pretreated cells. In an A-kinase deficient PC12 mutant, c-fos can be initially induced by PDBU or Ca^{++} and then reinduced by NGF (~50% of non-preinduced level). These data suggest a complex regulation of c-fos induction by NGF using both novel and established pathways.

302.9

MORPHINE INCREASES c-fos mRNA IN RAT CAUDATE-PUTAMEN. S.L. Chang* and R.E. Harlan (SPON: C. Dempsey). Dept. of Anatomy, Tulane University Medical Center, New Orleans, LA 70112; S.P. Squinto*, Dept. of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70119.

The c-fos is the cellular homologue of the oncogene v-fos carried by FBJ and FBR murine osteosarcoma viruses (J. Virol., '82, 114). The role of c-fos is postulated to be responsible for transduction of signal from cell surface to nucleus. Our preliminary results suggest that c-fos mRNA in rat caudate-putamen in vivo was increased by morphine administration to maximum at 36 min. and dropped back to control level at 49 min. A further confirmative study was then performed. A significant induction of c-fos mRNA (60%, $p < 0.05$) was observed by slot-blotting analysis after acute morphine treatment (10 mg/kg, 30 min). This effect was blocked completely by naloxone, the opiate antagonist, (10 mg/kg) simultaneously injected along with morphine. Naloxone alone did not have a significant effect on c-fos mRNA levels. Both morphine and naloxone are assumed to have high affinity for the μ-type opioid receptor. The caudate-putamen has been shown to have patches of μ-type receptor. We now are studying the cellular localization of the morphine inducible c-fos protein in the rat brain.

302.10

ESTROGENS MODULATE C-FOS AND PARVALBUMIN GENE EXPRESSION IN THE RAT CNS. E. Cattaneo*, M. Berchtold* and A. Maggi. M.P.L., Inst. of Pharmacol. Sciences U. of Milan I-20131 Milan Inst. Pharmacol. Biochem. U. of Zurich-Irchel CH-8057, Zurich.

A computer analysis of the rodent c-fos proto-oncogene indicates at 175 bps upstream from the TATA box the presence of the sequence GGTCCTAGGAGACC highly homologous to the reported "ERE" consensus sequence (GGTCAXXXTGACC). The measure of c-fos mRNA accumulation in the rat uterus and midbrain after estrogen (E) administration (75 µg/rats s.c.) at different times indicates an increase of c-fos mRNA within 24 hours. Since it has been reported that GABAergic neurons are a target for estrogens in the brain and that parvalbumin is a Ca^{++} binding protein tipically associated with these neurons, the time course of parvalbumin mRNA accumulation after E administration was observed. Preliminary results indicate a different profile of parvalbumin and c-fos induction. On the bases of the data obtained it is hypothesized that parvalbumin mRNA accumulation is secondary to c-fos induction. Therefore, parvalbumin could be involved in a feed back regulative mechanism preventing Ca^{++}-dependent c-fos stimulation.

302.11

EXPRESSION OF BRAIN SPECIFIC SRC(+) ONCOGENE IN RAT BRAIN, STUDIED WITH IN SITU HYBRIDIZATION. C.A. Ross, R.C.A. Pearson, G. Wright and S.H. Snyder. Johns Hopkins University School of Medicine, Department of Neurosci., Baltimore, MD 21205.

Brain Src proto-oncogene product (termed Src (+)) contains an 18 nucleotide insert not present in other tissues. In order to map its expression with in situ hybridization, we labeled antisense and sense 60 base oligonucleotide probes (to the 18 base insert plus 21 nucleotides on either side). Antisense labeling in brain could be blocked by pretreatment of sections with RNase, and could be predominantly blocked by coincubation with excess cold probe. By contrast, antisense labelling in peripheral tissues was fainter than in brain, and was little changed by coincubation with excess cold probe. Thus antisense labelling in brain appeared to be predominantly specific. Sense strand labelling was very faint. Antisense labelling in CNS was high at E 15, but still present at E 19 and in adult. In adult brain, antisense label was most dense in the cerebellar granule cell layer, hippocampal pyramidal layer and dentate gyrus, and olfactory bulb, with lower densities elsewhere. These studies suggest a general role for src(+) expression in nervous tissue, perhaps in maintainance of the neuronal differentiated state.

302.12

TEMPLATE SEQUENCE-DEPENDENT TERMINATION OF IN SITU TRANSCRIPTION. L.H. Tecott and J.H. Eberwine*. Nancy Pritzker Lab., Dept. of Psychiatry and Behavioral Sciences, Stanford University, Stanford, California 94305.

We have described a novel method, in situ transcription (IST), involving the oligonucleotide-directed reverse transcription of specific mRNAs within fixed tissue sections (Tecott, L.H., et al., Science, in press). Gel electrophoresis of radiolabeled IST transcripts primed by a 36mer (P1) complementary to rat proopiomelanocortin (POMC) mRNA in fixed pituitary sections revealed a complex pattern of bands. To determine whether the bands resulted from priming at a single site along POMC mRNA or at multiple messages, P1-primed IST transcripts were compared with those primed by P2, an overlapping POMC oligonucleotide. The P2-primed bands were shifted, but in register with the P1-primed bands, consistent with the relative positions of these primers along POMC mRNA. This suggested that the oligonucleotides primed single sites on POMC mRNA and that the bands resulted from termination of reverse transcription at discrete sites along the message. Further analysis revealed a striking correspondence between IST banding patterns and template sequences. Transcription terminated predominately at guanine residues. The IST banding patterns of 90% homologous regions of rat and mouse POMC mRNA were clearly distinguishable, demonstrating the specificity provided by banding pattern analysis. Such "fingerprints" of template sequences may aid in distinguishing among members of gene families, viral subtypes and alternate splice species of mRNA.

302.13

DEVELOPMENTAL EXPRESSION OF THE NEURON-SPECIFIC MuBr8 mRNA AND PROTEIN. M.C. Wilson,[1] G A. Oyler,* J.W. Polli,* G.A. Higgins,[1] and M.L. Billingsley. Hershey Medical Center, Hershey PA 17033; and Scripps Clinic and Res. Foundation[1], La Jolla, CA 92037.

The developmental expression of the neuron-specific gene product MuBr8 in rodent brain was studied using Northern and Western blots, in situ hybridization and immunocytochemistry. In situ hybridization detected MuBr8 in entorhinal and neocortex, olfactory bulb, and cerebellum at embryonic day 15 (E15); in adults, MuBr8 was highest in hippocampus and neocortex. Immuno-reactivity to MuBr8 synthetic peptide antisera was detected from E6 to adult in rat brain synaptosomes and cytosol; immunoreactivity increased 10-fold during this period, with the greatest changes occurring between postnatal day 3 and 11. MuBr8 mRNA and protein were expressed at low levels in PC-12 rat pheochromocytoma and human SMS-KCNR neuroblastoma. The regulation and posttranslational modification of MuBr8 protein in response to differentiating agents is being examined in these cell lines. These results suggest that MuBr8 is regulated during development, and may be a marker of neuronal differentiation. Supported by grants from March of Dimes (GAO) and PHS (M.C.W. and M.L.B).

ION CHANNELS: MODULATION AND REGULATION IV

303.1

ELECTROPHYSIOLOGY OF FOUR CLONED MUSCARINIC RECEPTOR SUBTYPES EXPRESSED IN A9 L CELLS. S.V.P. Jones*, J.L. Barker, N.J. Buckley*, T.I. Bonner*, R. Collins* and M.R. Brann* (SPON: R. Nelson). Lab. Neurophysiology, Lab. Molecular Biology, NINCDS, Lab. Cell Biology, NIMH, Metabolic Diseases Branch, NIDDK, NIH, Bethesda MD 20892. The function of four cloned muscarinic receptor subtypes (m1-m4) were investigated in A9 L cells transfected with each of the different muscarinic receptor subtypes. Electrophysiological responses to cholinergic stimulation of each muscarinic receptor subtype were recorded using the tight-seal whole-cell recording technique. Cells transfected with m1 and m3 muscarinic receptors were hyperpolarized by acetylcholine (ACh), while m2- and m4- transfected cells did not respond to ACh concentrations of up to 1mM. ACh induced outward currents in m1- and m3-transfected cells voltage-clamped at -50mV, that were blocked by the muscarinic receptor antagonist, atropine (1μM), but not by the nicotinic receptor antagonist, tubocurarine (50μM). Reversal potentials of ACh-evoked currents in m1- and m3-transfected cells, changed about 50mV per 10-fold increase in K^+ concentration, indicating a Nernstian dependence on K^+. In K^+-free solutions, outward responses to ACh stimulation in m1- and m3-transfected cells could still be detected, showing the response was also contributed to by Cl^-. The Cl^--dependent conductance was consistently smaller than that recorded in K^+ containing solutions. Responses to ACh in m1- and m3-transfected cells were blocked by barium (1mM) and cobalt (5mM) applied extracellularly and by buffering the intracellular Ca^{2+} to low levels with 5mM BAPTA. Thus a role for intracellular Ca^{2+} may be indicated in the generation of the m1- and m3-evoked K^+ and Cl^- conductances. m3-transfected cells respond to ACh in a manner that is qualitatively similar to that seen in m1-transfected cells, whilst m2- and m4-transfected cells are not coupled to electrically detectable responses in A9 L cells.

303.2

MUSCARINIC RECEPTOR SUBTYPES MEDIATE SEPARATE COMPONENTS OF CHOLINERGIC RESPONSES IN HIPPOCAMPAL PYRAMIDAL CELLS. T.A. Pitler and B.E. Alger. Dept. of Physiol., Univ. of Maryland School of Medicine, Baltimore, MD 21201.

Using intracellular techniques in the rat hippocampal slice we examined several components of muscarinic receptor activation, including the rate of rise of the carbachol induced depolarization, the maintained depolarization, the increase in input resistance, and the ability of carbachol to inhibit the action of the G-protein associated neurotransmitter adenosine. Using the specific M_1 receptor antagonist pirenzepine (PRZ), and the M_2 receptor antagonists AF-DX 116 and gallamine, we attempted to determine if different aspects of carbachol's action were mediated by the separate muscarinic receptor subtypes.

The M_1 antagonist PRZ blocks the fast rate of rise of the carbachol induced depolarization and blocks the ability of carbachol to inhibit the adenosine response, but does not affect the maintained depolarization or the increase in input resistance. On the contrary, the M_2 receptor antagonists both reduced the maintained depolarization and increase in input resistance, while having little effect on the rate of rise of the muscarinic depolarization or the ability of carbachol to block the adenosine response. AF-DX 116 was more consistent and selective than gallamine. Experiments are underway to identify the intracellular second messenger systems related to activation of each of the receptor subtypes.

303.3

NON HYDROLYSABLE GTP-ANALOGUES MAY FAVOUR AGONIST CONFORMATION OF DIHYDROPYRIDINE BINDING SITES.

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Inst. of Pharmacol., Univ. of Milano, ^Chair of Toxicology, II Univ. of Rome, +Dept. of Pharmacobiology, Univ. of Bari, Italy.

Recent studies hypothesize a direct coupling of G-proteins to ion channels including voltage-dependent calcium channels. The present work studies the effect of non-hydrolysable GTP-analogues on [3H]-PN 200-110 binding to rat cortex membranes. GTP- -S and GMP-PNP increased the amount of PN 200-110 displaceable by BAY K 8644 but not by nitrendipine.Using 0.3 nM ligand and 0.6 uM BAY K 8644 as competing drug the binding was 140±12 and 99.7±9 fmoles/mg protein, respectively in presence or absence of GMP-PNP. GTP exerted similar effects but at higher concentrations. Other nucleotides did not modify the binding significantly.Qualitatively different results were obtained when using [125 I]-omega-Conotoxin as ligand and the cold toxin as displacer. The effect of GTP-analogues on PN 200-110 binding, when an agonist dihydropyridine is used as displacer, is reminiscent of electrophysiological data indicating that activation of G-proteins may promote agonist responses to calcium channel ligands.

303.4

MUSCARINIC MODULATION OF Ca CURRENT IN A PARASYMPATHETIC NEURON.

A.Tse, R.B. Clark and W. Giles, Dept. of Physiology, Faculty of Medicine, University of Calgary, Calgary, Canada.

Bullfrog (Rana catesbeiana) intracardiac parasympathetic neurons were isolated with an enzymatic dispersion procedure (Tse et al, Soc. Neurosci. Abst. 13, 535). The Ca currents of these neurons were recorded with the whole cell variant of the gigaohm seal method. Voltage-clamp results suggested the presence of L-type and possibly N-type Ca currents. Since these neurons are known to receive cholinergic input from the vagus nerve, the effect of ACh on the total Ca current was investigated. ACh (0.2-5 μM) or carbachol (1-5 μM) caused a reversible, 30-50% decrease of the Ca current amplitude at depolarizations to about +10 mV, near the peak of the Ca current-voltage relation. Two lines of evidence suggested that this was a muscarinic effect: (1) dl-muscarine (0.5-2 μM) mimicked the ACh response. (2) atropine (10-50 uM) antagonized the ACh effect. Since the inclusion of the non-hydrolysable GTP analog, GTPγS (50-100 μM) in the pipette mimicked the ACh response, a GTP-binding protein is likely to be involved.

The action potential afterhyperpolarization (AHP) in these neurons was generated mainly via a Ca dependent K current. Muscarine (1-2 μM) reduced the AHP amplitude by 4.4 ± 1.6 mV (n=7). When 2-3 ms voltage clamp steps (from -60 to +10 mV) were used to simulate action potentials, muscarine reduced the magnitude of the Ca current and the Ca-dependent K tail current concomitantly. This suggested that the reduction of AHP by muscarine in these cells was at least partly a secondary effect of the muscarinic modulation of Ca current.

303.5

ACETYLCHOLINE ENHANCES CALCIUM CURRENT IN A SINGLE IDENTIFIED SNAIL NEURON.

H.M. Gerschenfeld* and D. Paupardin-Tritsch* (SPON: European Neuroscience Association). Lab. Neurobiol., Ecole Normale Supérieure, 75005 Paris, France.

In cell F1 of the right parietal ganglion of Helix aspersa bathed in a TTX/Ba-containing saline, acetylcholine or carbachol (CCh, 10 uM) induces an increase in inward current. This is an enhancement in Ca current because: 1) CCh does not affect any outward currents in F1 neurons bathed with Co-containing saline and 2) the response to CCh is not affected by intracellular Cs-loading which blocks the outward currents. This CCh effect shows marked desensitization. It is not altered either by D-tubocurarine or hexamethonium application (20 uM), but is blocked by atropine (5 uM). Extracellular application of either forskolin or zaprinast,a cGMP-dependent phosphodiesterase inhibitor, does not affect the Ca current in the F1 neuron. In contrast, intracellular injection of EGTA prior to CCh application blocks the CCh-induced increase in Ca current.

It is concluded that CCh enhances the Ca current of neuron F1 by activating a muscarinic-like receptor different from the three types of ACh receptor described in molluscan neurons (Kehoe, 1972). Moreover, this response is not mediated by cAMP or cGMP but cytosolic Ca appears to play a role in its intracellular mechanism.

303.6

APLYSIA NEURONS POSSESS A FAMILY OF G PROTEIN-LINKED NEUROTRANSMITTER RECEPTORS MEDIATING BOTH ACTIVATION OF 'S'-LIKE K CURRENT AND SUPPRESSION OF Ca CURRENT.

V. Březina* (SPON: P. O'Lague), Dept. of Biology, UCLA, Los Angeles, CA 90024.

Aplysia neurons exhibit three principal types of response to many transmitters: 'fast' Na and Cl currents, and a 'slow' K current. The K-current responses to acetylcholine, histamine and the neuropeptide FMRFamide are mutually non-additive; all represent activation of the same K current identical or similar to the 'S' current[1-5] The transmitters act at separate receptors (distinct from those responsible for the 'fast' responses), each of which also mediates suppression of the voltage-dependent Ca current[4-6]. These 'slow' (but not the 'fast') effects are mimicked by GTP-γ-S[3,6,8] and blocked by pertussis toxin[3,7], suggesting involvement of G protein(s). It has been suggested that these activate a phospholipase to generate metabolites of arachidonic acid which act as second messengers mediating the responses[9,10]. Synergistic activation of 'S'(-like) K current and suppression of Ca current by members of this family of receptors may underlie presynaptic inhibition in Aplysia[2,5].

1: J. Physiol. 382, 267. 2: Nature 325, 153. 3: Nature 325, 259. 4: Neurosci. Abstr. 13, #196.8. 5: J. Neurophysiol. 55, 131. 6: J. Physiol. 388, 565. 7: Neurosci. Abstr. 13, #169.10. 8: Neurosci. Abstr. 13, #401.5. 9: Nature 328, 38. 10: Neurosci. Abstr. 13, #169.9.

303.7

G PROTEINS RECONSTITUTE INHIBITION OF Ca^{2+} CURRENTS BY BRADYKININ (BK) IN PERTUSSIS TOXIN-TREATED RAT DORSAL ROOT GANGLION (DRG) NEURONS

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[2]Dept. of Pharm., U. of Texas Health Sci. Cntr., Dallas TX 75234

Using the whole-cell patch clamp technique on cultured rat DRG neurons, Ca^{2+} currents were evoked at 0 mV from -40 mV (sustained current) and from -80 mV (sustained plus transient currents). 100 nM BK inhibited both sustained and transient currents (% inhibition ± S.E.: 66.4 ± 7.6 and 66.6 ± 5.2, n=7). Pretreatment with pertussis toxin (PTX) blocked this inhibition (3.0 ± 6.9 and 0.6 ± 8.1, n=5). We tested the ability of the alpha subunits of three PTX-substrate G proteins isolated from brain to reconstitute the inhibitory effects of BK in PTX-treated cells by including 100 nM alpha subunit plus 1 mM GTP in the normal intracellular solution. Partial reconstitution of BK inhibition was achieved with all three. The alpha subunit of G_o (39 kD) was most effective (50.0 ± 3.4 and 48.0 ± 5.2, n=5) and that of G_i (41 kD) was least effective (36.8 ± 2.5 and 34.8 ± 6.7, n=4). The 40 kD alpha subunit of a G protein of unknown function was intermediate (39.8 ± 3.1 and 43.3 ± 2.5, n=7). This lack of specificity for a particular G protein was in contrast to our results with neuropeptide Y (NPY) in which reconstitution of Ca^{2+} current inhibition was selective for the alpha subunit of G_o. Unlike reconstitution of BK inhibition, the inhibition of Ca^{2+} currents by NPY was completely reconstituted (45.1 ± 5.1 and 54.4 ± 5.8, n=10) compared to that of untreated cells (51.1 ± 3.1 and 61.4 ± 5.7, n=8). We are investigating the dose dependence of reconstitution of BK inhibition to determine whether the relative lack of specificity and efficacy are due to less selective and lower affinity binding of the alpha subunits to the BK receptor or due to the presence of multiple G protein pathways in untreated cells.

303.8

AGONISTS THAT SUPPRESS M-CURRENT IN FROG SYMPATHETIC NEURONS INCREASE PHOSPHOINOSITIDE TURNOVER.

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Acetylcholine (ACh), substance P (SP), and teleost luteinizing hormone releasing hormone (t-LHRH), acting at distinct receptors all suppress the voltage dependent, non-inactivating potassium current I_M. Previous work has suggested that products of PI turnover may be involved in this suppression. Although ACh and numerous other neurotransmitters have been shown to increase PI turnover in mammalian neurons, this has not been shown in the frog. Using whole, desheathed frog (*Rana pipiens*) sympathetic chains preincubated in [^{3}H]myo-inositol we show that muscarine, SP, and t-LHRH all stimulate the formation of inositol phosphates, when compared to paired, untreated control chains.

TREATMENT	% OF CONTROL	N	
1 mM muscarine	181*	4	
+ 10 μM atropine	103	2	
10 μM t-LHRH	154*	8	
0.3 μM SP	126*	5	(* $p < 0.05$)

Neither increased intracellular calcium, nor phorbol ester-induced activation of protein kinase C, can fully mimic the agonist-mediated suppression of I_M (Pfaffinger, et al. Biophys. J. 53:637a). Thus, while activation of phospholipase C does not mediate suppression of I_M by agonists, it could play a role in modulating other currents (e.g. $I_{K(Ca)}$) or in other receptor mediated phenomena. Supported by NIH grants NS08174, HL30639, and training grant GM07270. M.D.L. is an FESN scholar in "Transduction of neuronal signals".

303.9

THE ROLE OF PROTEIN KINASE C IN DESENSITIZATION OF PEPTIDE RECEPTORS INVOLVED IN M-CURRENT SUPPRESSION. Martha Bosma* and Bertil Hille (SPON: M. Spencer). Dept. of Physiol. Biophys., Univ. of Wash., Seattle, WA 98195.

We have studied suppression of M-current (I_M) by peptide agonists and desensitization of peptide receptors in isolated internally dialyzed frog (Rana pipiens) sympathetic neurons using whole-cell voltage clamp. 1 uM substance P (SP) or chicken II LHRH (cII-LHRH) suppresses I_M, and in the continued presence of agonist, the receptors desensitize and I_M recovers within 1-3 min. After agonist washout, the receptors are refractory to a second application of the same peptide for at least 30 min. There is no cross-desensitization between the two peptides. 10 nM of either peptide suppresses I_M without receptor desensitization. 1 uM teleost LHRH suppresses I_M but desensitizes LHRH receptors only slightly in 3 min. It does not suppress I_M after a desensitization by cII-LHRH. Some of these agonist actions are altered by activators of protein kinase C (PKC). Addition of phorbol ester (1 uM PMA) causes a partial (60%) reduction of I_M by itself, after which the agonist actions of SP are occluded. However, cII-LHRH can still suppress I_M and desensitize its receptor. We investigated the role of PKC using two inhibitors. First we added to the pipette solution a peptide that acts as a pseudosubstrate of PKC and inhibits its phosphorylating activity (kindly provided by Dr. Bruce Kemp of Melbourne). Second, we used bath application of H7, which reversibly inhibits PKC. In experiments with either of these inhibitors, SP and cII-LHRH were still able both to suppress I_M and to desensitize their receptors fully. Apparently the inhibitors did block PKC since PMA reduced I_M less under these conditions and no longer diminished the response to SP. Hence, PKC is not an essential mediator either in agonist-induced suppression of I_M by these two agonists, or in desensitization of the receptors by the agonists. Supported by NS08174. MB is an MDA fellow.

303.10

G_o DIRECTLY ACTIVATES K^+ CHANNELS IN HIPPOCAMPAL NEURONS. A.M.J. VanDongen*, J. Codina*, L. Birnbaumer*, and A.M. Brown, Physiology and Molecular Biophysics, Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Guanine nucleotide binding (G) proteins couple numerous neurotransmitter-activated receptors to K^+ and Ca^{2+} channels in neurons. G_o is the major G protein in brain and its function is unknown although it can reconstitute opioid inhibition of Ca^{2+} channels (Nature 325:445, 1987). We tested whether G_o gates neuronal channels directly. G proteins purified from bovine brain (J. Biol. Chem. 261:8182, 1986) and preactivated with GTPγS were applied to inside-out patches of membrane excised from neonatal rat hippocampal neurons. The patch pipette contained (in mM): K or Na MES 130, $MgCl_2$ 1, EGTA 5, HEPES 10, and Tris to pH 7.4. KMES was used in the bath. G_o activated K^+ channels having a conductance of about 40 pS and, in some cases, the channels were inwardly rectifying. This activation began at picomolar concentrations and was concentration-dependent. AMPpnP at 100 μM had no effect, ruling out G_o-mediated phosphorylating mechanisms. Activation of these channels by G_o was specific because pre- or post-G_o, 2 G_o-poor fractions purified from bovine brain, either had no effect or were effective only at much higher concentrations. G_o also activated cation-conducting channels having a smaller conductance of 10-20 pS. We conclude that G_o directly activates a variety of neuronal K^+ channels and we propose that membrane channels are the main targets of G_o. (Supported by NIH-HL37044 to A.M. Brown and L. Birnbaumer)

303.11

SUBSTANCE P MODULATES POTASSIUM CHANNELS ON T-LYMPHOCYTES THROUGH A GTP-BINDING PROTEIN. M.A. Schumann* and P. Gardner. Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305.

Substance P (SP) is implicated in the mediation of hypersensitivity reactions, but its molecular mechanism of action on T-lymphocytes has not been established. We have used a tight seal-whole cell patch-clamp recording to investigate the action of SP on Jurkat cell line with voltage-dependent K^+ channels. Perfusion of the bathing medium with SP (1 nM-10 μM) induced in 1-3 mins a dose-dependent depression (2.4±1.7%-44.7±6.7%, n=30) of the K^+ current peak amplitude elicited by depolarizing pulses. The relative magnitude of the effect was greater with 11 mM than 1.1 mM EGTA in the pipette. The latter revealed an apparent Ca^{2+}-dependent desensitization. In all cases, SP produced in 1-3 mins a nearly irreversible increase (70 ± 20.3%, n=10) in the rate at which the current inactivates. Substance P antagonist [D-Arg^1,D.phe^5,D-$Trp^{7,9}$,Leu^{11}]-SP at 50 μM blocked SP (10 nM) action by 50%. When the cells were dialysed for 5 mins with Guanosine-5-O (2.thiodiphosphate) (GDP-β-S) which competitively inhibits the binding of GTP to G protein, SP action was blocked in a dose-dependent fashion. 500 uM GDP-β-S produced a more than 3-fold reduction in SP (5 μM)-induced depression of current amplitude. Our findings provide a direct demonstration of the G-protein-mediated inhibition by SP of voltage-dependent potassium channels in T-cells. NIH Grant #R01 EY06478.

303.12

RAS CHANGES TTX SENSITIVITY OF VOLTAGE-DEPENDENT NA^+ CURRENT IN AtT-20 CELLS. R.E. Flamm, N.C. Birnberg*, and L.K. Kaczmarek. Depts. of Pharmacol. and Cell. Molec. Physiol., Yale Univ. Sch. Med., New Haven, CT 06510.

Transfection of the cHa-ras oncogene into the mouse anterior pituitary cell line, AtT-20, results in morphological and electrophysiological changes in cell phenotype. Transformed cells (ras-AtT-20) are larger and have more extensive neurite growth than untransformed (AtT-20) cells. AtT-20 cells fire spontaneously with prolonged (200-400 msec) action potentials, whereas only short-duration (10 msec), stimulus-evoked spikes are recorded in ras-AtT-20 cells. We report that ras-induced transformation generates changes in the tetrodotoxin (TTX) sensitivity of the voltage-dependent Na^+ current. TTX sensitivity in untransformed AtT-20 cells is biphasic. 10 nM TTX produces a 60% reduction in mean Na^+ current and no further reduction is observed until cells are exposed to 5 uM TTX. In contrast, in ras-AtT-20 cells (3 different clones) a 70-95% reduction in mean Na^+ current is observed with 100 nM TTX and Na^+ current is entirely abolished with 1 uM TTX. These results indicate that the introduction of activated ras causes the disappearance of TTX-insensitive Na^+ channels. Whether ras modifies the expression of different Na^+ channel species or alters post-translational modifications of the Na^+ channels in AtT-20 cells is currently under study.

NEURAL CONTROL OF IMMUNE SYSTEM II

304.1

DIFFERENT IMMUNE COMPETENCE AND "ANXIETY LEVEL" IN LATERALIZED MICE. **E. Fride, R.L. Collins & P. Arora (SPON:P. Skolnick). NIDDK/LN, NIH, Bethesda MD 20892 and Jackson Laboratory, Bar Harbor ME 04609.**

Previous studies have found a relationship between lateralization of the left and right cortex and both response to stress and modulation of immune function. Hence, the level of anxiety, cerebral asymmetry and regulation of the immune system may all be interrelated. Mice of the high (HI), low (LO) and heterogeneous (HET) lines were examined for both handedness and performance in a "plus-maze". Less time spent in the open as opposed to that in the closed arms was taken as an indication of a higher level of anxiety. Three weeks later, the animals were sacrificed and single cell suspensions from spleens prepared. The immune functions studied were: CTL response, MLR and mitogen-stimulated T and B lymphocyte proliferation. HI mice spent less time in the open arms of the plus-maze than did LO or HET mice ($p<0.01$). CTL response and MLR of the HI mice were lower ($p<0.03$) compared to both HET and LO lines. Further, the proliferative response to PHA was reduced in the LO mice. Low cell numbers in the spleens of HI and LO mice were found, whereas basal [^{3}H]thymidine uptake of these cells was much higher compared to HET mice. Although the degree of paw preference correlated positively with the level of "anxiety" in the plus-maze ($p<0.002$), it did not correlate with immune function. In summary, we found that strongly lateralized mice were more "anxious" and displayed lower immune competence than weakly lateralized mice.

304.2

MATERNAL SEPARATION ALTERS PERIPHERAL BLOOD LYMPHOCYTE CD4/CD8 RATIOS AND POKWEED MITOGEN (PWM) BUT NOT PHYTOHEMAGGLUTININ (PHA) RESPONSES IN JUVENILE BONNET MACAQUES. L. Bostom*, G. Sunderland*, H. G. Durkin*, M. R. Murali*, L. Rosenblum* and A.R. Gintzler (Spon. K. Fukada). SUNY Health Science Center at Brooklyn, N.Y. 11203, U.S.A.

The effects of maternal separation on juvenile Bonnet Macaque (female, 9-14 months) CD4/CD8 ratios and mitogen responses were determined (PHA,.125-1.0 ug/ml; PWM, 1:50-1:400)using immunofluorescence (FACS) and ^{3}H-thymidine uptake, respectively. Prior to the 14 day separation, 4-5 baseline values were obtained from each of six primates. Thereafter, the offspring continued to be housed together during the separation. Increased CD4/CD8 ratios were evident within 24h of separation and progressively increased reaching maximal values (2-3 fold) at day 10-14 of separation. Ratios returned to pre-separation values within 14 days after reunion. PHA responses during separation did not differ from control values. In contrast, PWM responses became substantially depressed (3-5 fold) 7-14 days into separation and did not return to pre-separation values through day 14 after reunion. These data suggest that maternal separation selectively affect certain aspects of immune competence in primates and support the use of these animals to investigate the psychosocial parameters of immune function and their neural and hormonal mediators.

304.3

THYROTROPIN RELEASING HORMONE (TRH) RECEPTORS IN THE IMMUNE SYSTEM. D.V. Harbour*, E.M. Smith*, and W.J. Meyer, III* (SPON: J. Calverley). Dept. of Psychiatry, Univ. of Texas Medical Branch, Galveston, TX 77550

We have identified TRH receptors on T cells of the immune system. Scatchard analysis of competetive binding studies show two specific binding sites: a unique high affinity binding site with approx. Kd 4 X 10-11 M and Bmax of approximately 35 fmol/mg membrane protein and a lower affinity binding site that is similar to the pituitary with approx. Kd of 2 x 10-8M and Bmax of approximately 4 fmol/mg membrane protein. The receptors are presumed to be bioactive since release of immunoreactive (ir)-Thyrotropin (TSH) occurs after treatment of the cells with TRH. We are currently conducting calcium uptake studies to determine the second messenger pathway of the receptors. Preliminary studies incubating the Molt4 cells with the thyroid hormone T3 show a 32-60% downregulation of the TRH receptor number. This cell line is a model to study regulation of the TRH receptor on the cells of the immune system. In addition, the finding of these receptors on normal peripheral blood mononuclear cells provides a mechanism of interaction between cells of the immune system and peripheral sources of TRH which may result in modulation of a local immune response.

304.4

CNS LOCALIZATION OF THE IMMUNOSUPPRESSIVE EFFECT OF OPIATES. R.J. Weber* A. Hagan, and A. Pert. (SPON: D. Lozovsky). LN, NIDDK, NIH, and BPB, NIMH, Bethesda, MD 20892.

Central administration of neuroactive substances such as morphine and CRH result in alterations of immune function, and have provided strong evidence for a role of the CNS in control of immunity. In order to determine the origin of the CNS effects of morphine on immune function, rats were implanted with bilateral cannulae guides aimed for various brain structures. One week following surgery the animals received bilateral injections of morphine (5 ug in 1 ul saline) in each structure. Three hours following administration of morphine the rats were sacrificed, spleens removed, and natural killer(NK) cell activity measured. Injection of morphine into the periaqueductal gray matter (PAG), but not other brain regions, produced a rapid and dramatic suppression of NK cell activity, when compared to saline injected animals, and this suppression was blocked by prior peripheral administration of naltrexone. Electrical stimulation of the PAG reveals similar effects on immune function. The activation of in the PAG may be relevant for understanding the ability of certain forms of stress to alter immune function through opiate- dependent mechanisms.

304.5

MURINE SPLENIC LYMPHOCYTES DEMONSTRATE CON-A-INDUCIBLE PROLACTIN-LIKE IMMUNOREACTIVITY AS DETERMINED BY IMMUNOCYTOCHEMISTRY (ICC). J.R. Kenner*, P.F. Smith*[1], E.W. Bernton*, D. Hartmann*[2] and J.W. Holaday, Dept. Med. Neurosci., WRAIR, Wash., DC 20307; [1]Dept. of Anatomy, USUHS, Bethesda, MD 20814 and [2]Dept. of Pathology, Georgetown Univ. Sch. Med., Wash., DC 20007.

Circulating pituitary prolactin (PRL) has been implicated as a critical modulator of humoral and cellular immune function (Bernton et al. Science, 239:401,1988). An extra-pituitary source of PRL may also have an immunological role, as suggested by in vitro studies demonstrating inhibition of mitogen-induced lymphocyte proliferation by anti-PRL antibodies (Hartmann et al. J. Lympho. Biol., 42:335,1987). The present study demonstrates that mouse splenic lymphocytes contain immunoreactive PRL (IR-PRL) as measured by ICC, and that the quantity of IR-PRL is increased by T- [Concanavalin-A (Con-A)], but not B- [lipopolysaccharide (LPS)] cell mitogens. Spleen cells from 5-6 wk old C3H/HeN male mice were incubated 48 hrs in control media or mitogen and fixed with B-5. ICC was performed using both polyclonal and monoclonal anti-PRL antibodies and detected with horseradish peroxidase via the ABC method. 37% of unstimulated lymphocytes exhibited a lactotrope-like characteristic unipolar immunoreactivity which was increased to 87% by Con A- (but not LPS-) mitogen. Presorption of the monoclonal antibody by 24Kd PRL prevented detection by ICC. IR-PRL was also detected in lymphocytes stimulated 48 hrs in serum-free medium, and in EL4 (mouse) and H9 (human) T-cell lymphoma lines. Results indicate that lymphocyte expression of IR-PRL is enhanced by T-cell mitogens.

304.6

TUMOR NECROSIS FACTOR-α STIMULATES CORTICOTROPIN RELEASING HORMONE SECRETION BY EXPLANTED RAT HYPOTHALAMI IN VITRO. R. Bernardini§, A.E. Calogero§*, T.C. Kamilaris*, P.W. Gold* and G.P. Chrousos§ DEB§, NICHD and PBP, NIMH, NIH, Bethesda, MD 20892

The hypothalamic-pituitary-adrenal (HPA) axis is activated during the inflammatory/immune response. Interleukin (IL)-1, -2 and -6 have been shown to act as HPA axis activators in vivo. We have shown that this effect of IL-1 and IL-2 is directed towards the CRH neuron. In this study we examined the ability of tumor necrosis factor-α (TNF-α), a major macrophage-derived cytokine secreted following macrophage activation, to stimulate CRH secretion by in vitro cultured rat hypothalamic explants.

After explantation and overnight incubation in medium 199 (M199), single hypothalami were incubated for 60 min with M199, followed by a 40 min stimulation with graded concentrations of TNF-α (Dr. M. Palladino, Genentech, South San Francisco, CA) ranging between 10^{-13}M and 10^{-7}M. The viability of each explant was tested in a final step of the experiment, examining the CRH response to 60mM KCl-induced depolarization. Immunoreactive rat corticotropin releasing hormone (iCRH) was assayed directly in the media by a specific radioimmunoassay. Statistical analysis was performed by ANOVA followed by Duncan multiple range test. TNF-α stimulated iCRH secretion by explanted rat hypothalami at doses ranging between 10^{-11}M and 10^{-7}M ($p<0.01$; $ED_{50}=2x10^{-11}$M).

We conclude that TNF-α stimulates rat hypothalamic CRH secretion in vitro. Thus, this cytokine may be included in the glucocorticoid-mediated counterregulatory mechanism via which the immune system restraints its own response.

304.7

INTERLEUKIN-1 AS AN IMMUNOTRANSMITTER: PERIPHERAL IL-1 ADMINISTRATION ACTIVATES BRAIN NOREPINEPHRINE METABOLISM Adrian J. Dunn
Dept of Neuroscience, Univ of Florida, Gainesville, FL 32610-0244

Immunologic activation is associated with increased concentrations of plasma corticosterone, as well as increases in the firing rate and norepinephrine (NE) metabolism of hypothalamic neurons. Interleukin-1 (IL-1) is a cytokine produced by macrophages after antigen presentation. Recent reports indicate that IL-1 administration elevates plasma corticosterone, which may account for the elevation of plasma glucocorticoids observed following antigenic stimulation.

Purified recombinant IL-1 injected intraperitoneally (ip) markedly activates cerebral noradrenergic systems as indicated by increased concentrations of the NE catabolite, 3-methoxy,4-hydroxyphenyl-ethyleneglycol (MHPG) in several brain regions. The increase of MHPG is largest in the hypothalamus. Tryptophan concentrations are also increased throughout the brain. These effects are dose-dependent with a minimum effective dose of 25 ng. They peak 2-4 hours after IL-1 administration, roughly parallelling the increased plasma corticosterone. The activity of IL-1 is lost after heating. Several different preparations of both IL-1α and IL-1β are effective. These changes resemble those observed following infection of mice with Newcastle disease (NDV) or influenza viruses, or endotoxin administration.

Because noradrenergic neurons with terminals in the paraventricular hypothalamus are known to regulate the release of corticotropin-releasing factor (CRF), these results suggest that IL-1 stimulates the hypothalamic-pituitary-adrenal (HPA) axis by activating these neurons. The effect of IL-1 on hypothalamic MHPG explains both the activation of hypothalamic NE metabolism and that of the HPA axis during immune responses.

[Supported by the National Institute of Mental Health (MH25486)].

304.8

THE ORGANUM VASCULOSUM LAMINA TERMINALIS (OVLT) AND THE PREOPTIC ANTERIOR HYPOTHALAMUS AREA (POA) ARE PRIMARY SITES FOR INTERLEUKIN-1-INDUCED ACTH RELEASE. G. Katsuura*, K. Koves*, P.E. Gottschall* and A. Arimura (SPON : P. Guth), US-Japan Biomed. Res. Labs, Tulane Univ. Hebert Center, Belle Chasse, LA 70037.

We recently demonstrated that both systemic and central administration of interleukin-1 *beta* (IL-1 *beta*) induced ACTH release in conscious rats which appears to be mediated by an increased release of endogenous CRH. The present study was conducted to examine the primary site of action of circulating IL-1 *beta* in the brain. The OVLT, which has fenestrous endothel, was first examined as the possible port of entry of circulating IL-1 into the brain. Adult male rats were lesioned in the OVLT by high frequency current. Sham-operated animals served as the control. The magnitude of ACTH response to *iv* administration of IL-1 *beta* increased 1.5 ~ 1.7 times one week following lesion and returned to the control levels 3 weeks later. Lesion of the subfornical organ (SFO), another circumventricular organ, did not alter the magnitude of the response. On the other hand, bilateral lesion in the POA diminished ACTH response by 50%, as compared with sham-operated animals. These findings may be interpreted as follows: Lesion of the OVLT may have caused a post-surgical inflammatory reaction which resulted in an increased entry of IL-1 *beta* into this area, thereby eliciting a greater response. Three weeks post-surgery such inflammation may have subsided and the amount of IL-1 *beta* which had entered into the brain returned to the pre-surgical level. Lesion of the SFO may also have resulted in the increased entry of IL-1 *beta* into this area which, however, lacks the neuronal components which respond to IL-1 *beta*. The POA appears to contain the critical neuronal elements which convey the signal of IL-1 *beta* to the CRH neurons. (Supported by NIH grant DK09094).

304.9

ASTROCYTE-MEDIATED IMMUNOSUPPRESSION. M. Borgeson* and R. W. Keane. Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33101.

Using primary cultures of murine central nervous system (CNS) cells, we demonstrate that astrocytes suppress antigen-stimulated T helper cell proliferation and alloantigen- or mitogen- (Concanavalin A, phytohemagglutinin (PHA), lipopolysaccharide) stimulated spleen mononuclear cell (SMC) proliferation by 70-100%. As few as 0.4 % astrocytes present in the total cell population of proliferation assays suppressed PHA-stimulated lymphocyte proliferation by 65-70%. Astrocytes did not suppress the proliferation of a cytotoxic T cell line (CTLL-2), but did suppress the formation of alloantigen-reactive cytotoxic T cells in a mixed lymphocyte culture. Astrocyte-mediated suppression was not sensitive to indomethacin, a blocker of prostaglandin E production, and was not species-specific. Pure cultures of CNS microglia also suppressed mitogen-stimulated SMC proliferation, but microglial-mediated suppression was completely reversed by indomethacin treatment. Neither CNS neurons nor C3H fibroblasts suppressed lymphocyte proliferation. Astrocyte-mediated immunosuppression may limit immune reactions in the CNS and thus contribute to the maintenance of the immune privileged status of the CNS. Supported by NIH grant NS21728 and the National Parkinson's Foundation.

304.10

INNERVATION OF T-CELL COMPARTMENTS IN *XENOPUS LAEVIS* SPLEEN. S.Y. Felten and N. Cohen * Depts. of Neurobiology and Anatomy & Microbiology and Immunology, Univ. of Rochester Sch. Med., Rochester, NY 14642.

Studies of sympathetic postganglionic innervation of rodent spleen from our laboratory and others have described innervation of specific lymphoid compartments in addition to extensive innervation of the vasculature, capsule, and trabeculae. In rodents, this non-vascular innervation is found in the periarteriolar lymphatic sheath (PALS), a T-cell compartment, at the inner edge of the marginal zone among T- and B- cells and macrophages, in the parafollicular zone, with occasional fibers entering the follicle itself. In the PALS, direct contacts between tyrosine hydroxylase (TH) positive nerve terminals and lymphocytes have been noted.

The Xenopus spleen has a different arrangement of immune cells than the rodent since germinal centers are generally absent, and the T-dependent area is analogous to the marginal zone of the rodent. We examined the adult Xenopus spleen using glyoxylic acid histofluorescence (HF) to demonstrate catecholamine innervation patterns and HPLC to determine whether the postganglionic sympathetics innervating the spleen were adrenergic as has been reported in other anurans or noradrenergic as in rodents.

HF staining showed specific compartmentation of CA containing nerve fibers in the white pulp areas. There was little or no staining in any other compartment. Within the white pulp, there was prominent staining around the edge, associated with the marginal zone. In addition, there were fibers associated with the blood vessels that branched into the parenchyma of the PALS. HPLC with electrochemical detection for monoamines and their metabolites showed only norepinephrine (2.54 pMol/mg wet wt.). Epinephrine and serotonin were not present in measurable amounts.

304.11

THE ADRENAL MEDULLA AND SYMPATHETIC β_2-ADRENOCEPTORS INFLUENCE THE SEVERITY OF EXPERIMENTAL ARTHRITIS (EA) IN THE RAT. T.J. Coderre, M.F. Dallman*, A.I. Basbaum, and J.D. Levine*. Division of Neurobiology, UCSF, San Francisco, CA 94143.

In previous studies we demonstrated that β_2-adrenergic antagonists attenuate the severity of EA in the rat (Levine et al., PNAS 1988, in press). This study addressed the β_2-agonist contribution of the adrenal medulla to the severity of EA. Arthritis was induced by intradermal tail injection of 0.1 ml of 10 mg/ml Mycobacterium butyricum in mineral oil, and joint injury was assessed radiologically 28 days later. Bilateral adrenal medullectomy (ADMX) was performed 3 weeks prior to the induction of EA, and/or reserpine (0.25 mg/kg, i.p.) was given every 2 days, to produce a chemical sympathectomy (SYMX). Both ADMX and SYMX significantly attenuated EA compared to no treatment or sham-ADMX controls. Another group of ADMX rats received a slowly released preparation of either epinephrine (0.5 µM/kg) or the β_2-agonist, salbutamol (0.165 µM/kg). The drugs were dissolved in 0.2% ascorbic acid and mixed with paraffin oil and arlacel (4.25:5.0:0.27 v/v) and were administered s.c. every 3 days to restore the β_2-agonist effect of epinephrine normally secreted by the adrenal. Chronic treatment with salbutamol, but not epinephrine, produced a significant increase in EA (compared to untreated, ADMX rats). Salbutamol, however, did not increase EA in ADMX rats which were also SYMX. We conclude that EA is influenced by catecholamines which are released by the adrenal medulla. The catecholamines act, in part, at β_2-adrenergic receptors located on sympathetic postganglionic neurons. Supported by NIH grants AR32634, NS14627, 1F05TW03980 and the Arthritis Foundation.

304.12

THE DISTRIBUTION OF ANS RECEPTORS ON CELLS OF THE IMMUNE SYSTEM. K. Bulloch and T. Radojcic. Neuroimmune Physiology Lab., Wellcome Foundation/Depts. of Psychiatry and Pediatrics, University of Cal. San Diego, CA 92093

Lymphoid tissues and organs receive a rich ANS innervation. In order to determine the role of the ANS with regard to immune function we are now evaluating the temporal distribution of the ANS receptor fields within the lymphoid tissues. Herein we report the pattern of ANS receptors on isolated thymocytes and splenocytes as well cloned cell lines of the immune system. Our results show binding of both adrenergic and muscarinic antagonists to an immature thymocyte cell line are comparable to those seen in isolated thymocytes. A mature activated T-helper cloned cell line had a greater number of B-AR receptors than did immature cloned or normal thymocytes, but did not express any m-ACH receptors. However, T-killer cells are known to bear functional m-ACH receptors. These data are consistent with reports that ANS receptors vary in number with functional maturity of different subtypes of T lymphocytes. A cloned B cell line expresses a unique type of m-ACh receptor. Several reports have demonstrated that cholinergic agonists increase the secretion of immunoglobulins. Our data supports these findings and indicate that cholinergic agents may mediate their effect directly on B cell muscarinic receptors. The effect of ANS agonists on second messengers on immune function will be discussed. Supported by: grant #N00014-85K-0528 ONR and a grant from Joan B. Kroc Foundation of Psychoneuroimmunology.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CEREBELLUM II

305.1

INTERCONNECTING AN ANALOG SIMULATOR WITH AN OLIVARY NEURON. Y. Yarom* and A. Adan* (SPON: I. Parnas). Dept. of Neurobiology, The Hebrew University of Jerusalem, Israel.

The membrane potential of inferior olivary neurons occasionally oscillates with an almost sinusoidal waveform (Llinas & Yarom, 1986). An analog simulator consisting of four similar interconnected units was constructed. When one of the units receives a trigger signal, the system can either generate sustained oscillations or briefly oscillate and then slowly relax to resting conditions. This behavior is determined by the degree of coupling between the elements. Once the system is oscillating it will remain in this state, independent of any external inputs. In order to stop the oscillations, one or two of the units has to be disconnected from the rest. Thus, the four damped oscillators can generate sustained oscillations provided that they are interconnected. In order to test the hypothesis that olivary neurons function similarly to the simulator, the four units were interconnected to an olivary neuron in a slice preparation. Under these conditions the hybrid system behaves in the same way as the 4 units by themselves; it can generate sustained oscillations only when all 5 elements are connected. The simulator and the hybrid system differ in that a lower gain setting is sufficient in the hybrid system to generate sustained oscillations. Furthermore, in the hybrid system, briefly depolarizing the olivary neuron to a level which completely inactivates the somatic Ca spike turns off the sustained oscillations. The same effect can be generated by briefly hyperpolarizing the olivary neuron to below the threshold of the somatic Ca spike. We conclude that olivary neurons operate on principles similar to those characterizing the oscillating units of the simulator and that the somatic Ca spike is the main factor responsible for generating the sustained oscillations in olivary neurons.

305.2

DOES STIMULATION OF THE INFERIOR OLIVE PRODUCE MOVEMENT? Alan R. Gibson and Richard Chen*, Barrow Neurological Inst., Phoenix, AZ 85013. Production of movement by climbing fiber activity has important implications for models of cerebellar function. Mauk et al. (Proc. Natl. Acad. Sci. 83, 1986) report that stimulation of the rabbit rostral dorsal accessory olive (rDAO) in the face region produces blinks whereas stimulation in other rDAO regions produces head movements. Three cats were prepared for chronic recording and stimulation. Eyelid movements were monitored photoelectrically and limb movements were monitored with accelerometers. We identified regions of rDAO by mapping cutaneous receptive fields. The peak neural response of eye rDAO to an air puff occurred after the onset of the blink. Stimulation in rDAO could produce a blink, but thresholds decreased by more than 50% 1.0 mm below rDAO. Blinks were as easily elicited from limb rDAO as from eye rDAO, and none of the lowest threshold points for blinking were in the IO. Limb movements elicited by olivary stimulation showed no specificity for the region of the olive being stimulated and required 2 to 3 times as much charge as .9 to 1.8 mm above rDAO. No qualitative differences could be discerned between movements elicited within IO and the lower threshold movements elicited near IO. Our results do not support the concept that electrical activation of the inferior olive produces movements.

305.3

CELLS IN THE INFERIOR OLIVE, BUT NOT THE CEREBELLAR VERMIS, ARE RHYTHMICALLY ACTIVATED BY HARMALINE IN THE GENETICALLY DYSTONIC RAT. S.E. Stratton and J.F. Lorden, Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The genetically dystonic (dt) rat fails to develop a tremor following administration of harmaline. Because this mutant responds to other tremorogenic agents, an abnormality has been sought in the olivo-cerebellar pathway activated by harmaline. Single unit recordings in the cerebellar vermis showed that in normal animals, harmaline produced a sustained, rhythmic increase in complex spike activity in 67% of the cells. In the dt rat, only 9% of the cells showed this response. The present study was conducted to determine whether the reduced effectiveness of harmaline in the dt rat was due to a failure to activate the inferior olive, the site thought to be the pacemaker for the harmaline tremor. Pre- and post-harmaline (10 mg/kg, i.v.) single unit activity was recorded in 6 normal and 12 dt rats anesthetized with urethane (1.9 g/kg). In all cells recorded in the subnucleus b of the medial accessory olive (5 normal and 11 dt), a sustained, rhythmic increase in frequency from about 1 Hz to 4 Hz was obtained. Two cells that did not respond were located outside the inferior olive. These results suggest that the failure of the dt rat to show harmaline tremor is the result of a cerebellar defect. (Supported by NS18062).

305.4

SENSORY INPUT TO THE FORELIMB INFERIOR OLIVE AND ITS RELATIONSHIP TO MOTOR PATHWAYS. M.L. McCurdy*, C. Melton*, J.C. Houk and A.R. Gibson. Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611 and Division of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013

Stimulation of cat magnocellular red nucleus (RNm) inhibits responses of rostral dorsal accessory olive (rDAO) neurons to peripheral input (Soc. Neurosci. Abs.11:182,1985). Since the RNm does not project directly to rDAO, the neural connections subserving the olivary gating may involve sensory pathways to the rDAO. Our studies show that afferent input to the forelimb region of rDAO arises from a column of cells in the caudal cuneate nucleus and cervical spinal cord (Soc. Neurosci. Abstr. 12:326, 1986). We determined whether this column receives input from RNm by injecting WGA-HRP into the forelimb areas of both RNm and rDAO.

The dual injection protocol demonstrated that labeled column cells were within a larger zone comprising the rubral terminal field. Branches of rubrospinal tract axons were seen coursing toward the area of the column. The results identify a likely collateral pathway that could gate rDAO activity. Motor cortex projects to similar cuneate and spinal regions and is also reported to gate olive activity (Somatosens. Res 1:169,1983; J. Physiol. 228:619,1973).

A second question we addressed was whether the RNm-column-rDAO pathway was topographically organized. Topographic specificity could provide a basis for somatotopic specificity in the gating mechanism. Injections in spinal or cuneate parts of the column revealed no specific topography for RNm cells that project to the column. Small injections in somatotopic regions within the forelimb area of rDAO and in trunk rDAO demonstrate that the trunk and proximal forelimb are represented in the cuneate part of the column and the distal forelimb is represented in both spinal and cuneate parts. Although the somatotopy in rDAO is more detailed than that in the column, both nuclei emphasize the distal forelimb.

305.5

OPTOKINETIC STIMULATION INCREASES CORTICOTROPIN RELEASING FACTOR mRNA IN INFERIOR OLIVARY NEURONS OF RABBITS. N.H. Barmack and W.S. Young, III. R.S. Dow Neurol. Sciences Inst., Good Samaritan Hosp. & Med. Ctr., Portland, OR 97209 and Lab. Cell. Biol., NIMH, Bethesda, MD 20892.

Neurons located in the caudal dorsal cap of the inferior olive are excited by low velocity optokinetic stimulation in the posterior-anterior direction of the contralateral eye. These visual olivocerebellar neurons have been implicated in the regulation and possible plasticity of eye movements. A neuropeptide, corticotropin releasing factor, CRF, has been identified in all olivary neurons. Using in situ hybridization histochemistry we have examined the influence of prolonged, unidirectional, binocular opto-kinetic stimulation on the level of CRF mRNA in neurons of the caudal dorsal cap. Rabbits were partially restrained within an optokinetic drum which rotated at an angular velocity of 5°/sec for 6-150 hr. Frozen brain sections were hybridized with an S^{35}-labelled oligonucleotide probe for CRF. The x-ray film images of these sections were subsequently analyzed with a computer-based microdensitometer. Six hours of optokinetic stimulation was sufficient to cause an increase in CRF mRNA in neurons of the "stimulated" dorsal cap relative to the background activity of the rest of the inferior olive. After 140 hours of optokinetic stimulation there was a 10-fold increase in the level of CRF mRNA. This elevated level of CRF mRNA lasts 24 hours. These data suggest that CRF may play an important role in olivocerebellar function.

305.6

DEVELOPMENT OF THE OPTOKINETIC REFERENCE FRAME OF FLOCCULAR PURKINJE CELLS IN RABBIT. R. E. Soodak*, L. J. Croner* and W. Graf. The Rockefeller University, New York, NY 10021.

Purkinje cells of the rabbit flocculus encode retinal slip velocity with an internal coordinate system roughly aligned with the axes of the semi-circular canals and extra-ocular muscles. In addition, the simple and complex spike responses of a given purkinje cell are usually reciprocally related, with the simple spikes increasing in activity when the complex spikes decrease, and vice-verca. To examine the role of visual experience in the development of these highly specific neuronal responses, we recorded simultaneously the simple and complex spike activity of floccular purkinje cells from dark-reared rabbits. Recordings were made from adult animals that had been reared in total darkness from before eye opening. Our visual stimulus was a planetarium projector that simulated the retinal slip resulting from rotational self-motion.

As in normal rabbits, purkinje cells of dark-reared rabbits encoded rotational self-motion with an approximately sinusoidal tuning curve, similar to the encoding of angular acceleration by the semi-circular canals. In addition, all three visual response types found in normal animals, i.e., Vertical Axis, Anterior (45 deg) Axis and Posterior (135 deg) Axis, were found in dark-reared rabbits as well.

The reciprocal relationship between simple and complex spike activity was also observed in dark-reared rabbits. This suggests that simple spike-complex spike reciprocity is not the result of an instructive process in which the climbing fiber "teaches" the parallel fiber inputs through a mechanism of heterosynaptic plasticity.

Supported by NIH grant EY-04613

305.7

KINEMATIC ANALYSIS OF NICTITATING MEMBRANE RESPONSES IN CEREBELLAR LESIONED RABBITS. J. P. Welsh. Dept. of Psychology, University of Iowa, Iowa City, IA 52242

Cerebellar lesions reduced the frequency and amplitude as well as increased the rise time of nictitating membrane extensions (NMEs) elicited by tones or low intensities of corneal air puff. These deficits were most prominent after lesions in the anterior interpositus nucleus (aINT). Over a range of air-puff intensities, the NME of normal rabbits consists of one peak of velocity and acceleration. The tone-elicited NME of normal rabbits consists of one peak of velocity but consists, however, of 2 peaks of acceleration. Cerebellar lesions producing a continuum of deficits in frequency, amplitude and rise time of tone-elicited NMEs progressively reduced the peak velocity and acceleration as well as increased the time of peak velocity and acceleration of tone-elicited NMEs. In contrast to the normal NME elicited by a tone, only one peak of acceleration was observed in NMEs following cerebellar lesions that reduced NME amplitude. Lesions of aINT reduced peak velocity and latencies to peak velocity and peak acceleration of the NME elicited by air puff, especially at low intensities. The different acceleration profiles of tone-elicited and air puff-elicited NMEs may be a function of differential patterns of motoneuronal excitation for this behavior. These analyses suggest that cerebellar lesions may disrupt NME performance by fundamentally disrupting patterned motoneuronal activity. Supported by USPHS MH Grants 16841 & 15773.

305.8

SPATIAL AND TEMPORAL PATTERNS OF SPONTANEOUS AND EVOKED CALCIUM DEPENDENT EVENTS IN THE DENDRITES OF GUINEA PIG CEREBELLAR PURKINJE CELLS IN VITRO. W.N.Ross and R.Werman* Dept. Physiology, New York Med.Coll.,Valhalla,NY 10595 and Dept. Neurobiology, Hebrew Univ., Jerusalem, Israel.

We have used the indicator dye arsenazo III in conjunction with a 64 element photodiode array to measure dendritic calcium changes induced by spontaneous or somatically-stimulated electrical events. The recording system had a time resolution of 1 msec and sufficient sensitivity to detect calcium changes at each location without signal averaging. Calcium transients corresponding in time with somatically recorded action potentials could be detected from all locations on the dendritic arborization. Individual calcium action potentials in a burst often had different spatial distributions. In general, transients that were only detected from secondary branches were associated with small electrically recorded events in the soma. Calcium transients which were detected from the main trunks had large somatic electrical potentials whether or not there were corresponding transients over the finer dendritic branches. Somatically stimulated transients were larger in the proximal dendritic region while spontaneous events were larger distally. Calcium increases corresponding to plateau potentials were also detected without averaging. These had a heterogenous spatial distribution, but were generally larger nearer to the soma. Supported by USPHS grant NS16295 and the U.S.-Israel Binational Foundation.

305.9

SYNAPTICALLY EVOKED CALCIUM TRANSIENTS IN THE DENDRITES OF CEREBELLAR PURKINJE CELLS IN VITRO. H. Miyakawa*, V. Lev-Ram*, and W.N. Ross. (SPON: F. Horvath). Dept. of Physiology, New York Medical College, Valhalla, NY 10595.

We have used the same preparation and optical recording apparatus as Ross and Werman (preceding abstract) to record calcium increases in the dendrites of Purkinje cells in response to white matter stimulation which predominantly activates the climbing fiber synapse. These calcium transients could be detected in a single trial from all locations in the dendritic field, but were usually distributed closer to the soma when compared with transients evoked by spontaneous calcium-dependent action potentials in the same cell. No signals were detected from the soma. The amplitudes of the synaptically activated and action potential activated transients were of the same order of magnitude. When the two potentials were activated at about the same time their transients did not sum. The recovery time course of the transients was complex but the initial component was rapid, often having a time constant of less than 100 msec. Repetitive stimulation could increase this recovery time but the increase was not uniform at all dendritic locations.

Supported by USPHS grant NS16295. V. Lev-Ram is a Chiam Weizmann Postdoctoral Fellow.

305.10

DENDRITIC CALCIUM-DEPENDENT EVENTS IN PURKINJE CELLS DETECTED WITH FURA-2. V. Lev-Ram, H. Miyakawa, and W.N. Ross. (SPON: K. Hubbard). Dept. of Physiology, New York Medical College, Valhalla, N.Y. 10595.

Purkinje cells in sagittal cerebellar slices were injected with the impermeant calcium indicator fura-2 and excited at 395±20 nm with a xenon lamp. Epifluorescence at >510 nm from a 70 um diameter spot in the dendritic field was measured with a photodiode. When the cell fired a series of calcium-dependent action potentials, each spike caused a transient reduction in fluorescence which could be detected without averaging. Climbing fiber synaptic activation also caused a reduction in fluorescence detected in a single trial. A burst of action potentials often caused a reduction in fluorescence of over 35%. The time course of these transients was approximately the same as those recorded from similar cells using absorbance changes of arsenazo III. These experiments show that fura-2 and a photodiode array could be used to measure the spatial, temporal, and amplitude characteristics of calcium-dependent dendritic events in Purkinje cells.

Supported in part by USPHS grant NS16295. V. Lev-Ram is a Chiam Weizmann Postdoctoral Fellow.

305.11

SPATIAL AND TEMPORAL PATTERNS OF PURKINJE AND GRANULE CELL ACTIVITY RECORDED SIMULTANEOUSLY IN THE RAT CEREBELLUM USING SILICON MICROELECTRODES. M.E. Nelson[1], B. Rasnow[2], J.J. Banik*[3], and J.M. Bower[1]. Depts. of Biology[1], Physics[2], and Electrical Engineering[3], Caltech, Pasadena, CA 91125.

We have developed a technique which allows us to make simultaneous extracellular recordings from a planar array of cerebellar locations using a multiprong, multisite, silicon-based recording probe. This new technique is more powerful than earlier multi-single neuron recording methods (Bower and Llinas, Soc. Neurosci. Abstr., 9: 607, 1983) which were limited to recording one site per inserted probe and which did not provide a constrained and easily reproducible geometry between recording sites. The key to the new technique is our development of a miniature 30-channel silicon-based microelectrode with excellent recording characteristics. Using photolithography and anisotropic etching techniques, we are able to shape the silicon substrate into a structure which supports a high density of recording sites while at the same time being no larger than standard glass pipettes. The probes are a comb-like structure with 10 teeth per comb. At its widest point, the shank of each tooth is approximately 24μ x 12μ in cross-section and tapers to a sub-micron point at the end of its 1.5 mm length. The teeth are spaced 150μ apart and there are 3 recording sites on each tooth. For these cerebellar studies, we have positioned the recording sites on each individual tooth to simultaneously recording climbing fiber responses at the dendritic level, simple spikes at the somatic level, and granule cell responses in the underlying granule cell layer. We have used the probes in Crus IIa of the rat cerebellar cortex to record spontaneous activity and responses to tactile stimulation of the face. (This work supported by NIH Grant NS22205, the Joseph Drown Foundation, and the Whitiker Foundation.)

305.12

SPATIAL PATTERNS OF CEREBELLAR CORTEX ACTIVITY IN VIVO USING VOLTAGE SENSITIVE DYES AND IMAGE ANALYSIS. S.A. Elias, H. Yae*, J.H. Kim and T.J. Ebner. Depts. Neurosurgery and Physiology, Neuroscience Grad. Prog., Univ. of MN, Mpls., MN 55455.

We recently reported that voltage sensitive dyes and image processing can be used to investigate the spatial features of cerebellar circuitry. This technique has been further used to study (1) the three-dimensional nature of parallel fiber activation and (2) the spatial activation of the cortex to peripheral input. Rats were anesthetized with ketamine and the cerebellar cortex stained with RH414. Using a epifluorescence microscope, images were recorded using a CCD video camera. The video signals were filtered, windowed and the control/stimulus averaging and subtraction (up to 1000 times) performed on line. Using a microelectrode to provide surface stimulation, the evoked optical images were recorded at depths of focus from surface to 500 um. The resulting images were organized longitudinally, and at greater depths showed increasing parasagittal spread. Images to repetitive stimulation of the face and forelimb were made up of two components (1) areas of cortical activation and (2) increased blood flow in the surface blood vessels. The preliminary data suggests that this technique will be useful for the study of somatosensory input and properties of intrinsic cortical activity. Supported by Minnesota Med. Found. and NIH/NS-18338.

SOMATOSENSORY CORTEX III

306.1

EFFECTS OF SELECTIVE REMOVALS OF THE POSTCENTRAL HAND REPRESENTATIONS ON THE RESPONSIVITY OF NEURONS IN THE SECOND SOMATOSENSORY AREA (SII) OF RHESUS MONKEYS. T.P. Pons, P.E. Garraghty and M. Mishkin, Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

We previously reported that elimination of all four hand representations in the postcentral strip (i.e. areas 3a, 3b, 1, and 2) eliminated the hand representation in SII. Two of these postcentral areas (3b and 1) process predominantly cutaneous inputs, whereas the other two (3a and 2) process mainly "deep" somatic inputs. We have now found that following selective removal of the 3b and 1 hand representations the SII hand representation remains intact, but activation of its neurons requires a substantially higher-than-normal amplitude of somatic stimulation. We used a pressure aesthesiometer to quantify the force required to elicit a consistent neuronal response from recording sites in different representations in SII 6-8 weeks after the selective ablations. Whereas a stimulation force of < 0.5 gr was adequate to produce a response at a majority of recording sites outside the hand region of SII, a stimulation force of $>$ than 2 gr was necessary to evoke a response at sites within it, presumably because of the need to activate "deep" somatic inputs from the hand. These findings suggest that modality-specific information is relayed from the postcentral strip to SII along parallel channels, with cutaneous inputs being transmitted via areas 3b and 1 and "deep" inputs via areas 3a and 2.

306.2

CORTICAL RESPONSES TO PARESTHESIAS. C.N. Applegate* and P.T. Fox SPON:(S.G. Eliasson) Washington University School of Medicine, St. Louis, MO 63110

Paresthesias are associated with ectopic impulses in peripheral and spinal axons, but the role of cortex is not known. Using positron emission tomography (oxygen-15 water), we identified multiple areas of cortical activity during paresthesias that follow prolonged (60 min) hand vibration. Three conditions were aquired in each of 6 subjects: rest, hand vibration, and paresthesias (after stimulus termination). Image analysis used paired subtraction (stimulus - rest, paresthesias - rest), inter-subject averaging and a search routine that identified foci of blood flow change. Left hand vibration increased blood flow in: right post-central ("S1"), 23%; right medial frontal ("SMA"), 12%; right parietal opercular ("S2"), 8%; left superior lateral parietal ("S3") 7%, right insular ("S4"), 7%; and bilateral orbitofrontal, 8% cortices. During paresthesias the blood flow increases were: right S1, 12%; right S2, 8%; left S3, 6%; right S4, 9% and bilateral orbitofrontal, 8%. SMA was not active during paresthesias. Thus cortical areas activated by a physiologic stimulus were also actived during paresthesias. In fact, extraprimary responses were of equal or even greater magnitude during paresthesias than during vibration.

306.3

RIGHT-HEMISPHERIC DOMINANCE FOR SOMATOSENSORY PROCESSING IN HUMANS. P.T. Fox, C.N. Applegate* Washington University Medical School, St. Louis, MO 63110

Human hemispheric specialization for language, skilled movement, and attention is well known. Hemispheric domiance for modality-specific sensory processing is not well described. Studying right-handed normal volunteers with positron emission tomography (oxygen-15 water), we found a right-hemispheric response predominance in 3 extraprimary somatosensory areas.

A vibratory stimulus was applied bilaterally to lips, fingers, and toes and also unilaterally (right and left) to fingers on separate trials in 9 subjects. The control state was eyes-closed rest. Images of average blood-flow change were formed using paired-image subtraction and intersubject averaging.

No evidence of lateral dominance was found for responses in primary somatosensory cortex or supplementary motor area. Right-hemispheric dominance was found for three extra-primary somatosensory responses: buried posterior sylvian ("S2"), lateral superior parietal ("S3"), and anterior insular ("S4") cortices. This effect was most clearly demonstrated with bilateral stimuli. Responses to bilateral stimuli were predominately or exclusively on the right. Responses to unilateral stimuli were contralateral, but were weaker or absent on the left.

306.4

THE SPATIAL AND TEMPORAL ORGANIZATION OF THE PRIMARY SOMATOSENSORY EVOKED POTENTIAL P.B. Hoeltzell*, R.W. Dykes, C.E.Chapman Dept. Physiol. McGill Univ., Mtl. QU.

The primary somatosensory evoked potential consists of a biphasic waveform that is initially positive at the surface. With increasing depth, this potential reverses polarity, becoming initially negative. Traditionally it is modelled by current dipole.

Data was obtained from pentobarbital anesthetized cats. A computer-controlled punctate mechanical stimulus was delivered to the cat forepaw. Recordings were made using 200 um steps in the AP and Ml planes. Vertical data were collected using 100 um steps. The waveform profiles were analyzed both as a function of time and space. A current source density (csd) analysis was also performed.

Three distinctly different waveform profiles were found. The first consisted entirely of initially negative, non-inverting waveforms. The second profile was composed of initially positive waveforms. Waveforms found at the evoked potential focus were more complex and followed the classic description.

Laminar analysis in conjunction with the csd data showed that one waveform produced a strong source-sink couplet in layer II and another produced a weak sink-source couplet in layer IV. The classical evoked potential was shown to be the result of the summation of these two separate neuronal populations.

(Supported by MRC and NIH)

306.5

AN HYPOTHETICAL MODEL OF CORTICAL SENSORY PROCESSING BASED UPON STUDIES OF THE SOMATOSENSORY-EVOKED RESPONSE IN AWAKE MONKEYS AND CONSIDERATION OF CORTICAL CONNECTIVITY.

L.J. Cauller*, A.T. Kulics* and T.J. Teyler (SPON: T.J. Voneida). Department of Neurobiology, Northeastern Ohio Universities' College of Medicine, Rootstown, OH 44272.

A series of experiments has been undertaken to identify the neural events underlying N1, the surface-negative component (50ms poststimulus) of the somatosensory-evoked potential (SEP) (Kulics,A.T. and Cauller,L.J. *Exp. Brain Res. 62*:46, 1986). The amplitude of N1 predicts the discrimination response of behaving monkeys (Kulics, A.T. *EEG Clin. Neurophysiol. 53*:78, 1982). Current source-density analysis has identified current sinks in superficial layers I/II of postcentral Area 1 associated with multiple unit activity (MUA) in layers II-V. The excitatory nature of N1 is indicated by the prominence of late MUA which extends into subcortical white matter. In contrast to the awake response, the N1 interval of the response during slow-wave sleep is associated with MUA inhibition and large current sources through layer III. As the principal source of excitatory input to superficial layers, the "backward" projection which converges upon Area 1 from secondary somatosensory areas is thought to be the origin of N1 input (Pandya D.N. and Seltzer B., *J. Comp. Neurol. 204*:196, 1982). A model is developed which considers the functional significance of the "backward" cortical system for conscious sensory processing that emphasizes the role of distal apical dendrites of the population of pyramidal neurons which engage superficial layers I/II. The unconscious state of cortical sleep is viewed as the blockade of superficial inputs.

306.6

INTRAHEMISPHERIC CORTICO-CORTICAL AFFERENTS TO AREA 5 AND SsA IN THE CAT. C. Avendaño, E. Rausell* and A. Verdú*. Dept. Morphology, Medical School, Autónoma Univ., 28029 Madrid, Spain.

The intrahemispheric cortical afferent connections of area 5 and the adjoining "anterior suprasylvian area" (SsA) were studied in the cat by placing single or multiple injections of retrograde tracers (HRP, WGA-HRP or fluorescent dyes) into different divisions of areas 5a, 5b, 5m and SsA. Labeled cells were plotted on projection drawings of coronal sections of the brain, and on two-dimensional "maps" of the cerebral cortex.

The major findings of this study are: 1. Areas 3a, 3b, 1 and 2 project to area 5 and to SsA. These projections, however, show marked differences in amount and topographical distribution depending on the medio-lateral and rostro-caudal location of the injections. 2. Areas 4 and 6 also project heavily to area 5 and SsA in a well organized topographic fashion: Area 4 projects mainly upon areas 5a, 5m, SsA, and the medial part of 5b; area 6 projects mainly upon the lateral part of 5b, and SsA. Moreover, the upper bank of the cruciate sulcus (areas 4β and 4δ) projects to medial parts of area 5, and the lower bank (areas 4β, 6aα and 6aβ) projects to lateral parts of area 5. 3. SIIm, SII and SIV are connected mainly with medial parts of area 5 (particularly 5a), and SsA. 4. Areas 7 and 7m, and a number of visual areas (19, SVA, AmLS, PmLS, 21, 20, 18, AILS and PILS) project in varying degrees to lateral parts of area 5b. Some of these areas also send weak to moderate projections to the medial part of 5b and the lateral part of 5a. 5. Sparse projections arising from the dorsolateral prefrontal, cingular, retrosplenial, granular insular, and suprasylvian fringe cortices were found to distribute particularly in lateral portions of 5b. 6. Quite abundant intrinsic connections were also found, which were loosely organized according to a complex topographic pattern.

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306.7

CELLULAR LOCALIZATION OF CHOLINERGIC AND OPIATE RECEPTORS IN RAT SENSORIMOTOR CORTEX. M. Sahin*, W.D. Bowen and J.P. Donoghue. Div. of Biology and Medicine and Center for Neural Science, Brown University, Providence RI 02912.

Both cholinergic and opiate systems are thought to be modulators of synaptic function in cerebral neocortex. In the present study, we sought to dissociate those receptors which are localized to afferent fibers from those that are on intrinsic cortical elements. We examined the cellular location of nicotinic and muscarinic cholinergic and μ-opiate receptors by combining *in vitro* receptor binding techniques with lesions of thalamic or cortical neurons. Lesions were made electrolytically or by injecting kainic acid (KA) in the thalamus or by injecting quinolinic acid (QA) into the cortex. Ligands to each receptor type (tritiated nicotine, QNB, DAGO, muscimol) were applied to adjacent sections taken from these animals. Autoradiograms were analyzed using a computer densitometry system.

In controls, *nicotinic* receptor labeling was most dense in mid-cortical layers where thalamic fibers terminate. Following thalamic lesions, labeling within the cortical projection zone of the damaged thalamic neurons decreased 50% compared to the contralateral control side. In contrast, normal labeling remained after cortical lesions. Together, these results suggest that nicotinic receptors are largely located presynaptically on thalamo-cortical fibers. *Muscarinic* receptors were dense throughout cortex except for layer V. This normal pattern was unchanged by thalamic lesions, but was reduced up to 85% after cortical QA lesions, indicating that muscarinic receptors are located on cortical neurons. In sensorimotor cortex, *μ-opiate* labeling resembled the projection pattern from the posterior nucleus (PO). Opiate labeling decreased significantly after thalamic lesions, but a considerably greater decline (up to 75%) was observed after cortical QA lesions. Thus, μ-opiate receptors appear to be located both presynaptically on PO fibers, and postsynaptically on cortical neurons.

Based on iontophoretic studies, presynaptic nicotinic receptors may account for suppression and postsynaptic muscarinic receptors may account for enhancement of thalamic transmission observed after ACh applications in rat sensorimotor cortex (Donoghue and Carroll, '87). The role of μ-opiate receptors in cortex is not clear, but may have diverse functions based on their separate locations. Supported by NIH NS22517 and March of Dimes.

307.1

CENTRAL, NOT PERIPHERAL, ANGIOTENSIN II SYNERGIZES WITH ALDOSTERONE IN RAT SALT APPETITE. A.N. Epstein & R.R. Sakai*, Dept. of Biology & Institute of Neurological Sciences, Univ. of Pennsylvania, Phila. Pa. 19104-6018.

Rats that were pretreated with systemic DOCA (5 days at 500 µg/rat) and then received 6 hr of intravenous angiotensin II (ANG II) at a dipsogenic dose (64 ng/min/rat) did not drink 3% NaCl, in contrast to others that were also pretreated with a mineralocorticoid but were given intracerebroventricular ANG II (0.6, 6.0, or 60 ng by pulse injection) which evoked reliable and conspicuous salt intake. The failure of peripheral ANG II to evoke salt intake in synergy with aldosterone (ALDO) is consistent with our report at last year's meeting (Epstein, et al; Soc. Neurosci. Abst., 13, 1168, 1987) of the contrast between the effects of central and peripheral ANG II receptor blockade on depletion-induced salt appetite. Sarile (a specific ANG II receptor antagonist) reduced salt intake generated by sodium depletion in a dose-related fashion when it was given into the rat's cerebral ventricles, but had no effect on the same behavior when it was given intravenously.

Thus, these data suggest that peripheral ANG II of renal origin does not participate directly in the arousal of the rat's salt intake. It contributes to the behavior by releasing ALDO which then synergizes within the brain with ANG II of cerebral origin.

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307.2

FOURTH VENTRICULAR (4V) EFFECTS OF PEPTIDE YY (PYY) AND NEUROPEPTIDE Y (NPY) ON INGESTIVE BEHAVIORS. ES Corp*, LD Melville*, D Greenberg, J Gibbs and GP Smith. Dept. Psychiatry, NYH-Cornell Med Ctr, White Plains, NY 10605

PYY and NPY produce dose-related increases in food intake after 4V injections. We now report that these peptides act with different potencies and different effects on feeding and non-feeding behaviors. PYY was about 4X as potent as NPY for eliciting a half-maximal feeding response (1.25 ug PYY vs. 5ug NPY). Moreover, PYY elicited eating after a shorter latency (4±1 min and 10±2 min for PYY 5 and 10 ug, respectively vs. 19±1 min for both NPY 5 and 10 ug). A dose (10 ug) of PYY and NPY that produced equivalent, maximal food intakes produced differential behavioral effects (Table of mean % time, 60-min test, n=4).

	VEH	PYY10	NPY10
FEEDING	8.5	45.0*	52.5*
GROOMING	9.8	2.1*	1.1*
RESTING	26.7	30.0	7.9*#
SLEEPING	42.3	0.0*	27.8*#
EXPLORING	11.9	17.3	10.2
DRINKING	0.8	5.5	0.5

*p<.05 compared with VEH # p<.05 compared with PYY.

The differences in potency and behavioral effects suggest PYY and NPY are not acting through identical sites and/or receptor mechanisms in the hindbrain.

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307.3

NEUROPEPTIDE Y (NPY) INCREASES FOOD INTAKE COMPARABLY WHEN RATS RECEIVE FOOD EITHER IMMEDIATELY OR AFTER 4-HR OF FOOD DEPRIVATION. Pamela K. Green*, Alfred J. Sipols, Paige Israel* and Stephen C. Woods. Dept. of Psychology, NI-25, Univ. of Washington, Seattle, WA 98195.

NPY, like insulin, causes increased food intake when administered acutely to rats. Rats given insulin and food-deprived for several hours show comparably increased food intake as when given immediate access to food. The present study sought to determine whether NPY has a similar latent effect on food intake. Six naive male Long-Evans rats (260-380 g) were maintained on ad lib food (pelleted chow) and water. Each animal received an intraventricular (ivt) cannula aimed at the lateral cerebral ventricle. After recovery, rats were given ivt injections (1 ul) of saline or 9.5 ug NPY in saline during the light portion of the day. One-hr food intake was measured either immediately after the injection, or else after a 4-hr interval with no food. Each rat was run in 3 of the 4 conditions (ie, saline vs NPY, and 0 vs 4-hr deprivation). Mean food intake was increased following NPY when animals had immediate access to food (saline = 0.4 ± 0.7 g; NPY = 2.7 ± 1.2 g). After 4-hr food deprivation, saline animals ate slightly more (ns) food (1.4 ± 1.4 g). NPY injected animals, however, again ate increased food (2.5 ± 1.4 g), the amount being comparable to that consumed with immediate access tp food. These findings suggest that NPY causes long-term changes in the neural mechanisms that regulate food intake.

307.4

NEUROPEPTIDE Y PRODUCES CONDITIONED TASTE AVERSIONS IN LEAN BUT NOT OBESE ZUCKER RATS. A. J. Sipols, D. Figlewicz Lattemann*, and S. C. Woods. Department of Psychology, University of Washington NI-25, Seattle, WA 98195.

Neuropeptide Y (NPY) elicits large and rapid increases of food and water intake when administered in microgram quantities into either the cerebrospinal fluid or the paraventricular nuclei of many animals. We previously found that obese (fa/fa) Zucker rats display a diminished food intake response to intraventricular (IVT) NPY in comparison to that of their their lean (Fa/Fa) and heterozygous (Fa/fa) siblings. We have also described the formation of conditioned taste aversions to novel flavors following IVT NPY in Long-Evans rats. The present experiment examined the possible association of Zucker rat genotype with susceptibility to NPY-induced taste aversions. Female Zucker rats were surgically implanted with cannulas aimed at the third cerebral ventricle. The animals were adapted to a schedule of ad lib food with water available only 20 min/day. Following stabilization of daily water intake (8 days), a novel aqueous solution of non-caloric lime-flavored Kooladid was offered instead of water. After 20 minutes, half the rats of each genotype were injected IVT with saline (1 µl) and half with 9.5 µg NPY. On the following day, all subjects were presented with a two-bottle choice of lime Kooladid vs. water. Lean (Fa/Fa) animals receiving NPY drank significantly less Kooladid (24.8 +/- 13.7%) than lean animals receiving saline on the previous day (58.1 +/- 9.6%, p<.05). Total liquid intake was comparable for the two groups. Kooladid intake of obese Zucker rats receiving NPY did not differ significantly from that of their saline controls (36.8 +/- 17.4 vs. 46.2 +/- 8.3%). This was also true of the heterozygous rats. Therefore, obese Zucker rats neither eat more food nor form conditioned taste aversions in response to IVT NPY.

307.5

THE KAPPA OPIOID ANTAGONIST, NOR-BINALTORPHIMINE, DECREASES STARVATION- AND OPIOID-INDUCED FEEDING. A.S. Levine, M. Grace, W. Welch, C.J. Billington and P.S. Portoghese. VA Medical Center and Univ. of Minn., Minneapolis, MN, 55417.

Agonists of the mu, delta and kappa opioid receptors increase short term feeding and blockade of the opioid receptor with non-selective antagonists decreases feeding. Recently, the highly selective kappa opioid antagonist, nor-binaltorphimine (nor-BNI) has been reported. In the present study, 50 male rats were injected with nor-BNI (100 nmol) or vehicle intraventricularly (ICV) following a 24 hour starvation period. Nor-BNI was injected either two hours, one hour, or immediately prior to placement of food in the home cage. There was a main effect of nor-BNI ($F(2,44)=4.60$, $p<0.05$) on food intake, but no main effect of time of injection of nor-BNI. Nor-BNI decreased food intake (3.8±0.6 g/hr and 3.9±1.9 g/hr) compared to vehicle control rats (5.5±.5 g/hr and 6.9±.6 g/hr, $p<0.05$) when the drug was injected immediately before access to food. Twenty-four hour food intake was similar in the nor-BNI group (19.4±3.1 g) and the vehicle group (23.6±2.2 g). The kappa agonist U-50,488H (100 µg) was administered ICV to 7 rats and increased feeding (2.9±.9 g/hr) compared to 6 rats receiving the vehicle (.8±.5 g/hr, $p<0.05$). ICV injection of nor-BNI (100 nmol) + U-50,488H blocked the ability of the kappa agonist to induce feeding (.3±.2 g/hr). These data indicate that the kappa antagonist nor-BNI decreases food intake induced by food deprivation and by administration of a kappa agonist.

307.6

NEUROPEPTIDE K SUPPRESSES FEEDING IN THE RAT. S.P. Kalra, A. Sahu*, M.G. Dube* and P.S. Kalra (SPON: M.F.NOLAN). OB/Gyn, Univ. Fla. Col. of Med., Gainesville, FL 32610

Neuropeptide K (NPK), a 36 amino acid residue peptide belonging to the tachykinin family is localized immunocytochemically in the rat brain, especially in hypothalamic sites implicated in the control of ingestive behavior in the rat and in many neurons in the gastrointestinal tract. In addition, high concentrations of NPK and other tachykinins have been detected in plasma and tumor tissue from patients with carcinoid tumors. We studied the effects of NPK on feeding behavior in 3 experimental paradigms. In Expt. 1, intraperitoneal (i.p.) injection of NPK (1.25 and 3.14 nmols) to food-deprived (24 h) rats significantly delayed the onset of feeding and decreased 1 and 2 h cumulative food intake. Similarly in Expt. 2, NPK (3.14 nmols, i.p.) 15 min before onset of the dark phase (lights on 0500-1900 h) significantly delayed the occurrence of ingestive behavior and cumulative food intake was markedly suppressed. Since intraventricular (Ivt) injection of neuropeptide Y (NPY) stimulates feeding in rats, in Expt. 3 the effects of NPK on NPY-induced feeding in satiated rats were assessed. NPY (0.47 nmol, Ivt) elicited a vigorous feeding response while NPK treatment i.p. 15 min prior to NPY, significantly suppressed food intake and delayed the onset of ingestive behavior. Thus, NPK can acutely and consistently suppress normal as well as evoked feeding behavior. These findings suggest that NPK may play a role in the control of feeding behavior in rats and some of the clinical gastrointestinal tract symptoms may be mediated by the reportedly high circulating concentrations of NPK in carcinoid patients. (NIH DK 37273).

307.7

CIRCADIAN RHYTHM OF PLASMA CORTICOSTERONE IN DIABETIC RATS. **M.H. Oster*, T.W. Castonguay, C.L. Keen*, and J.S. Stern*. (SPON: B. Wong). Dept. of Nutrition and Food Intake Laboratory, Univ. of Calif-Davis. Davis, CA 95616.**

Abnormal corticosterone rhythms have been implicated in several models of hyperphagia. We hypothesized that the circadian rhythm of corticosterone in diabetic (DIAB) rats would have a different pattern than that of non-DIAB, control rats. To test this hypothesis, 20 Sprague-Dawley male rats (mean body wt.:118g) were given ad libitum access to a stock diet (Purina rat chow) and housed individually in a light and temperature controlled room (14/10 l/d: 22°C). Rats were made diabetic by 2 subcutaneous injections of streptozotocin (40 mg/kg). Rats which were not injected served as controls (CON). Ten days after induction of the diabetes, tail blood samples were taken every 4 h for 24 h. Plasma corticosterone levels (determined by RIA) were significantly higher in DIAB rats than in CON rats during the light cycle; however, concentrations were similar during the night cycle. To determine if the altered corticosterone rhythm of DIAB rats was a function of hyperphagia, rats were divided into two groups and fed either a diet with 21% of calories from fat (low fat or LF) or a diet with 64% of calories from fat (high fat or HF). Food intake of DIAB rats fed the HF diet was not different from controls. While the peak level of plasma corticosterone achieved was comparable in these 3 groups, the high level was maintained over a longer period of time in the DIAB-HF rats. (DK/HL07355, DK35747)

307.8

FEEDING PATTERNS IN YOUNG ANIMALS IN RELATION TO BODY WEIGHT AND SEX. G. Shor-Posner, G. Brennan*, R. Jasaitis*, B. Leibowitz* and S.F. Leibowitz. The Rockefeller University, New York, N.Y. 10021

Our understanding of eating patterns in young animals may help us to identify predisposing factors in the development of eating disorders. In this study, the natural feeding behavior and dietary selection patterns of 20 weanling rats, maintained on pure macronutrients, were examined.

Analyses revealed striking differences in diet selection as a function of sex and age. In weanling animals: females tended to consume more of the carbohydrate diet than did the males (27 vs 21 Kcal), who ate significantly more fat (16 vs 8 Kcal). During puberty and adolescence: an early rise in protein intake was observed, followed by a sharp decline in young adults of both sexes. Fat intake increased over time and preference for this diet was positively related to body weight. Rats heavy initially (82g at 28 days) ate 54% of their diet as fat 6 weeks later; this is compared to leaner rats (68g at 28 days) that subsequently ate only 38% as fat. A similar correlation became apparent in females only after the onset of puberty. These findings indicate that initial body weight and sex of the young animal may be critical factors in determining natural patterns of eating and body weight gain.

307.9

EFFECTS OF GOSSYPOL ON FOOD INTAKE AND BODY WEIGHT M.Chitcharoenthum* and J.E.Cox.(SPON:S.Mangel) Dept. Psychol.Univ.Alabama at Birmingham,Birmingham Al 35294.

Decreases in food intake (FI) and body weight (BW) are side effects of chronic treatment with gossypol,a polyphenolic compound from cotton plant. We examined how gossypol depresses BW. In the first experiment, gossypol acetic acid (GAA) was injected (10 mg/kg BW;sc) into young 5-wk old rats daily for 6 days. BW and FI were decreased significantly. BW remained depressed after the termination of treatment even though FI recovered. Carcass analysis showed that body fat content of GAA-rats was less than half that of controls. Behavioral observations of young rats during feeding (three meals/day and 2h/meal) showed that the GAA- and control rats had similar feeding latency and duration but differed significantly in daily FI within the first week of treatment. In young rats, GAA and thyroidectomy depressed basal oxygen consumption similarly (measured at 0,4,and 8 days of treatment). Free thyroxine levels in GAA-rats were significantly lower than in controls within 2 days of treatment. These results suggest that gossypol affects metabolism by depressing thyroid function and this action may be responsible for the effect of GAA on BW. However, daily sc injection of T4 (800 ng/rat) or T3 (3 ng/rat) did not prevent the effect on BW. This suggests the involvement of other mechanisms in the BW-depressant effect of gossypol. Similar results were also observed in adult rats.(supported by NSF BNS-8606768)

307.10

RELATIONSHIP BETWEEN PLASMA OSMOLALITY AND EXTRACELLULAR FLUID NEUROCHEMICAL CONCENTRATIONS IN THE LATERAL HYPOTHALAMUS AS MEASURED BY IN VIVO DIALYSIS. P.A. Mason, D. Bhaskaran, and C.R. Freed. Depts. of Med. and Pharm., Univ. Colo. Health Sci. Ctr., Denver, CO 80262.

Previously, we have reported that plasma osmolality is inversely related to extracellular fluid catechol concentration in the lateral hypothalamus (LH) as measured by in vivo electrochemistry (EC) (J. Neurochem., June 1988). Since the composition of the EC signal is ambiguous, we have repeated the experiments using in vivo dialysis. Male Sprague-Dawley rats had guide cannulae implanted above the LH. One week after surgery, rats were water-restricted for 48 hr. On the test day, a 300 u x 1.5 mm dialysis probe was inserted and perfused with Ringer's solution (1.5 µl/min) as the rats drank distilled water or 0.9% saline. Dialysate was assayed by microbore HPLC to determine the concentrations of ascorbic acid, uric acid, epinephrine, norepinephrine, and dopamine. Results showed that the ascorbic acid concentration increased in response to drinking distilled water, but not 0.9% NaCl. No consistent change was observed in the uric acid concentration. Results for epinephrine, norepinephrine, and dopamine are pending. These data suggest that ascorbic acid release from the LH is related to changes in plasma osmolality.

307.11

POTENTIATION OF TRANSMITTER-RELATED FOOD INTAKE REDUCTION BY CYCLO(HIS-PRO). L.-M. Kow, K. Shiosaki,* A. Nadzan* and D.W. Pfaff. The Rockefeller University, New York, N.Y. 10021, and Abbott Laboratories.

Our electrophysiological evidence suggests that cyclo(His-Pro) [cHP], a metabolite of TRH, can inhibit food intake (FI) by modulating actions of neurotransmitters (Exp Brain Res 67:93, '87). To test this, we investigated effects of intracerebroventricular infusion of cHP or saline combined with IP injection of d-Amphetamine (Amph. 0.4 mg/kg), d-Fenfluramine (Fenf. 3.0, 1.5, or 0.75 mg/kg) or saline on the FI of food-deprived male rats. Amph treatment inhibited FI to 56, 73, and 94% of the baseline level at 1, 2, and 7 hrs, respectively, after food presentation. cHP alone at 1 umole/rat had no effect on FI, but when combined with Amph, potentiated the inhibition of FI to 28%, 40%, and 70% respectively. Such a potentiation was also observed, in a dose-dependent fashion, with cHP at 0.5 and 0.05 umole/rat. Similarly, combined cHP (1 umole/rat)-Fenf caused twice as much inhibition of FI as saline-Fenf treatment at every Fenf dose and time point. Thus, cHP potentiates FI reduction by Amph and Fenf, probably by modulating neurotransmitters released with Amph or Fenf treatment.

307.12

DEFECTIVE BRAIN GLUCOSE UTILIZATION IN OBESITY-PRONE RATS. B.E. Levin and A.C. Sullivan*. Neurology Svc., VA Med. Ctr., E. Orange, NJ 07019, and Dept. Pharmacol. and Chemotherapy, Hoffmann-LaRoche, Nutley, NJ 07110.

Only half the male Sprague-Dawley rats develop diet-induced obesity (DIO) when fed a high energy (HE) diet. Resistance to developing DIO can be predicted before exposure to the HE diet by the presence of lower glucose-induced plasma norepinephrine (NE) levels (AJP 253:R476, 1987). Here, chow-fed diet resistant (DR; n=10) and DIO-prone (n=10) male rats were prospectively identified by the areas under their glucose-induced (1g/kg, i.v.) NE curves (DR<4000 and DIO>4000 pg/ml/60min). DR- and DIO-prone rats (4h) were then trained to drink 1ml of 50% glucose in 1min and then were tested for local cerebral glucose utilization (LCGU). Rats were fasted for 4h and then given a bolus of ^{14}C 2-deoxyglucose (30µCi, i.v.). Control rats (C) received no solution while S rats received 1ml of oral 0.15% saccharin in place of glucose. Cerebral autoradiograms of DR-prone rats showed 60-190% increases in medullary (a. postrema, n. tr. solitarius, dorsal motor n. X) LCGU in the S versus C conditions. This increased forebrain LCGU to S but DR-prone rats had 25% greater LCGU in the ventromedial hypothalamus than DIO-prone rats. Thus, DIO-prone rats had specific defects in brain LCGU which might be associated with their tendency to become obese on HE diets.

308.1

ANGIOGENESIS IN INTRACEREBRAL GRAFTS OF CNS AND PITUITARY TISSUES.
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The vascularization of solid grafts from Lewis rat fetal CNS and adult anterior pituitary tissues placed stereotaxically into the third cerebral ventricle of AKR nude mice was investigated immunohistochemically with biotinylated primary antisera against rat or mouse class I major histocompatibility complex (MHC). HRP conjugated to avidin was used to localize and identify the antigen-antibody reaction. Host mice were given intraperitoneal injections of gamma interferon (50,000 units) for 3 days before sacrifice. Brains of host mice were frozen, and 5u-thick cyrostat sections were prepared for immunostaining. Both types of donor tissue contained blood vessels at the time of grafting; however, blood vessels supplying CNS grafts were not perfused with host blood until after 7 days post-transplantation, whereas blood vessels of anterior pituitary grafts received host blood prior to 3 days post-transplantation. H & E stained sections contained cells within grafts. At 21 days, immunostaining for rat class I MHC revealed that vessels indigenous to the donor tissues were sustained within the grafts and that these graft vessels failed to enter host mouse brain parenchyma. Immunostaining for mouse class I MHC demostrated HRP-reactive blood vessels throughout the host brain and within the grafted rat tissues. The grafted CNS tissue exhibited a blood-brain barrier to blood-borne HRP; grafted anterior pituitary tissue did not. Ultrastructural inspection of blood vessels supplying CNS grafts disclosed typical blood-brain barrier endothelia; endothelia in anterior pituitary grafts were fenestrated. The results suggest that blood vessels supplying intracerebral grafts of CNS and peripheral tissues are derived from both the host and donor and that the grafted tissue dictates the type of endothelial cell inhabiting the graft.

308.2

CHARACTERIZATION OF THE ENDOTHELIAL BARRIER IN NEURAL TRANSPLANTS AND RELATED TISSUES.
J.M. Rosenstein, L.A. Sternberger and N.H. Sternberger. (Spon. J. Bernstein). Depts. of Anatomy, George Washington Univ., Wash.,D.C. 20037 and Univ. of MD Sch. of Med., Baltimore, MD 21201.

A monoclonal antibody, endothelial barrier antigen (EBA) recently described (PNAS 84:8169) can be used to determine both the location and morphology of blood-brain and blood nerve barriers in vivo and has the potential for elucidating the biochemical basis of the endothelial cell barrier. We have utilized anti-EBA to characterize barrier properties during neovascularization of neural transplants and correlated observed barrier dysfunction as depicted by protein exudation with potential vascular changes. Normally, in aldehyde fixed paraffin sections all microvessels, including pial, not related to permeable regions stain strongly with anti-EBA. Fetal CNS grafts in lateral or IV ventricle are vascularized by choroidal vessels which did not stain for anti-EBA and neither did many pial vessels incorporated into CNS grafts. Vessels within autonomic tissue grafts as expected did not stain since they are rarely invaded by CNS vessels. Interestingly, vessels of the iris, like pial vessels which are barrier-competent lacking direct astrocytic contact, stained strongly for anti-EBA. The EBA antibody can be a useful probe for determining barrier properties in neural grafts and adjacent host tissues. (Supported by NS-17468 and NS-24185).

308.3

THE BLOOD-BRAIN BARRIER IN INTRACEREBRAL ALLOGRAFTS OF HYPOTHALAMUS AND NEOCORTEX.
S.J. Wiegand and J.T. Hansen. Dept. of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

Recently, it has been reported that grafts of fetal neocortex exhibit a marked and essentially permanent blood-brain barrier (BBB) dysfunction (Science, 235:772, 1987). Longstanding disruption of the BBB could adversely affect the function, and ultimately the survival of CNS allografts. Therefore we have reexamined this issue using our standard grafting protocol. Donor tissues were obtained from the neocortex or anterior hypothalmus of Long-Evans fetuses (E17) and transplanted to the periventricular hypothalamus/third ventricle of adult male Long-Evans rats. Experimental and control animals received an injection of horseradish peroxidase (HRP, 200 mg/kg, IV) between 15 min. and 12 hrs. prior to sacrifice. Light microscopic analysis of TMB and DAB reacted sections revealed that the parenchyma of the transplants were largely free of diffusely distributed HRP reaction product, except where the grafts were adherent to the median eminence or subfornical organ of the host brain, or where other host tissues having a 'leaky' vasculature (choroid plexus, dura mater) became intimately associated with the transplants. Diffuse labeling, similar in extent and intensity to that observed within the grafts, also was noted in areas of host and control brains that were contiguous with circumventricular organs (eg basal hypothalamus), suggesting that intravenously injected HRP entered the grafts only by way of the host brain and not by extravasation from vessels inherent to the transplants. Ultrastructural analysis confirmed that capillaries in all regions of the transplants exhibited features of normal CNS vasculature (non-fenestrated endothelia with occluding junctions and low to moderate levels of endocytosis). These observations support the contention that CNS allografts develop a characteristic and competent BBB.

Supported by NIH grants NS 19900 and NS 25778.

308.4

VASCULAR MORPHOLOGY AND PERMEABILITY IN FETAL CNS GRAFTS TO THE SUBCAPSULAR KIDNEY SPACE.
J.F. Naradzay and J.M. Rosenstein. (Spon. K. Peusner). Department of Anatomy, George Washington Univ. Sch. of Med., Wash. D.C. 20037.

Recent studies have shown that the BBB to protein in fetal neocortical tissue is altered when grafted into the brain ventricle. According to conventional neovascularzation theory, due to local tissue factors, any CNS graft would be expected to have a blood-brain barrier (BBB). In order to determine if grafted CNS would alter the phenotype of ingrowing peripheral vessels we have utilized a relatively uncomplicated model. We transplanted rat fetal cortex which already has a BBB to protein, to the subcapsular space of the rat kidney. After postoperative times between 2 weeks and 3 months HRP was injected systemically for periods between 1 and 4 minutes to determine permeability characteristics. Correlative EM depicted vascular morphology. Each graft contained protein exudate particularly around large vessels in the neuropil. At the EM level these vessel endothelium had fenestrations, were invested with collagen and were not contacted by glia; capillaries indigenous to the CNS graft were also found. The presence of non-CNS blood vessels with certain peripheral vessel ultrastructural and permeability characteristics would appear to contradict angiogenesis dogma in that CNS grafts can receive and do not alter vessels of a different phenotypic or physiologic nature. (Supported by NS-17468).

308.5

EFFECTS OF HIPPOCAMPAL TRANSPLANTS WITH OR WITHOUT NERVE GROWTH FACTOR (NGF) ON SEPTAL CHOLINERGIC NEURON SURVIVAL.
G.V. Smith & L.F. Kromer, Dept. of Anat. & Cell Biol., Georgetown Univ., Washington, D.C.

Previous studies have demonstrated that implants of fetal hippocampal (HPC) tissue can promote the regeneration of injured axons in the septohippocampal pathway and that intraventricular infusions of NGF prevents the death of medial septal neurons following transection of the fornix-fimbria (F/F). Thus, the present experiments were conducted to determine whether the combined use of NGF infusions plus embryonic transplants optimizes the long-term survival of septal neurons after axotomy. For these studies adult rats were randomized into 4 experimental and 2 control groups. Group I received a simultaneous F/F lesion and HPC transplant. Group II received NGF for 2 weeks following the F/F lesion. Group III received both NGF for 2 weeks and was also given a HPC transplant 1 week following the lesion. Group IV was lesioned 1 week prior to receiving the HPC transplant, but was not given NGF. Group V received only the F/F lesion. Group VI received a simultaneous F/F lesion and an "inappropriate" neural transplant. Eight weeks after the F/F lesion the specimens were evaluated for the survival of choline acetyltransferase-positive cells. Statistical analyses of our preliminary data suggest several interesting findings. Most importantly, there were no differences between groups receiving NGF alone or in combination with a HPC transplant. However, both of these groups differed from control groups and groups receiving only HPC transplants. These preliminary results support earlier reports that NGF may be critical for septal cholinergic neuronal survival following axotomy and suggest that embryonic transplants alone may not provide as strong a survival effect. (Supported by NIA grant #AG-06648)

308.6

MODULAR ORGANIZATION OF FETAL STRIATAL GRAFTS
F.-C. Liu*, A. M. Graybiel and S. B. Dunnett. (SPON: B. Seltser) Dept. Brain & Cognitive Sciences, MIT, Cambridge, MA 02139

Intrastriatal grafts were made by injecting dissociated cells of E15 ganglionic eminence into adult rat striatum pretreated 7 days before with ibotenic acid. Some donors had been exposed to 3H-thymidine at E11-E15. After 9-16 month survivals, the grafts, as reported by Walker et al '87, Isacson et al '87 and Graybiel et al '87, displayed a modular organization in which acetylcholinesterase (AChE)-positive patches (P) appeared in an AChE-poor surround (nonpatch, NP). We tried to determine whether (a) both P and NP were donor tissue; (b) both P and NP were striatal tissue; (c) the P regions resembled striosomes, matrix or both. The 3H-thymidine study showed that the graft tissue was donor tissue. Many markers characteristic of normal striatum were expressed in the P regions: tyrosine hydroxylase, perikaryal met-enkephalin (ENK) and calcium binding protein (CaBP, Gerfen et al, PNAS '85) immunoreactivities, dopaminergic and muscarinic binding and binding for choline uptake sites. None of these markers were expressed strongly in NP regions. By contrast, cells uncharacteristic of striatum were found in the NP regions: large CaBP-positive neurons and similar large somatostatin-positive neurons. These findings raise the possibility that the NP regions were not striatal. The NP regions were probably not pallidal either because they lacked epidermal growth factor-like immunoreactivity (Fallon et al, '84). Most of the P tissue resembled striatal matrix in having CaBP- and ENK-positive medium size neurons and (2 cases) lacking high 3H-naloxone binding. We conclude that the modular organization of the grafts may represent a modular admixture of striatal and nonstriatal tissue. *We thank Drs. Gerfen, Fallon and Loughlin for antisera, and NSF BNS83-19547 & BNS-8720475.*

308.7

TRANSPLANTS OF FETAL HIPPOCAMPAL TISSUE IMPAIR ACQUISITION OF THE MORRIS WATER MAZE BY RATS WITH HIPPOCAMPAL ABLATIONS. M.L. Woodruff, A.J. Nonneman, R.H. Baisden and D. L. Whittington*. Dept. of Anatomy, Col. of Med., East Tenn. St. Univ., Johnson City, TN 37614 and Dept. of Psych., Univ. Kentucky, Lexington, KY 40506.

Transplantation of fetal hippocampal tissue produces recovery of behavioral function in hippocampal damaged rats in the Rabinovitch-Rosvold mazes (Kimble et al. Brain Res. 363:356, 1986) and in the DRL task (Woodruff et al. Brain Res. 408:97, 1987). This study was conducted to determine whether such transplants would also reduce the impairment in acquisition of the Morris water maze that follows hippocampal lesions in rats. Rats with hippocampal lesions were compared to normal rats or rats with either grafts of fetal hippocampus or cerebellum/brainstem. Testing in the water maze began 120 days postoperatively. The dependent variables included the swim time to find a submerged platform over 10 daily trials and the amount of time spent in the area of the platform on two probe trials with no platform in the tank. Statistical analysis indicated that the rats with hippocampal lesions were impaired in the water maze relative to normals, but that the transplanted rats were more impaired than the rats receiving just hippocampal lesions. These results indicate task differences in the ability of transplants to produce recovery. (Supported by USPHS Grant #ES04070 to MLW.)

308.8

FETAL NEOCORTICAL TRANSPLANTS IMPROVE SPATIAL BEHAVIOR IN OLD MICRENCEPHALIC RATS. M.H. Lee, A. Rabe and P. Wang*. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314

Transplantation of fetal brain tissue has been shown to partially restitute impaired function associated with aging. Using an animal model of micrencephaly, we sought to determine whether functional deficits associated with congenital brain abnormalities could also be ameliorated by neural transplants. Transplants consisted of solid neocortical tissue (10-20 mm^3) of normal E18 fetuses, grafted onto the posterior neocortex of 10-day old micrencephalic rats. Micrencephaly was induced during fetal development by injecting the pregnant dam with methylazoxymethanol acetate (30mg/kg, E15). The offspring has been shown to be permanently impaired on a variety of spatial problems, including Morris water maze in which both immature (1 mo) and mature (6 mo) micrencephalic rats showed profound deficit in locating a hidden platform. At 2 years of age, transplant-bearing micrencephalic (MT) rats were tested in the Morris maze with micrencephalic (MC) and normal (NC) controls. As compared to the NC, the MC rats were again impaired in terms of both the time spent to locate the platform ($p<.01$) and the number of trials required to reach the criterion (10 sec) performance ($p<.01$). The transplants did help the MT rats improve their performance: they were not only significantly superior to the MCs on both measurements ($p<.05$), but also indistinguishable from NC rats on the criterion measure.

308.9

Polymer Brain Implants for Controlled Release of Dopamine: in vitro and in vivo observations.

B.A. Sabel, A. Freese[1], M. During[2], M. Saltzman[1] and R. Langer[1]. Dept Med Psychol, Univ Munich Medical School, Fed. Rep. Germany; Dept Chem Eng, M.I.T., Cambridge[1]; Dept Pharmacol, Yale Univ, New Haven, USA[2].

Brain tissue implants can reverse some of the behavioral and biochemical deficits associated with brain lesions. It has been proposed that these effects may be in part due to a non-specific release of neurotransmitters. Therefore, as an alternative to tissue implants, we have developed a controlled-release polymer matrix which can release a neurotransmitter, dopamine (DA), at a controlled rate for an extended period of time.

A variety of DA-containing matrices were prepared and release kinetics were studied in vitro. DA-release was monitored by spectophotometry (280 nm) and confirmed by HPLC. The release in vitro depended on the geometry and loading of the polymer matrix, and in some configurations, linear release of DA occured for at least 65 days.

Matrices were then implanted unilaterally into the brain of rats onto the dorsal surface of the caudate nucleus after aspiration of overlying neocortex. Intrastriatal microdialysis revealed that DA-levels in extracellular fluid (ECF) were constant from 20 days throughout the length of the experiment (65 days) and reached unprecedented elevations to 7.2 µM of DA (normal is 26 nM).

The possibility that these polymers may be effective in reversing some of the behavioral deficits associated with brain lesions is now under study. However, we believe that the controlled release polymer technology provides a novel method for delivering substances into the brain.

308.10

IMPLANTABLE MICROENCAPSULATED DOPAMINE (DA): A NOVEL MEANS FOR SLOW-RELEASE OF DA INTO BRAIN TISSUE. A.McRae[1], S.Hjorth[2], L.Dillon[3]*, D.Mason[3]* & T.Tice[3]*. University of Göteborg, Departments of [1]Histology and [2]Pharmacology, S-40033 Göteborg, Sweden, and [3]Southern Research Institute, Birmingham, AL 35205, USA.

Biodegradable controlled-release systems constitute an exciting new technology for drug release in the CNS. DA was encapsulated in a thermoplastic polyester excipient: poly [DL-lactide-co-glycolide], which is of the same class of material used to make resorbable sutures. Ten male rats were unilaterally lesioned in the ascending median forebrain bundle using 6-OHDA (8µg/4µl). When a stable baseline apomorphine rotational response for all rats was established - 4-7 weeks after 6-OHDA lesion - they were sterotaxically injected under ether anaesthesia with 3µl of a DA microcapsule suspension into 2 sites in the denervated lateral striatum. Controls received empty capsules. Thirty minutes following the injections rats were placed into the rotometers and their behavior was recorded for the subsequent 2-4 hours. Rats with DA microcapsules exhibited contralateral rotation with an amplitude comparable to that of apomorphine. Control rats did not show any rotational behavior. Furthermore, preliminary results indicated that rats receiving DA microcapsules showed attenuated rotational responses to apomorphine (mixed D1/D2 agonist) and (-)-3PPP (D2 agonist/antagonist) up to 3 weeks following implantation. Histological analysis with anti-DA antiserum confirmed the presence of DA in the capsules 3-4 weeks following the injection. The present data indicate that it is possible to attain functional significant amounts of DA for prolonged periods of time by injecting microencapsulated DA directly into brain tissue. This novel "slow-release" approach may become useful not only in basic neuroscience research but also in the clinical management of neurodegenerative disorders.

308.11

MAGNETIC RESONANCE IMAGING OF RAT BRAIN FOLLOWING FETAL STRIATAL TISSUE TRANSPLANTS. A.B. Norman, S.R. Thomas*, R.G. Pratt*, R.C. Samaratunga*, M. Kolmonpunporn and P.R. Sanberg. Lab. of Behavioral Neuroscience, Depts. of Psychiatry and Radiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0559.

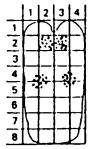
Magnetic resonance imaging (MRI) provides an excellent method for visualizing brain pathologies. Kainic acid (KA) lesions of rat striatum produce a degeneration of the striatum with a concomitant dilation of the lateral ventricles. Fetal striatal tissue transplants into the KA-lesioned striatum reverse the functional deficits produced by the lesion. However, it is generally necessary to kill rats in order to correlate the survival, growth and location of the transplants with functional recovery. We examined the ability of MRI to visualize lesions and transplanted tissues in vivo.

Twelve male rats received unilateral striatal KA lesions (5 or 10 nmol). Four weeks later rats were anesthetized, their heads placed into a 5.1 cm diameter radio frequency coil for subsequent imaging at 0.15 Tesla. T_1 weighted scans using 3.5 mm coronal sections clearly showed the enlarged ventricles on the lesioned side. Hippocampal damage was also observed in some of the rats. These lesions were confirmed by histological techniques in five rats. T_2 weighted scans demonstrated that the remaining lesioned striatal tissue was abnormal compared to the contralateral side. Four weeks following transplants of E17 fetal striatal tissue in the lesioned striatum, two rats were imaged. Changes were evident in the MR images, possibly as a result of the transplanted tissue. Histological verification will be necessary to confirm this conjecture. MRI with its excellent discrimination of tissues and CSF appears to provide a non-invasive method for monitoring lesions and subsequent transplants in vivo.

308.12

FETAL (E.17) CATECHOLAMINERGIC BRAINSTEM NEURONS FOR GRAFTING: LOCALIZATION, CHARACTERIZATION AND VIABILITY. Yves Sauvé*, J.W. Commissiong & G. Toffano (Spon. D.G. Watt). Dept. of Physiol., McGill Univ., Montreal, Canada H3G 1Y6 and Fidia Res. Lab. 35031 Abano Terme, Italy.

The present report deals with the characterization of fetal (E.17) rat catecholaminergic (CA) neurons used for grafting in the CNS. The fetal brainstem was dissected as illustrated (32 x by 1.0 mm^3), and cell suspensions prepared from the shaded areas, were smeared on coated slides (1% gelatin, 1% albumin), and then processed for histofluorescence. A high population of CA neurons was found in 2-2/2-3 (MES) and 4.5-1.5/4.5-2.5 (LC) (Brain Res. 380: 205-215, 1986). A high proportion of the neurons from the MES were morphologically intact with somatic dendrites and complete axons. The brainstem was perfused with a 0.1% paraformaldehyde solution and 16 µm sections processed for histofluorescence. The MES appeared as two cell clusters located medially and rostrally (2-2, 2-3) and the LC was located more caudally (4.5-1.5, 4.5-2.5). The viability of both the MES and LC cells in suspension, as assessed by the vital stains acridine orange and ethidium bromide (in a 0.8% NaCl/0.6% glucose; 307 mOsm), was > 60% for up to 24 h. These results provide reproducible methods for obtaining brainstem catecholaminergic cells for grafting.



309.1

THE EFFECT OF COCAINE AND SCH 23390 ALONE AND IN COMBINATION ON MILK INTAKE IN RATS. D. Rapoza* and W. L. Woolverton. Drug Abuse Research Center, The University of Chicago, Chicago, IL 60637

Recent research suggests that D1 dopamine (DA) receptors may play an important role in the behavioral effects of psychomotor stimulants. The present study examined the effects of cocaine and the D1 DA antagonist SCH 23390, administered alone or in combination, on milk intake in rats. Ten male Sprague-Dawley rats were given access to a milk solution (2:1 tap water:Bordens sweetened condensed milk) for 15 min each day. When intake was stable, dose-response functions were determined for cocaine (4.0 - 32 mg/kg, i.p., 10 min pre-session) administered alone or in combination with SCH 23390 (0.12 - 0.5 mg/kg, i.p., 30 min pre-session). The mean baseline intake was 95 ml/kg. Both cocaine (4.0 - 32 mg/kg) and SCH 23390 (0.12 - 0.5 mg/kg) caused dose-dependent decreases in milk intake. Intake that had been reduced to 7 ml/kg by 16 mg/kg cocaine was restored by SCH 23390 (0.12 [N=7], 0.25 [N=6], and 0.5 [N=5] mg/kg) to 42, 37, and 50 ml/kg, respectively. Similarly, intake that had been reduced to 43 ml/kg by 0.5 mg/kg SCH 23390 was restored by cocaine (4.0 or 8.0 mg/kg) to 65 or 62 ml/kg, respectively. Thus, there was a mutual attenuation of the effects of cocaine and SCH 23390 on milk intake when the two drugs were combined. These results suggest that D1 receptors play a role in the effects of cocaine on milk intake in rats, and are consistent with the hypothesis that D1 receptors are permissive in the expression of the behavioral effects of cocaine. (Supported by NIDA grant DA-00250)

309.2

EFFECTS OF (+)-MDMA AND (-)-MDMA ON THE THRESHOLD FOR REWARDING BRAIN STIMULATION: FURTHER EVIDENCE FOR DOPAMINERGIC MEDIATION OF MDMA'S REINFORCING EFFECTS. M. Bird and C. Kornetsky. Laboratory of Behav. Pharmacol., Boston University School of Medicine, Boston, MA 02118.

We recently reported inhibition of the threshold lowering effects of rewarding brain stimulation of (+,-)-MDMA (3,4-methylenedioxymethamphetamine) by D2 but not 5-HT2 antagonist, and that reinforcing doses of (+,-)-MDMA (ED50=0.70 mg/kg) increased striatal dopamine (DA) turnover without significantly altering 5-HT metabolism (Soc. Neurosci. Absts. 13:1323, 1987). Current evidence indicates 5-HT stereoselectivity for (-)-MDMA, (Psychopharmacol. 88:525, 1986), while (+)-MDMA has comparatively greater DA releasing activity (Eur. J. Pharmacol. 132:269, 1986). The relative effects of MDMA's enantiomers on the reward threshold in F-344 male rats with electrodes in the medial forebrain bundle were determined to further assess the contribution of 5-HT and DA in (+,-)-MDMA's euphoriant properties. Although both isomers lowered the threshold, (+)-MDMA had greater efficacy and potency than (-)-MDMA (ED50=0.30 and 1.70 mg/kg, respectively). These results offer additional evidence that (+,-)-MDMA activates central reward pathways via dopaminergic processes. [(Supported in part by NIDA grant DA 02326 and Research Scientist Award DA 00099 (CK)]

309.3

GBR BINDING IN THE NUCLEUS ACCUMBENS. P.S. Berger*, J.D. Elsworth, J. Arroyo*, and R.H. Roth. (Spons: D.L. Rosin). Depts. Pharmacol. & Psychiat., Yale Univ. Sch. Med., New Haven, CT 06510.

Some of the behavioral actions of cocaine may be dependent on dopaminergic activity in the nucleus accumbens (NAc), since 6-hydroxydopamine lesions of this area abolish both the reinforcing effects of cocaine in the self administration paradigm and the increase in locomotor and rearing behaviors produced by low doses of cocaine. However, it has been reported (J. Neurochem., 1985, 45: 51-56) that cocaine does not inhibit dopamine (DA) uptake in the NAc and that Na^+-dependent ^{3}H-cocaine binding is absent in the NAc. In striatum GBR-12935 has a much higher affinity for the DA uptake carrier (Kd=1 nM) than cocaine (Kd=400 nM). Therefore we have characterized ^{3}H-GBR binding in the NAc and re-examined the effect of cocaine on DA uptake in the NAc. ^{3}H-GBR was found to bind with high affinity in the NAc with an apparent Kd of approximately 1 nM. The IC50 values for the displacement of ^{3}H-GBR by a series of compounds including cocaine, methylphenidate, DA, nomifensine, GBR-12935, phenylcyclidine, mazindol and benztropine in the NAc correlates well with their IC50 values in striatum (r=.99). In a P2 preparation of NAc, cocaine inhibited DA uptake potently with an IC50 value of 400 nM. These biochemical data are consistent with the hypothesis that interaction of cocaine with the DA uptake carrier in NAc may be involved in the reinforcing effects of cocaine. Supported by NIH Grant DA 05119.

309.4

$5HT_3$ ANTAGONIST SPECIFICALLY BLOCK MORPHINE AND NICOTINE-REWARD. G.Di Chiara, E.Acquas, P.Leone and E.Carboni. Inst. of Exp. Pharmacology and Toxicology, Univ. Cagliari, 09100 Cagliari, Italy.

The effect of two potent and specific antagonists of $5HT_3$ receptors, ICS 205-930 and MDL 72222, on the reinforcing properties of amphetamine, morphine and nicotine was studied in rats. Drug-induced reinforcement was assessed by measuring drug-conditioned place-preference. ICS 205--930 and MDL 72222 dose-dependently reduced the place-preference induced by morphine (1.0 mg/kg s.c.). At doses of 30 μg/kg s.c. the two antagonists completely blocked morphine-induced place-preference while doses of 15 μg/kg s.c. significantly reduced it. ICS 205-930 and MDL 72222, at doses of 30 μg/kg s.c. also prevented the place-preference induced by nicotine (0.6 mg/kg s.C.). In contrast, ICS 205-930 and MDL 72222, up to doses of 30 μg/kg s.c. failed to modify the place-preference conditioning elicited by amphetamine (1.0 mg/kg s.c.). The results indicate that $5HT_3$ receptors are specifically involved in the reinforcing properties of morphine and nicotine.

309.5

THE EFFECTS OF NICOTINE ON BRAIN-STIMULATION REWARD. D.J. Lyons, G.T. Bain and C. Kornetsky Laboratory of Behavioral Pharmacology, Boston Univ. Sch. Med., Boston, MA 02118.

The purpose of this experiment was to determine if nicotine increases sensitivity of animals to rewarding electrical intracranial stimulation, a model of drug-induced euphoria. Bipolar electrodes were stereotaxically implanted into the medial forebrain bundle-lateral hypothalamic area of male F344 rats. Doses of nicotine from 0.25 to 0.75 mg/kg consistently increased the subject's sensitivity to electrical stimulation, i.e., lowered the threshold, in a fashion similar to that seen with other drugs of abuse. In addition, co-administration of a low, ineffective dose of nicotine with an ineffective dose of morphine (0.25 mg/kg) or amphetamine (0.06 mg/kg) resulted in significant increases in sensitivity to the stimulation. These results suggest that the reinforcing effects of nicotine acts through the same neural systems as these other drugs. However, in striking contrast to naloxone's ability to block the threshold lowering effects of morphine, cocaine or amphetamine, naloxone failed to attenuate the threshold lowering effects of nicotine in 3 of 4 animals. These results suggest that although nicotine may act in part through neural systems that support opiate and psychomotor stimulant reward there also may be important differences. [(Support in part by NIDA grant DA 02326 and Research Scientist Award DA 00099 (CK)].

309.6

RIMCAZOLE (RMZ), A NOVEL ANTIPSYCHOTIC, BLOCKS THE ACTIVATION OF A_{10} DOPAMINE NEURONS BY PHENCYCLIDINE AND (+)SKF 10,047, A σ-AGONIST. E.D. French, M. Smith* and A. Ceci*, Dept. Pharmacol., Univ.Arizona, Coll.Med., Tucson, AZ. 85724

RMZ's preclinical and clinical antipsychotic profile has been suggested to be due to its selective affinity for central σ-binding sites. Since both σ-agonists and PCP activate the mesocorticolimbic dopamine neurons, we assessed the ability of RMZ to attenuate these actions.

Single-unit extracellular recordings from VTA A_{10} neurons in chloral hydrate anesthetized rats showed that RMZ pretreatment (2.5 or 10 mg/kg) significantly attenuated the approximate 43% increase in firing rate elicited by both PCP and (+)SKF; the former appearing more sensitive to antagonism. RMZ did not alter spontaneous activity or apomorphine-induced slowing of these dopamine cells. Also, the naloxone-sensitive activation of A_{10}'s by morphine was not affected by RMZ, nor did naloxone affect the stimulatory effects of either PCP or SKF, thus indicating an apparent selective blockade of PCP and SKF by RMZ. Interestingly, in behavioral studies RMZ effectively antagonized only the locomotor stimulatory effects of (+)SKF, but not PCP. These latter findings might best be explained by the fact that PCP's behavioral effects, unlike those of (+)SKF, are preferentially mediated through presynaptic dopamine mechanisms. Notably, RMZ also did not attenuate morphine- or d-amphetamine-induced hyperactivity. Moreover, the electrophysiological and behavioral effects of (+)SKF were antagonized by the selective σ-agonist, DTG.

These results suggest that RMZ's moderate efficacy in ameliorating schizophrenic symptoms may be related to its ability to antagonize PCP &/or σ-sites on mesocorticolimbic dopamine neurons.

309.7

ACUTE NEUROENDOCRINE RESPONSE TO CANNABINOID TREATMENT IN THE MALE RAT. L.L. Murphy*, R.W. Steger*, M.S. Smith* and A. Bartke* (SPON: P. Consroe). Depts of Physiology, Southern IL Univ Sch of Med, Carbondale IL 62901 and Univ of Pittsburg, Pittsburg, PA 15261.

The acute neuroendocrine responses to low doses of delta-9-tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD) administered alone or in combinations were evaluated by measuring plasma luteinizing hormone (LH) and prolactin (Prl) levels and norepinephrine (NE), dopamine (DA) and serotonin (5-HT) dynamics within the hypothalamus at specific times after oral cannabinoid treatment. Adult male Sprague-Dawley rats were given p.o. a sesame oil vehicle (controls) or a low dose (0.5 mg /kg b.w.) of THC, CBN, CBD, THC+CBN or THC+CBD and were sacrificed by decapitation 30, 60 or 120 min after treatment. Only the dosing regimens of THC+CBN and THC+CBD significantly reduced plasma LH at every interval tested to 50-60% of values measured in vehicle controls (30 min, $p<0.05$; 60 min, $p<0.05$; 120 min, $p<0.01$). In addition, plasma LH levels were reduced to 55% of control values 60 min after THC administration ($p<0.05$). There were no changes in plasma Prl in response to cannabinoid treatments. All the cannabinoid treatments dramatically affected NE turnover in the median eminence (ME) and medial basal hypothalamus (MBH). There was complete suppression of NE turnover at 30 min post-THC and 120 min post-THC+CBN in the ME and 120 min post-THC+CBD in the MBH. Cannabinoids did not significantly affect MBH-DA turnover or 5-HT content. These data demonstrate that treatment with a low dose of THC alone or in combination with CBN or CBD suppresses LH secretion and further suggests that alterations in hypothalamic noradrenergic activity may be the mechanism of cannabinoid action. (Supported by DA 03875).

309.8

CHRONIC COCAINE REDUCES ALPHA-2 RECEPTOR ELICITED MYDRIASIS AND INHIBITION OF LOCUS CERULEUS. J. Marwah, A. L. Curtis and D. Pitts. Wayne State University, Detroit, MI 48202.

The effects of chronic cocaine (50 mg/kg/day for two weeks) administration on alpha$_2$ adrenoceptor mediated responses were studied in rats. Chronic administration of cocaine significantly (compared to sham controls) attenuated the alpha$_2$ adrenoceptor mediated inhibition of noradrenergic locus ceruleus (LC) neurons as well as alpha$_2$ adrenoceptor elicited mydriasis. Noradrenergic LC neurons from the cocaine treated and sham groups differed significantly in their responsiveness to the inhibitory effects of clonidine (ED_{50} values ug/kg were sham 7.35 +/- 1.13 and cocaine treated 17.17 +/- 4.40 $p < 0.05$). The ED_{50} values for the mydriatic response were sham 5.71 +/- 0.49 and cocaine treated 16.42 +/- 0.69 ug/kg respectively $p < 0.001$. No differences in cardiovascular responses to systemically injected clonidine between the chronic cocaine and sham treated groups were observed. Chronic cocaine treatment attenuates the two alpha$_2$ adrenoceptor mediated responses most likely via an interaction with central catecholaminergic neurotransmission. (Supported in part by a NIDA grant DA 04158.)

309.9

EFFECTS OF COCAINE ON ACQUISITION AND RETENTION OF ETHANOL TOLERANCE. D.L. Hjeresen. Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545

The frequent combined use of abused substances remains a significant drug abuse treatment problem. Due to the complexity of multiple drug interactions, little research exists to indicate the effects of various drugs on the development of tolerance, a key measure of drug dependence. Recent research has demonstrated that nicotine accelerates and maintains tolerance to the hypothermic and ataxic effects of ethanol (EtOH) *(Hjeresen & O'Donnell, Neurosci Abst 12:49, 1986)*. The present research was conducted to determine the effects of cocaine (Coc) on these same measures.

Male Long-Evans rats (mean body weight=332.1± 3.8 g) were tested in four treatment groups (N=6 ea): 1) EtOH (2.5 g/kg, 15% v/v, i.p.) and Coc (1.0 mg/kg in NaCl vehicle), 2) EtOH and NaCl (1 ml/kg), 3) NaCl (equivalent in volume to EtOH injections) and Coc and, 4) NaCl and NaCl. For 11 consecutive days a colonic temperature was taken, rats were injected with both drugs and returned to their home cages for 10 min. Rats were then tested in a locomotor activity apparatus for 30 min. Additional colonic temperatures were taken 40, 70 and 100 min following drug injections. Rats treated with NaCl+Coc had significantly higher colonic temperatures and locomotor activity than NaCl+NaCl rats. Rats treated with EtOH+Coc had significantly higher locomotor activity but lower colonic temperatures compared to EtOH+NaCl treated rats. The EtOH+Coc treatment delayed acquisition of tolerance to the hypothermic but not ataxic effects of EtOH. On Days 12 to 17, rats were all treated with NaCl+NaCl to extinguish tolerance. On Day 18, all rats were tested with an EtOH+NaCl injection to determine any retention of tolerance. Groups previously treated with EtOH were significantly more tolerant to the hypothermic effects of EtOH than NaCl rats regardless of previous Coc treatment. Rats in the NaCl+Coc group were more active than rats in all other groups. Thus, Coc appears to interact differently with independent effects of EtOH.

309.10

DESIPRAMINE ENHANCES BROMOCRIPTINE BUT NOT AMANTADINE IN COCAINE WITHDRAWAL. A. J. Giannini. Department of Psychiatry, Ohio State University and Northeastern Ohio Medical College, Youngstown, Ohio 44504.

Fifty cocaine abusers were divided into 5 groups of 10 volunteers each. The first group was treated with placebo (P), the second with bromocriptine 2.5 mg. q.i.d. (B), the third with amantadine 100 mg. q.i.d. (A), the fourth with desipramine 200 mg. q d and bromocriptine 2.5 mg. q.i.d. (DB), the fifth with desipramine 200 mg. q d and amantadine 100 mg. q.i.d. (DA). All groups were superior to placebo ($p < .01$) after 20 days. There was no difference between B and A after 30 days. DB, however, was superior to DA after 30 days ($p< .02$) as well as B ($p <.05$) and A ($p < .05$).

It is hypothesized that desipramine enhances the catecholamine agonist effects of bromocriptine by changes in the presynaptic auto-receptor.

309.11

FLUOXETINE BLOCKS DEPRESSION IN PHENCYCLIDINE BUT NOT COCAINE WITHDRAWAL. R.H. Loiselle, A. James Giannini. Dept of Psychiatry, Northeastern Ohio Medical College and Ohio State University, Youngstown, OH 44504

Fluoxetine 20 mg. was given to 10 male cocaine and 10 male phencyclidine abusers in withdrawals. Placeboes were given to age matched controls also in withdrawal. Fluoxetine was more effective than placebo ($p < .01$) in phencyclidine but not cocaine ($p > .10$) withdrawal. This supports a serotonergic role for phencyclidine but not cocaine withdrawal.

309.12

ELEVATED SERUM PROLACTIN LEVELS IN NEWLY ABSTINENT MALE COCAINE ABUSERS. J.N.Wilkins*, D.A. Gorelick, B.R. Bamshad*, D.Y Setoda*, Research Service, W.L.A. V.A. Medical Center, Brentwood Divn., 11301 Wilshire Blvd., Los Angeles, CA 90073.

We measured serum prolactin (HPrl), as a peripheral marker of central nervous system (CNS) dopamine (DA) activity, by radioimmonassay, and cocaine and benzoylecgonine (BE) levels by high pressure liquid chromatography, with diode array detection, in 23 male inpatients receiving treatment for cocaine dependence (DSM IIIR) at various stages of abstinence (from 0 to 25 days). Four subjects (group 1) were positive for blood BE and urinary cocaine, evidence of very recent exposure to cocaine, in addition to being positive for urinary BE. Fifteen subjects were positive only for urinary BE, and 4 subjects were negative in the blood and urine tests (group 2). The mean admission HPrl level for group 1 was 13.7 ± 1.9 ng/ml (± S.D.; mean self-reported last cocaine use of 0.75 days), compared to a mean of 11.93 ± 2.6 for group 2 (mean self-reported last cocaine use of 18.25 days). Both groups were elevated above our laboratory mean for similarly aged-matched drug-free controls (6.8 ± 1.5 ng/ml). After 1 week of inpatient treatment, HPrl levels for both groups fell approximately 20 to 25%, while after 2 weeks of inpatient treatment HPrl levels in group1 became normal (7.3 ± 4.8 ng/ml) while HPrl levels from group 2 rose to levels slightly higher than on admission (12.6 ± 3.7 ng/ml). Although preliminary, these results are consistent with decreased CNS DA activity in newly abstinent cocaine abusers, possibly lasting as long as 2-5 weeks.

310.1

TROPHIC ACTIVITIES OF BRAIN MACROPHAGES. M. Mallat*, E. Hétier*, R. Houlgatte*, P. Denèfle*, J. Ayala*, A. Bousseau*, P. Brachet* & A. Prochiantz. U.114 INSERM, Collège de France, 11 pl.M.Berthelot, 75231 Paris cedex 05, France.

Amoeboid microglial cells have been purified from rodent brain primary cultures. Cell purity has been estimated to 95% with the help of specific biochemical markers such as:

1. Presence of iC3b receptor and CD4 antigen.
2. Activity of non-specific esterases.
3. Expression of MHCII antigens.
4. Strong phagocytic behaviour.

These amoeboid microglial cells, when stimulated with LPS in vitro, synthesize high amounts of interleukine 1 and TNF mRNAs. In addition, LPS stimulation provokes the release of Nerve Growth Factor. These findings confirm the hypothesis that amoeboid microglial cells constitute an important source of trophic and neurotrophic molecules in the mammalian brain.

310.2

NON-NEURONAL CELLS REGULATE SOMATOSTATIN EXPRESSION IN CULTURED SYMPATHETIC NEURONS. L.A. Kessler, Y. Wong and K. Spiegel. Depts. Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Neuronal interactions with non-neuronal cells may influence neurotransmitter phenotypic expression. The present study examines the role of non-neuronal cells in regulating somatostatin (SS) expression in cultured sympathetic neurons of the neonatal rat superior cervical ganglion (SCG). SS levels were unchanged or reduced when SCG neurons were co-cultured with ganglion non-neuronal cells. Further, the amount of SS released into the medium remained unchanged or only slightly increased in co-cultures. Surprisingly, however, levels of SS mRNA were significantly increased in co-cultures, suggesting that post-transcriptional mechanisms prevented a concomitant increase in peptide levels.

Effects of non-neuronal cells could be mediated by either the release of soluble factors or by direct cell-cell contact between non-neuronal cells and neurons. To study the role of non-neuronal cell derived soluble factors, SCG neurons were treated with medium conditioned by exposure to ganglion Schwann cells, ganglion fibroblasts, RN22 Schwannoma cells or rat skin fibroblasts (RFCM). Treatment with any of these conditioned media increased levels of both SS mRNA and SS peptide. However, when neurons were co-cultured with non-neuronal cells, treatment with RFCM incresed SS mRNA levels but not levels of the mature peptide. Thus the presence of non-neuronal cells prevented an increase in SS peptide concomitant with the increase in mRNA.

To study the role of cell-cell contact, neurons were either cultured on top of non-neuronal cells which had been lightly fixed with 10% trichloroacetic acid or were treated with a preparation of non-neuronal cell membranes. Each of these treatments increased SS mRNA levels. Our observations suggest that non-neuronal cells may influence SS expression both by the release of soluble factors and by contact-mediated mechanisms. Although non-neuronal cells regulate levels of SS mRNA, post-transcriptional regulation may be rate-limiting so that peptide levels remain unchanges even though mRNA levels are greatly increased.

310.3

DEPOLARIZATION AND INSULIN-LIKE GROWTH FACTOR-I (IGF-I) DIFFERENTIALLY REGULATE PRESUMPTIVE NEUROBLAST MITOSIS E. DiCICCO-BLOOM and I.B. BLACK. Div. of Developmental Neurolory, Cornell Univ. Med. Coll.,New York, NY 10021

The process of neurogenesis has been studied descriptively, yet regulatory mechanisms are unknown. Employing a recently developed, virtually pure neuronal culture system, we found that insulin growth factors specifically regulated entry of neuroblasts into the cell cycle (PNAS, in press). We now compare a wholly different signal, neuronal depolarization, with growth factor stimulation of mitogenesis.

Dissociated embryonic rat sympathetic neuroblasts were cultured for 48 hrs in fully-defined medium. [^{3}H]thymidine incorporation was assessed by scintillation spectroscopy (Inc) or by determination of the labeling index (LI), i.e. the percentage of identified neuroblasts incorporating nuclear thymidine, defined by autoradiography.

Neuronal depolarization mediated by elevated extracellular K^+ (30mM) or by the specific Na^+ channel agonist, Veratridine (Ver), both stimulated neuroblast Inc. This stimulation reflected an increased proportion of neuroblasts entering the cell cycle, since the LI increased from approximately 12% in controls to 21% and 30% after K^+- and Ver-induced depolarization respectively. In contrast, equimolar Na^+ addition decreased Inc slightly.

To examine potential relationships between IGF-I and depolarization-induced DNA synthesis, we defined the role of the Na^+ channel by using the specific antagonist, Tetrodotoxin (TTX). Whereas Na^+ channel blockade completely prevented Ver stimulation, TTX had no effect on IGF-I-induced mitosis. We tentatively conclude that depolarization and growth factors mediate neuroblast mitosis via different mechanisms. (Supported by NIH Grants HD 00676, BRSG S07 RR05396, the Mellon Fdn. and a McKnight Research Project Award).

310.4

ROLE OF VOLTAGE SENSITIVE CALCIUM CHANNELS (VSCC) IN MITOGENIC STIMULATION OF NEUROBLASTS D.W. PINCUS, E. DICICCO-BLOOM, and I.B. BLACK Div. of Developmental Neurology , Cornell University Medical College, New York, NY 10021.

We have recently found that insulin growth factors (IGF) and depolarization differentially stimulate neuroblast mitosis *in vitro* (previous abstract). We now define the role of VSCC in this differential regulation.

Dissociated embryonic rat sympathetic neuroblasts were cultured for 48 hrs in fully-defined medium and assayed for [^{3}H]thymidine incorporation (Inc) by scintillation spectroscopy.

Depolarization by elevated extracellular K^+ (30mM) or the specific Na^+ channel agonist, Veratridine (Ver), increased Inc more than 2-fold. Increased Inc elicited by Ver was prevented by the specific Na^+ channel blocker Tetrodotoxin (TTX). In contrast, K^+ stimulation of Inc was unaffected by TTX, suggesting a Na^+ channel-independent mechanism. To determine whether Ca^{++} entry mediated the depolarization effect, cells were cultured in the presence of K^+ and the specific VSCC blocker, Nitrendipine. The drug preferentially blocked the K^+-induced increase in Inc, while having little effect on baseline or IGF-induced mitosis. To further elucidate the role of Ca^{++} entry, cells were cultured with or without the VSCC agonist, Bay K 8644, in the presence of submaximal doses of Ver or IGF. While BAY and Ver alone had little effect, the two combined significantly increased Inc, suggesting that Ca^{++} fluxes via VSCC mediate depolarization effects. Conversely, Bay did not augment IGF mitogenesis, indicating different underlying mechanisms. (Supported by NIH Grants HD 00676, HD 23315 and a McKnight Research Project Award).

310.5

PRODUCTION OF NERVE GROWTH FACTOR RECEPTOR IN RECOMBINANT EXPRESSION SYSTEMS. Alonzo H. Ross[1], Usha R. Reddy[2], V. Prabhakar[1], and G. Venkatakrishnan[1]. [1]Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545; [2]Childrens Hospital of Philadelphia, PA 19104.

The nerve growth factor receptor (NGF-R) is a 75,000-dalton glycoprotein with an N-terminus signal sequence and a single transmembrane domain. The availability of the receptor cDNA has allowed us to express recombinant receptor in several systems. NGF-R was prepared in bacteria as a fusion protein with B-galactosidase, but this material did not bind NGF, possibly because of incorrect disulfide bonding. The receptor was then expressed in monkey COS cells using a vector with a cytomegalovirus early promotor and an SV40 origin of replication which allows amplification of the plasmid in COS cells. The full-length receptor is strongly expressed and is transported to the cell surface. The extracellular domain of the NGF-R was also expressed in COS cells, but even though the signal sequence was correctly cleaved, this protein was inefficiently transported through the endoplasmic reticulum and, hence, poorly secreted. Both the extracellular domain and the full-length NGF-R bound NGF and were bound by a conformation-dependent anti-NGF-R monoclonal antibody. These studies suggest that there are multiple signals for the subcellular localization of NGF-R. We are also constructing plasmids for the preparation of large quantities of NGF-R using the recombinant Baculovirus system.

310.6

NERVE GROWTH FACTOR (NGF) REGULATES SYNAPTIC MOLECULES IN THE DEVELOPING RAT SUPERIOR CERVICAL GANGLION (SCG). K. Wu* and I.B. Black. Division of Devel. Neurology, Cornell Univ. Med. Coll., N.Y., NY 10021.

Our previous work indicated that presynaptic innervation is necessary for both the normal development of the ganglionic postsynaptic density protein (PSDp) and the normal maintenance of the protein in the adult SCG. Subsequent experiments suggested that sympathetic impulse activity itself regulates the adult rat ganglionic PSDp. In the present study, we examined the effect of NGF on the PSD protein in SCG. Treatment of neonates with NGF (daily dose of 10 g/g body weight) for 10 days increased calmodulin binding to the PSDp by 4.5-fold. The treatment elicited only a 1.8-fold increase in total synaptic membrane protein, suggesting that the effect on the PSDp was highly selective. The effect of NGF appeared to be highly specific since Cytochrome C, structurally similar to NGF, had no effect on the PSD protein. Finally, NGF treatment elicited a 7-fold increase in the PSDp in unilaterally denervated ganglia, indicating that the synaptic effects of NGF did not depend on the presence of presynaptic cholinergic nerve terminals. Our results suggest that the trophic protein NGF as well as impulse activity, may regulate synaptic function. (This work was supported by NIH Grants NS 10259 and HD 23315, IBB is the recipient of a McKnight Research Project Award).

310.7

NEURAL REGULATION OF MUSCLE GLUTAMINE SYNTHETASE EXPRESSION. S. R. Max, B. Feng*, C. Banner*, M. Konagaya*, Y. Konagaya*, and J. W. Thomas. Dept. of Neurology, Univ. of Md. Sch. Med., Baltimore, MD 21201 and Lab. of Molecular Biology, NINCDS, NIH, Bethesda, MD 20892.

The mechanism of glutamine synthetase induction in rat skeletal muscle following denervation (Soc. Neurosci. Abs. 12:1107, 1986) was investigated. Adult male rats were subjected to mid-thigh section of the sciatic nerve. At 1, 2, and 5 hours and 1, 2, and 7 days after denervation, rats were sacrificed, and denervated and contralateral control soleus and plantaris muscles were excised, weighed, homogenized and assayed for glutamine synthetase. Glutamine synthetase activity increased about 2-fold 1 hour after denervation in both muscles. This increase in enzyme activity was maintained for up to 7 days post-denervation. Immunotitration with an anti-glutamine synthetase antibody suggested that denervation causes an increase in the number of glutamine synthetase molecules in muscle. Nevertheless, enhanced enzyme activity probably does not reflect increased synthesis of glutamine synthetase molecules; cycloheximide did not block the denervation-mediated increase in enzyme activity, and Northern blot analysis revealed no increase in glutamine synthetase mRNA following denervation. A combination of denervation and dexamethasone injections resulted in additive increases in glutamine synthetase activity. Thus, the mechanism underlying increased glutamine synthetase following denervation appears to be post-transcriptional and is distinct from that of glucocorticoid-mediated glutamine synthetase induction previously described by us (Endocrinology 120:1179, 1987). (Supported by NIH, HD-16596).

310.8

GANGLIOSIDE GM1 MODULATION OF PROTEIN KINASE ACTIVITY IN PC12 CELLS. B.S. Hilbush and J.M. Levine, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794

Exogenous gangliosides exhibit neurotrophic effects, enhancing regeneration *in vivo* and neurite outgrowth *in vitro*. Ganglioside GM1 can potentiate the response to nerve growth factor (NGF) in the PC12 cell line, thereby enhancing neurite outgrowth (Dev. Brain Res. 8:215-221,1983).The molecular mechanisms responsible for GM1 action are unknown; however, a modulation of NGF-induced protein kinase activity might account for the trophic effects of exogenous gangliosides. Therefore, we have investigated the effects of exogenous GM1 ganglioside on protein kinase activity in PC12 cells.

The extent of ^{32}P incorporation into tyrosine hydroxylase (TH) was used to assess the effects of GM1 on kinase systems. Treatment of PC12 cells with GM1 alone resulted in no changes in basal levels of TH phosphorylation. GM1 together with NGF (50ng/ml) caused a 30-40% increase in TH phosphorylation compared to treatment with NGF alone. These stimulatory effects were maximal at concentrations of 1-10μM, while higher concentrations ($\geq$ 100 μM) were inhibitory. Pulse-chase studies demonstrated that the increases in TH phosphorylation were due to kinase activation rather than inhibition of phosphatase activity.

Two-dimensional tryptic peptide mapping of TH demonstrated that phosphopeptide T_2 undergoes a 2-fold increase in labeling upon treatment with GM1 and NGF. T_2 is phosphorylated in response to agents known to raise intracellular Ca^{2+} levels, suggesting that this phosphopeptide is a substrate for a Ca^{2+}/calmodulin-dependent protein kinase (J.B.C. 260:9047-9056,1985). Thus, GM1 may act synergistically with NGF to activate Ca^{2+}/calmodulin -dependent protein kinase. NGF has been shown to activate cAMP-dependent protein kinase and C kinase. The ability of exogenous GM1 ganglioside to recruit an additional protein kinase to the NGF response may provide a mechanism responsible for its neurotrophic effects.

310.9

INTRAOCULAR TRANSPLANTATION OF AN ENRICHED SCHWANN CELL SUSPENSION FACILITATES THE SURVIVAL OF RAT RETINAL GANGLION CELLS FOLLOWING OPTIC NERVE SECTION. L. Maffei*[1], G. Carmignoto*[2], G. Ferrari*[2], P. Candeo*[2] and R. Canella*[2] (SPON: J. Paolicchi). [1]Institute of Neurophysiology, CNR, Pisa, Italy and [2]Fidia Research Laboratories, Abano Terme, Italy.

Schwann cells (Scs) are known to play an essential role for the regeneration of mammalian peripheral nerves. The results here reported show that Scs transplanted intraocularly improve the survival of rat retinal ganglion cells following optic nerve section. Scs were derived from neonatal rat sciatic nerves. A single intraocular injection of a cell suspension containing at least 80% of Scs (about 10^6 Scs) was performed at the time of the optic nerve section. Surviving retinal ganglion cells were retrogradely labelled with HRP applied to the proximal stump of the optic nerve. In the retinae which received Scs, surviving retinal ganglion cells were much more numerous with respect to controls. Approximately 25% of the normal ganglion cell population was still viable 9 weeks after axotomy. Even 12 weeks after section, numerous viable retinal ganglion cells were present. The properties which make Scs active in peripheral nerve regeneration (source of trophic factors and/or supporting substratum for axon regeneration) could also be responsible for the survival of retinal ganglion cells after optic nerve section.

310.10

CONCENTRATION DEPENDENCE OF A NEURON SURVIVAL FACTOR FOR DORSAL LATERAL GENICULATE NUCLEUS (dLGN) NEURONS IN VIVO. K.L. Eagleson*, F. Haun, and T.J. Cunningham, Dept. of Anatomy, Med. Coll. of Pa, Phila., PA 19129,

Occipital cortex ablation in newborn rats produces near complete degeneration of the dLGN in 5 days. This degeneration is attenuated when embryonic cortical cells, first maintained in explant co-culture with embryonic diencephalon for 5 days, are transplanted into the lesion cavity. These transplants prolong for about a week the survival of a population of host dLGN neurons generated on embryonic days 15 and 16 (E15, E16). Cells generated earlier (on E14) are unaffected. A similiar effect is obtained when a macromolecular fraction of the culture medium conditioned by the transplant cells is entrapped in gel beads and then implanted into the lesion cavity. In this study we tested the effect of varying the concentration of this medium fraction, with the starting concentration of the fraction designated X. Increasing concentrations produced a progressive fall-off of trophic activity such that at 200X neuron survival was identical to unconditioned medium controls. Diluting the medium had the opposite effect, but largely due to increased survival of E14 generated neurons (e.g., 26% remain at 0.05X, compared to 5% in lesion only animals). The results may reflect the separate operation of neurotrophic and neurotoxic factors at different concentrations for different dLGN neuron populations. Or, it may be that the production of a single cortically-derived neurotrophic factor is controlled strictly during development such that the low concentrations required by E14 thalamic neurons are available early in development and the higher concentrations required by E15,E16 thalamic neurons are available later. Supported by NIH grant NS16487 (TJC).

310.11

PARTIAL NEUROTROPHIC MAINTENANCE OF FUNGIFORM TASTE BUDS. D.R. Riddle* and B. Oakley. (SPON: E. Valenstein). Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109.

Recent evidence indicates that fungiform taste buds are not completely dependent upon their nerve supply. We examined fungiform taste buds in rats 8, 23 and 180 days after unilateral transection of the chorda-lingual nerve. All fungiform taste buds deteriorated with time; 45% disappeared completely. However, some small remnants remained; many of the distorted remnants disappeared or regulated into well-shaped atrophic taste buds (small, generally lacking a taste pore: mean of 55% of the normal volume). Comparison of three rodent genera revealed the neurotrophic dependence of fungiform taste buds is least in golden hamsters (*Mesocricetus auratus*), intermediate in albino rats (*Rattus norvegicus*) and greatest in mongolian gerbils (*Meriones unguiculatus*).

The following data suggest that taste buds toward the front of the tongue are the least susceptible to denervation---apparently their stem cell progeny can differentiate and either form taste bud remnants or regulate into atrophic buds. Among fungiform taste buds, those at the tip of the tongue are twice as likely to survive denervation for months. In 10 rats the chorda-lingual nerve was transected unilaterally and the IX nerve bilaterally. By 8 days all vallate taste buds had completely disappeared, whereas 6% of the foliate and 71% of the fungiform taste buds were still present in some form. Thus, fungiform taste buds may survive denervation to a degree that depends upon the genera of the rodent and the locus on the tongue. Supported by NIH Grant NS-07072.

310.12

THE TRIGEMINAL NERVE LACKS THE NEUROTROPHIC CAPACITY TO MAINTAIN TASTE BUDS. B. Oakley and D.R. Riddle*. Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109.

We have tested the proposition that branches of the trigeminal (Vth) cranial nerve can maintain fungiform taste buds in rats. This is a sequel to a companion study on the effects of denervation upon rodent fungiform taste buds. We transected the chorda tympani nerve and caused the anterior part of the rat tongue to be innervated by the a) lingual nerve alone, b) mylohyoid nerve alone, c) lingual and mylohyoid nerves, or d) lingual and auriculotemporal nerves. We determined the number of missing taste buds and the number and volume of remnants of taste buds and atrophic buds (small, but well-shaped). Except for a small decrease in the number of missing taste buds, Vth nerve innervated taste buds fared little better than denervated controls (chorda-lingual nerve transected). However, Vth nerve innervation significantly decreased the marked tendency of a large filiform papilla to emerge from an empty fungiform papilla in denervated controls, suggesting that trigeminal axons suppressed the formation of ectopic filiform papillae.

Thus, the various branches of the Vth nerve showed no specific neurotrophic capacity to support fungiform taste buds, the number and size of which did not differ from denervated controls. These results extend to fungiform taste buds our conclusion from studies of vallate and foliate taste buds: only chemosensory axons will support taste buds on the tongue. This specificity of neurotrophic support of mammalian taste buds raises the possibility of specific neurotrophic agent(s) in chemosensory axons. Supported by NIH Grant NS-07072.

311.1

ADHESION MOLECULE BOUNDARIES DURING CNS PATTERN FORMATION AND SYNAPTIC STABILIZATION. D. Steindler, N. Cooper, A. Faissner* and M. Schachner. Dept. of Anat. and Neurobiol., Univ. of Tenn., Memphis, and Dept. of Neurobiol., Univ. of Heidelberg.

Previous lectin binding and GFAP immunocytochemistry (ICC) studies during postnatal development in the mouse CNS have shown that "hidden" boundaries exist around developing functional cytoarchitectonic arrangements during a critical period in the first postnatal week (Cooper and Steindler, J. Comp. Neurol. 249:157, 1986; Brain Res. 380:341, 1986; Steindler and Cooper, Dev. Brain Res. 36:27, 1987). Transient glial-glycoconjugate boundaries cordon off cortical layers, nuclei, and functional units (e.g. somatosensory barrels and barreloids, neostriatal striosomes), and they may be involved in specific pattern formation events including neuron and afferent sorting. In the present study, we have begun to characterize boundary constituents using nine different monoclonal antibodies directed against putative cell or substrate adhesion molecules, including the J1 glycoprotein, L2, and AMOG, in LM and EM ICC studies in the CNS of mice ranging in age from E18 to adult. LM ICC of the J1 glycoprotein, for example, reveals boundaries around developing barrels during the first postnatal week. Coincidental with the disappearance of these tissue boundaries in the second postnatal week, several of the Mabs reveal an intricate network on the surface of neurons that surrounds axosomatic and axodendritic synapses. We believe that glia and associated adhesion molecules may serve to sort and later corral young neurons within functional arrangements. Greater concentrations of glial processes and their increased expression of certain adhesion molecules within boundary sites actually bog down the young neurons, thus preventing their extension into adjacent, functionally different zones. Tissue boundaries disappear, and cell surface boundaries appear during synaptic stabilization when the business of the brain evolves from pattern formation to information processing and transfer. Supported by NIH grant NS 20856.

311.2

Development of Cat-301 expression on motor neurons requires muscle afferents. R. Kalb and S. Hockfield Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT

Previously we found that monoclonal antibody Cat-301 recognizes a postnatally expressed neuronal surface antigen on hamster spinal motor neurons. Motor neurons do not develop Cat-301 immunoreactivity following neonatal lesions which disrupt their normal pattern of activity, such as sciatic nerve crush or thoracic hemicordotomy. Other motor neuron antigens recognized by monoclonal antibodies are unaffected by these procedures. The same lesions in adults have no effect on Cat-301 immunoreactivity. In an attempt to determine what component of the segmental reflex arc is necessary for Cat-301 expression, we performed dorsal rhizotomies on hamster pups. The extent of deafferentation was assayed using calcitonin gene related peptide (a primary afferent marker). In neonatally deafferented cord segments Cat-301 immunoreactivity does not develop on motor neurons, while deafferentation in adults has no effect on Cat-301 expression. Neonatal or adult rhizotomy does not inhibit the expression of other motor neuron antigens. Next, to determine which dorsal root afferents stimulate Cat-301 development we depleted small diameter afferents by neonatal capsaicin treatment. The efficacy of capsaicin was assayed by the loss of dorsal root ganglia substance P immunoreactivity. Depletion of small diameter afferents has no effect on the development of motor neuron Cat-301 expression in neonates or adults. These results suggest that the development of motor neuron Cat-301 depends on non-nociceptive afferents, including larger diameter muscle afferents conveying muscle tension or joint position sense of normally active muscles

311.3

A POSITIONAL MARKER IN THE DORSAL EYE OF THE EMBRYO. Ursula C. Dräger and Sylvia A. Rabacchi. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

We are trying to identify molecules involved in the establishment of positional information in the eye of the early mouse embryo. In independent fusions we found two monoclonal antibodies that label strongly the dorsal retina in mouse, rat, chick and xenopus embryo; in addition, most cells in the body are weakly labeled. The two antibodies appear to recognize the same antigen but different epitopes on it. In the mouse embryo, strong labeling of the dorsal eye begins at the optic vesicle stage; at the early eye-cup stage the antigen becomes very abundant, filling diffusely the cytoplasm of all cells in the dorsal one-third of the neural retina. As differentiation advances from center to periphery, the dorsal eye antigen recedes toward the dorsal edge of the retina. Besides its presence in undifferentiated retina, the antigen appears transiently at high levels in axons originating from dorsal retina, and this dorsal bundle can be followed to the lateral geniculate nucleus. In addition to strong expression in the dorsal eye, high levels of the antigen are seen in various other places including the inner ear, early motor neurons and muscle.

Western blot analyses show a major band at 44kd and several minor bands; the antigen is present in all tissues and in all species we tested, including human, rodents, fish, lobster, drosophila and flat worms.

From these morphological and biochemical observations we conclude that we have identified a very conserved and widely distributed antigen; its very high expression in the embryonic dorsal retina suggests that it may be involved in the determination of the dorso-ventral axis of the embryonic eye. (Supported by EY01938)

311.4

MOLECULAR CLONING OF THE 'DORSAL EYE ANTIGEN': HOMOLOGY TO THE HIGH-AFFINITY LAMININ RECEPTOR. Sylvia A. Rabacchi, Rachael L. Neve and Ursula C. Dräger. Department of Neurobiology, Harvard Medical School and Department of Clinical Genetics, Children's Hospital, Boston, MA 02115.

Using a monoclonal antibody against an antigen expressed at high levels in the dorsal retina of the vertebrate embryo we isolated a cDNA clone from an embryonic rat brain library in λgt11. When this clone was used to probe RNA from embryonic mouse heads, it hybridized to a single prominent 1.3kb RNA species. Sequence analysis revealed a partial clone coding for the COOH-terminal portion of a protein with very strong homology to the partial sequence of the human laminin receptor described by Sobel et al. (PNAS, 1986). Antisera to purified laminin receptor and to a synthetic peptide from the COOH-terminal portion of the protein (generous gifts from L. Liotta), and an antiserum to the laminin-receptor β-galactosidase fusion protein (a generous gift from B. Segui) recognized in Western blots of our preparations the same bands as our monoclonal antibody, i.e. a prominent band at 44kd and weaker bands including one at 67kd; these bands were present in blots of whole tissue extracts as well as crude membrane preparations and high-speed supernatants. In addition, our monoclonal antibody reacted strongly with the fusion protein (courtesy of B. Segui). On sections through embryonic mouse eyes the antiserum to the purified receptor labeled the dorsal retina in a fashion similar to but weaker than our monoclonal antibody.

From these observations we conclude that the "dorsal eye antigen" is either identical or closely related to the high-affinity laminin receptor. (Supported by EY01938)

311.5

TRANSIENT PATTERNED EXPRESSION OF THE 1F8 ANTIGEN DURING DEVELOPMENT OF THE GRASSHOPPER NERVOUS SYSTEM. E.E.Ball, M.J.Bastiani#, H.G.deCouet*, J.M.A.Quinn*. Molecular Neurobiology, Res. School Biol. Sci., Australian National Univ. PO Box 475, Canberra City, ACT 2601, Australia; #Biology Dept., Univ. of Utah, Salt Lake City, Utah 84112, USA

The 1F8 MAB recognizes a cell surface protein (1F8a) of molecular weight ~105kD which is expressed with changing temporal and tissue specificity during the embryonic development of grasshoppers (Locusta, Schistocerca). The neuroepithelium first expresses 1F8a at the borders of thoracic segments 2-3 at 26-27% of embryonic development. Expression then spreads both anteriorly and posteriorly, beginning first at the margin of the neuroepithelium of each segment. Midline staining becomes less intense as the neuroepithelium begins to stain. By 30% most thoracic neuroepithelial cells express 1F8a. From 30-35% staining of the abdominal nervous system spreads posteriorly and the entire neuroepithelium stains by the end of this period. By 40% multiple transverse stripes in the dorsal part of each ganglion begin to stain, giving the CNS a ladder-like appearance. Each stripe consists of the progeny of only a few cells. At ~40% expression of 1F8a begins to disappear from the neuroepithelium in the same order as it appeared, and from 45-60% it is limited to a thin layer in the midline of each ganglion and to the median neuroblast. At 60% staining of the CNS again becomes widespread for a short time before becoming restricted to a smaller, segmentally repeated set of cells, some or all of which are glia.

311.6

ISOLATION OF A GENE EXPRESSED IN NEURONAL STEM CELLS. U. Lendahl*, L. Zimmerman*, K. Frederiksen and R. McKay. Depts. of Brain and Cognitive Science, Biology, Mass. Inst. of Technology, Cambridge, MA 02139, U.S.A.

The Rat.401 protein is a 200 kD protein specifically found in neuronal stem cells in the rat CNS, but absent from adult CNS. Immunostaining reveals an intermediate filament-like cytoplasmic distribution.

We here report the cloning of the gene encoding the Rat.401 protein. Immunopositive clones were obtained from a lambda gt11 cDNA expression library made from embryonic day 15 rat poly(A)$^+$ RNA. We now have cDNA clones spanning more than 4 kb (kilobase pair) of the Rat.401 gene. The size of the mRNA is approximately 6 kb. Rat.401 mRNA is found only in developing CNS regions and in amounts proportional to the protein levels. The close correlation between amounts of mRNA and protein suggests that Rat.401 gene expression is regulated at the mRNA level.

Preliminary DNA sequence analysis indicates that portions of the Rat.401 protein are repetitive, displaying a 9 amino acid motif of predominantly negatively charged amino acid residues. A search in the EMBL data bank reveals no significant homologies to other characterized genes.

Supported by grants from NIH, Whitaker Health Science Fund, the Rita Allen Foundation and an EMBO Fellowship to UL.

311.7

MOLECULAR CLONING OF A HOMEOBOX FROM THE LAMPREY. J.W. Pendleton, S. Chang*, M.J. Cohen, and F.H. Ruddle*, Department of Biology, Yale University, P.O. Box 6666, New Haven, CT 06511.

The CNS of the larval sea lamprey, Petromyzon marinus, has many advantages for the study of neural phenomena - large identified neurons, a hardy in vitro preparation, and well-characterized growth after CNS injury. Because of these attributes, studies of homeobox-containing genes and their expression in the lamprey CNS may be of interest.

We screened a lamprey genomic library (kindly provided by R. Doolittle) with probes containing the homeobox from the Drosophila antp and mouse Hox1.5 loci. Preliminary sequence analysis indicates that this lamprey homeobox is a member of the highly conserved antp group. Furthermore, the deduced amino acid sequence suggests that this homeobox shares a high degree of homology with the following class of homeoboxes: human Hu1, mouse Hox1.3 and Hox2.1, Xenopus XHox1B, and salmon pS12B (Hart et al 1987, Fjose et al 1988). This class of homeobox-containing genes is known to be expressed in the CNS, and we are currently examining expression of this homeobox-containing gene in the lamprey. From an evolutionary perspective, the lamprey is the most primitive vertebrate from which a homeobox has been cloned and sequenced.

311.8

THE LOCUS *ELAV* OF *D. MELANOGASTER* ENCODES AN RNA BINDING PROTEIN. S. Robinow*, A. R. Campos*, K.-M. Yao* and K. White* (SPON: L. I. Mortin). Department of Biology, Brandeis University, Waltham, MA 02254.

We are interested in the genetic basis which underlies the maturation of a neuron from its birth to its terminally differentiated state. In the fruit fly, *Drosophila melanogaster* a region at the tip of the X chromosome is required for the proper development of the nervous system. The locus *embryonic lethal-abnormal visual system* (*elav*) is one of at least two loci which map within this region that are required for proper neural development (Campos, A. R., *et al., J. Neurogenet.*, 2:197-218, 1985; Homyk, Jr., T., *et al., J. Neurogenet.*, 2:309-324, 1985; Jiménez, F. and J. A. Campos-Ortega, *J. Neurogenet.*, 4:179-200, 1987). This locus has been cloned and molecularly characterized (Campos, A. R., *et al., EMBO J.* 6:425-431, 1987). Transcripts from this locus are found in most, if not all neurons at all developmental stages while they are not found in neuroblasts and at least one class of glial cell (Robinow, S. and White, K., *Dev. Biol.*, 126:294-303, 1988).

We have identified the transcriptional and translational start sites for at least one of the three embryonic transcripts. A protein sequence homology search of the conceptual translation of the open reading frame found within an embryonic cDNA suggests that this locus encodes an RNA binding protein. However, at present we have no direct evidence that the *elav* gene product can bind RNA. We are in the process of raising antibodies against a series of *elav* fusion proteins. Antibodies will be used to test directly for the ability of this protein to bind RNA. We will also approach the question of whether this protein binds all or only a subset of transcripts.

311.9

POSITION-DEPENDENT EXPRESSION OF PEPTIDERGIC PHENOTYPE IN POST-MIGRATORY NEURONS OF THE INSECT ENTERIC NERVOUS SYSTEM. P. F. Copenhaver and P. H. Taghert. Anatomy & Neurobiology 8108, Washington University Medical School, St. Louis, MO 63110.

We have been examining the developmental regulation of peptidergic phenotype within the Enteric Plexus, a discrete region of the enteric nervous system (ENS) of the moth, Manduca sexta. Unlike many neurons in the insect CNS, the ~400 neurons of the Enteric Plexus (EP cells) do not appear to be uniquely identifiable but can be categorized by morphological and biochemical subtype: they arise by a process of placode evagination from the foregut epithelium and acquire their mature positions (along the foregut and midgut) by a multiphasic sequence of cellular migration via stereotyped pathways. The behavior of the EP cells during migration appears probabilistic and results in significant variability in their post-migratory distributions. However, the expression of substances related to the molluscan peptide Phe-Met-Arg-Phe-amide (FMRFamide) commences in the Enteric Plexus only after migration is complete, and then only in a subset of the EP cells that have successfully navigated onto the midgut surface. To test the role of this migratory process in regulating peptidergic phenotype, we have begun to manipulate the developing ENS in embryo culture. Animals isolated from their eggshells and embryonic membranes will nevertheless exhibit the normal sequence of cell migration and position-dependent expression of FMRFamide-like substances in vitro. If a subset of the EP cells are surgically prevented from contacting the midgut migratory pathways, none of these neurons successfully navigate onto the midgut. In addition, no detectable levels of FMRFamide-like material is found within this stranded group, suggesting a lack of peptidergic expression in the absence of the normal migratory process. We are currently testing the relative importance of specific migratory pathways and post-migratory environments in regulating peptidergic phenotype within this cell population both in situ and in primary culture. Supported by an NIH postdoctoral fellowship to P.F.C. (F32NS07957) and NIH grant # NS21749 (to P.H.T.).

311.10

CHANGES IN THE EXPRESSION OF A FMRFAMIDE-LIKE PEPTIDE IN THE PERIPHERAL NEURON L1 IN *MANDUCA* EMBRYOS J. B. Wall* and P. H. Taghert (SPON: A. Loewy) Dept. of Anatomy and Neurobiology, Washington University Medical School, Saint Louis, MO 63110

We are studying a peripheral neuron (L1) to ask what factors influence the biochemical differentiation of a neuron during development. The segmentally repeated cell called L1 extends a distal process towards the heart, and in posterior segments (A2-A8), contacts it. The L1 neuron also projects a varicose and highly branched process medially in the Transverse Nerve. The morphology and disposition of the axon suggests a neuroendocrine function. The cell is both SCP_B and FMRFamide immunoreactive (IR): such labelling is specifically associated with large (~200 nm) secretory granules. Using single cell dye fills and the anti-peptide antibodies, we have compared morphological growth with levels of IR material. There are two phases to the pattern. Peptide-IR is first detectable shortly after L1 extends its initial growth cone at 41% of embryogenesis. At this stage, IR is seen in the cell body, axon, growth cone and filopodia. L1 cells in all segments simultaneously begin such expression and no segment-specific differences are noted. Other neurons in *Manduca* embryos are also labeled by these same antibodies, but their initial expression follows that of L1 and they have extended considerably longer axons at these times. The L1 pattern changes when, at ~60%, L1 cells in anterior segments increase in IR levels, while the levels in homologous cells in the posterior segments gradually decline. These differences are not the result of cell death. We are currently studying the mechanisms that underlie this early cell-specific initial expression, and later segment-specific transient expression.
Supported by NIH Grant # NS21749 (PHT).

311.11

ISOLATION OF A GENE THAT IS SPECIFICALLY EXPRESSED IN A SUBSET OF GLIA FROM THE MOTH *MANDUCA SEXTA*. N. Plaut*, P.F. Copenhaver and P.H. Taghert (SPON: I.A. Ferguson). Dept. of Anatomy & Neurobiology,Washington Univ. Med. School, Saint Louis, MO 63110.

Whereas insect neuronal development has been extensively analysed in recent years, relatively few modern studies of insect glia have been reported. By means of a differential screen between distinct regions of the larval *Manduca* nervous system, we have isolated a cDNA clone of a gene (NPF) that is specifically expressed in a sub-class of glia. The clone is 450bp in length and about two thirds the size of the smallest transcript detectable with northern analysis. The DNA sequence reveals a single long open reading frame whose initial regions are not represented in the isolated clone; neither the nucleotide nor predicted protein sequence is homologous to any recorded sequences in the Genebank database.

NPF transcripts are expressed exclusively in the nervous system and at least two transcripts (~0.7 & ~1.0 kb) are present; the ratio of these vary during post-embryonic development. NPF transcripts are first detected during the latter half of embryogenesis. In larval stages, there are quantitative differences in NPF expression between the various ganglia of the CNS. In situ hybridisation with both DNA and RNA probes indicates that the NPF gene is abundantly expressed in those glial elements that separate the nerve cell bodies from the neuropil. Glial elements that constitute the neural sheath and that populate the neuropil do not express the gene. Weaker signals are also detected in the cell body layer of ganglia although these also appear due to non-neuronal cells. With a view to pursuing a genetic analysis of potential NPF function, we are cloning a *Drosophila* homologue of NPF. Supported by NIH grant NS 21749 (PHT).

311.12

CULTURED DROSOPHILA NEUROBLASTS USED TO IDENTIFY GENES IMPORTANT DURING NEUROGENESIS. L.A. Perkins, K. Zhang,* A.P. Mahowald,* and N. Perrimon* Case Western Reserve U., Cleveland OH 44106 & Harvard Med. School, Boston, MA 02115.

Utilizing a unique approach to isolate large populations of neuroblasts from early gastrula stage embryos it was possible to obtain RNA from undifferentiated neuroblasts and differentiated neurons. These RNAs were used to generate cDNA probes for differential screening of 2 embryonic cDNA libraries. Clones were selected if no hybridization was observed with the cDNAs derived from differentiated neurons and if hybridization was observed with the cDNAs derived from undifferentiated neuroblasts. One of our cDNA clones identifies the previously described neurogenic gene Delta. Northern analysis reveals 3 developmentally regulated transcripts; 2 of which are expressed during neuroblast segregation. In situ hybridization to sectioned embryos shows strong labelling over neuroblasts. Another of our clones identifies the heat shock cognate gene, hsc-4. Developmental Northern analysis reveals one major transcript not developmentally regulated, in situ hybridization to sectioned embryos shows an intricate pattern of tissue enriched expression. Segregating neuroblasts are particularly enriched for this transcript. Other of our cDNA clones do not identify previously described loci and reveal interesting temporal and spacial developmental profiles. From among these clones we hope to identify new loci important during neurogenesis.

312.1

APPLICATION OF A NEW AUTOMATED METHOD OF SCALING SOMATOSENSORY EVOKED POTENTIALS IN SEVERELY BRAIN INJURED PATIENTS. C.E. Dixon, S. Choi*, W. Burton*, C. Chan*, A. Marmarou*, P.G. Newlon, H. Lutz*, H.F. Young*. Div. of Neurosurgery and Dept. of Biostatistics, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Abnormalities in somatosensory evoked potentials (SEP's) are known to provide a powerful prognostic tool in severe human head injury. However, evaluation of an abnormal SEP had previously been dependent on human inspection. We describe herein the computerized application of a simple scale for analysis of SEP waveforms. The scale is based on the sum of the amplitudes of all the peaks occurring between 20-250 msec after stimulus. A total of 135 SEP waveforms were collected from 53 severely brain injured patients within 1 day post-injury. Each digitized waveform consisted of 512 sample points and was first processed through a peak detection program, then, a scaling program which provided a "grade" from I-III (Greenberg et al., 1977) and the probability of the waveform belonging to that grade. Grades produced by the automated technique were highly correlated with grades produced by human inspection. Differences between the two techniques were primarily attributable to errors of peak detection and may be resolved with further refinement. This method can be easily applied to computerized serial on-line monitoring of patients. Supported by NIH NS 12587.

312.2

Lower Limb Motor Evoked Potentials Elicited in Humans With a Magnetic Stimulator. I.Bell, R.D.Linden, C.H.Tator, R.D.G.Blair.

To determine the optimal scalp location for eliciting motor evoked potentials (MEP) with a magnetic stimulator, 11 normal subjects were stimulated at nine different sites on the scalp. The stimulation sites were: Cz; the midpoints between Fz-Cz, Cz-Pz, C3-Cz and Cz-C4; anterior and posterior to the midpoints of C3-Cz and Cz-C4 at 10% of the nasion-inion distance. Electromyographic activity was recorded from the extensor digitorum brevis muscles. The sweep time was 150 ms with a 30 ms delay (filter settings 10 Hz and 2000 Hz) and the interstimimulus interval was 10 sec. Stimulus intensity was set at threshold and 20% above threshold. Stimulation at each site was performed with the current flowing through the coil in a clockwise direction and in a counter clockwise direction.

MEPs elicited when the coil was placed over the anterior and central scalp locations elicited the largest responses. Threshold responses were recorded to stimulation at anterior locations in eight subjects (three left-sided, one central and four right-sided) and at central locations in three subjects (two left-sided and one right-sided). Responses could not be obtained from all 9 sites in each subject and the effect of current direction in the coil varied between subjects. This study demonstrates for the first time that MEPs can be easily recorded from the lower limbs in humans. Further, the optimal coil orientation to elicit a response from the lower limbs requires careful positioning.

312.3

ABNORMAL P1 POTENTIALS IN AUTISTIC SUBJECTS. *J.BUCHWALD, R.ERWIN, J.SCHWAFEL* AND P.TANGUAY*, (SPON: R. STRANDBURG. Dept. of Physiol.,Brain Res.Inst. and Ment.Ret.Res.Ctr., UCLA Med.Ctr.,Los Angeles, CA 90024.*

Previous work on the cat and the human has closely linked the generator substrate of the P1 potential to the ascending reticular activating system(ARAS) and to its relays in the intralaminar thalamus. A current theory of autism postulates that dysfunction of the ARAS may be fundamental to this syndrome. Thus we hypothesized that P1, a putative electrophysiological probe of the ARAS, might be abnormal in autism. To test this hypothesis, middle-latency responses(MLRS) were recorded in a group of 11 autistic subjects (mean age 25.7 years) with mean full scale IQ of 97. Each had a history of childhood autism and prior to recording was rediagnosed as autistic, utilizing DSM III criteria, by a UCLA child psychiatry team. A group of 11 normal adults (mean age 20.5 years) served as controls. MLRs from midline (Cz) electrodes were evoked by click stimuli presented at rates of 0.5,1,5,8, and 10/sec. Computer averages of 500 trails were analyzed for peak-to-peak Pa-Nb and P1-Nb amplitudes; Pa, the maximum 25-40 msec positive peak, Nb the maximum 40-50 msec negativity, and P1 the maximum 50-65 msec positivity. While Pa showed no difference between the two groups, the autistic P1 was significantly smaller at slow click rates and did not change with increasing rates. This is the first report of abnormal P1 responses in autism and indicates functional abnormality in the generator substrate of this component, postulated to be the ARAS.(Supported by HD05958 and HD04612.)

312.4

PONTINE RETICULAR FORMATION LESIONS IN THE CAT:EFFECTS ON P1 POTENTIAL AND BEHAVIOR. *J.Harrison, J.Buchwald, S.Song*, N.Woolf, L.Butcher. Brain Res.Inst.,Ment.Ret.Res.Ctr.,Dpts.of Physiol. and Psychol.,UCLA Med.Ctr.,Los Angeles, CA 90024.*

The midlatency auditory P1 evoked potential seems to reflect activity of the reticular formation(RF). It is present during wakefulness and REM sleep but absent during slow-wave sleep in cats and humans(Chen & Buchwald, Erwin & Buchwald). The cat P1 is positive with 20-30 msec latency, present at slow but not rapid click repetition rates and absent with barbiturate anesthesia. It is eliminated by scopolamine, a cholinergic antagonist (Dickerson & Buchwald). These features led us to propose that P1 is dependent on cholinergic neurons of the RF. With radio-frequency lesions we destroyed large cholinergic cells in the pedunculo-pontine tegmental nucleus in the cat RF. Early RF-lesion studies in cats resulted in a permanent sleep state(Lindsley et al.). Our cats with somewhat more posterior lesions were awake the day after surgery, but had behavioral deficits: lack of grooming, biting objects, circling to one side, tendency to bump into walls, and sometimes pain insensibility. Sham operations did not result in these effects, which are similar to deficits from lemniscal damage(Sprague et al.), although we did not lesion the lemnisci. The largest lesions resulted in permanent loss of P1. In some cases P1 remained, and some cholinergic neurons were spared. Post-lesion P1 potentials were eliminated by scopolamine and enhanced by physostigmine. These data support our hypothesis that P1 is dependent on cholinergic neurons of the RF.(Supported by USPHS Grants HD05958, and NS25400.)

312.5

THE PROPAGATION POTENTIAL. A.P. Rudell* and S.E. Fox. Dept. of Physiology, SUNY Health Sci. Ctr., Brooklyn, NY 11203.

Experiments with earthworm giant axons and bullfrog sciatic nerves have convinced us that there is a net longitudinal current flow so long as an action potential (AP) is propagating in an axon. Its extracellular component flows in a direction opposite to AP propagation. When recorded differentially from electrodes separated several cm along the outside of the axons, a negative peak appeared at the time the AP reached the first electrode and a positive peak when it reached the second. Between these events was a negative plateau (with respect to the first electrode), the propagation potential, averaging about one-third the magnitude of the peaks in the earthworm. The plateau in the earthworm was not absolutely flat, but had ripples, possibly due to differences in extracellular resistance along the nerve and to variation in its diameter. The ripple pattern was not the same for each preparation, so that an average response of 25 different axons approached absolute flatness for the duration of AP propagation. Differential intracellular recordings from giant axons with micropipets inserted in the head and tail regions showed depolarizing afterpotentials, but their decay was too rapid to account for the propagation potential. Several other hypotheses have been considered as possible explanations for the plateau, but the mechanism has not yet been established.

The implication of the findings for EEG and evoked potentials in humans is that axonal activity may be responsible for a larger portion of such recordings than previously supposed. Slowly conducting axons should produce long duration propagation potentials, which could summate to large values in spite of significant temporal dispersion.

312.6

AGE-RELATED CHANGES IN AUDITORY EVENT-RELATED POTENTIALS AS A FUNCTION OF TASK DIFFICULTY IN NORMAL HUMAN SUBJECTS. V.L. Rothenberger*, N.K. Squires*, and D.T. Arcieri*. (SPON: John Stamm). Psychology Dept., S.U.N.Y. Stony Brook, Stony Brook, NY 11794.

Event-related potential (ERP) studies have described consistent age-related changes in the P300 component, with older subjects having longer latencies than younger subjects. The current literature is inconsistent in reporting the nature of the aging/latency relationship; several studies report a linear relationship, while other studies describe a non-linear function. The current project addressed the problem of whether variation in task difficulty may explain the form of the aging/P300 latency relationship. Auditory ERPs were recorded in two three-tone auditory oddball tasks where subjects attended to two rare tones differing in the difficulty of the discrimination. Results indicated significant correlations between age and latency in the easy discrimination (r=.51) and the difficult discrimination (r=.49). Regression analyses revealed linear relationships in all task conditions. The slope of the regression line appears to increase as a function of task difficulty.

312.7

VEP CHANGES IN MS PATIENTS IN CORRELATION WITH THE STAGE OF THE DISEASE AND OTHER CLINICAL DATA. M. Ejma*, R. Podemski*, P. Olejniczak. Dept. of Neurol. Med. Sch. in Wroclaw, Poland.

Whole visual fields of each eye separately were stimulated with a reversible checkerboard pattern in 205 MS patients and 100 controls. The latencies of the P100 and amplitudes of the P100/N120 were analyzed with the Nicolet CA-1000 averager.

MS patients showed significant elongation of the latencies and reduction of the VEP amplitudes compared to the control group. VEP latencies' duration correlated positively with the certainty of the diagnosis, stage and duration of the disease. It did not depend on the actual patient's age, age at the onset of the disease or improvement obtained in the course of treatment. More marked elongation of the P100 latencies was accompanied by the fall of the amplitudes of the P100/N120 complex. Significant VEP changes were found not only in patients with optic disc decolorization, but also in cases with normal eye bottom pictures.

312.8

RELATIONSHIPS BETWEEN INTRA- AND EXTRACRANIAL RECORDINGS OF HUMAN EVENT-RELATED POTENTIALS. I. Kiss, P. Lordeon*, R. Brenner* and R. Dasheiff University of Pittsburgh Epilepsy Center and Western Psychiatric Institute and Clinic, Pittsburgh, PA 15213

Candidates for surgical treatment of epilepsy received bilateral frontal and temporal implants of 8-contact intracranial electrodes. Event-related potentials were generated using an auditory "oddball" paradigm and recorded simultaneously from sets of 16 intracranial and 4 surface midline sites.

ERP's recorded from frontal and scalp electrodes contained features of similar latency but opposite polarity. Recordings from temporal lobes were remarkable for their lack of N1-P2-like features despite the presence of N2-P3-like activity. In addition, anomalous features were recorded at intracranial regions shown to produce epileptiform discharges.

These preliminary results suggest that components of ERP's recorded at the scalp may be reflected in intracranially recorded ERP's and that N1-P2 and N2-P3 are generated in separate regions. It is also possible that intracranially recorded ERP's may aid in the location of seizure foci.

312.9

BIOMAGNETIC STUDIES FOR THE LOCALIZATION AND CLASSIFICATION OF CNS DISFUNCTION IN CHILDREN. N.C.Rasis and P.A.Anninos. Lab. of Med. physics and Neurophysiology,Univ. of Thrace, sch. of Med. Alexandroupolis,Greece.

Using the Biomagnetometer SQUID (Superconducting Quantum Interference Devise) we studied various CNS disfunctions in children and compared and classified them according to the different types that we found.With the above instrument,due to its better spatial resolution,we can localize different CNS disfunctions by measuring separetely the emitted magnetic field from 32 points in each hemisphere from the skull. These points were chosen around specified standart EEG origin positions as defined by the international 10-20 system from electrode placement.The emitted magnetic field from each measuring point is measured from a distance of 3 mm from the skull thus avoiding the junction potentials which usually develop with the use of electrode contacts.Our results were expressed in terms of "Iso-Spectral Amplitude" maps(ISO-SA)for band frequencies between 2-7Hz.In these studies we examined three groups of children.In each of these three groups we concerned with identified clinical general epilepsy,post-traumatic epilepsy and febrile convulsions respectively.In the first group using the above mapping technique we were able to indentify well extented regions of high magnetic emitted energy in the 2-7Hz band frequencies,whereas in the second ,we had focal regions near the traumatic area.Finally in the third ,we found for first time diffused regions of high emitted magnetic energy in the 2-7Hz band range located in the temporal hemispheres.

312.10

INTERLEUKIN-1 INDUCED INFLAMMATION IN THE CENTRAL NERVOUS SYSTEM: PHYSIOLOGICAL AND PATHOLOGICAL CORRELATES. M. Litwak*, C. Brosnan*, C. Schroeder and J. Arezzo, (SPON: M. Schroeder). Dept. of Neuroscience, Neurology and Pathology, Albert Einstein Coll. of Med., Bronx, N.Y. 10461

Interleukin 1 (IL1) is a protein secreted by activated macrophages. In vitro studies with endothelial cell cultures show IL1 to be involved in binding of T and B-lymphocytes and to act synergistically with interferon -gamma and tumor necrosis factor to promote migration of lymphocytes and neutrophils to the focus of inflammation. To evaluate IL1 induced inflammation on CNS function, we tested the effects of monocular injections of IL1 upon the latency and amplitude of visual evoked potentials (VEP) in 5 normal rabbits. Intraocular injection allows IL1 direct access to the myelinated fibers of the retina. The nearly complete crossing of the visual pathways permits the saline treated eye to function as an internal control.

IL1 induced an initial slowing of conduction at 3.0 hours which returned to normal by 72 hours. The effects were limited to the retina and distal portions of the optic nerve of the treated eye. At 3 hours the onset and peak of the cortical VEP were delayed by approximately 2.0 msec (10%). Histology revealed that IL1 affects the vasculature, altering the blood brain barrier resulting in the infiltration of inflammatory cells and fluids; there was no evidence of myelin pathology. Other cyokines, including: interleukin-2, interferon-gamma and tumor necrosis factor, produce similar alterations in conduction. These alterations differed principally in timing of the onset, the magnitude of the alteration and degree of reversibility. These results indicate that inflammatory mediators have an acute and direct effect on conduction within CNS pathways that may underlie the exacerbating/remitting characteristics of demyelinating diseases. Supported by NS-11920-14.

312.11

PEAK LATENCIES OF FELINE SPINAL CORD MOTOR EVOKED POTENTIALS IN RESPONSE TO ELECTRICAL, TRANSCRANIAL BRAIN STIMULATION. P.E. Konrad, W.A. Tacker, Jr.,* and D. Schooler.* Hillenbrand Biomed. Eng. Ctr., Purdue Univ., W. Lafayette, IN 47904.

Motor evoked potentials (MEPs) are bioelectrical potentials originating in efferent pathways as a result of brain stimulation. They can be used to diagnose brain or spinal cord injury which may result in motor dysfunction. The development of a standard feline model of the MEP test requires characterization of peak latencies at standard recording locations and under a variety of stimulus intensities and modalities likely to be used by investigators. To this end, 10 cats were anesthetized and prepared for paraspinal recording of MEPs at the level of $T_{9/10}$. Peak latencies of spinal cord MEPS were recorded in response to scalp-, dura- and cortex-to-palate transcranial stimulation. A broad range of stimulus intensities and durations (0 - 80 mA; 50 - 1000 μsec; 21 Hz; 200 repetitions per average) were used for each stimulus modality resulting in the genesis of nine distinct peaks. The range of average peak latencies was 1.57 - 9.75 msec. Statistical analysis using a general linear model procedure and studentized Newman-Keuls test showed that the average latency for each peak is statistically different ($p < 0.05$) from each other, independent of the effect of stimulus modality or cats. The results emphasize the absence of overlap of latencies for the peaks. The first four peaks occurred with relatively high frequency (>41%) within the range of stimulus intensities tested.

313.1

OXIDATION PRODUCT OF SEROTONIN: NEUROTOXIC PROPERTIES. E.P.Crino, E.A.Vogt, J-C.Chen, and L.Volicer. Dept. of Pharmacology, Boston Univ. Sch. of Med., Boston, MA. 02118 and GRECC, E.N.Rogers Mem. Vet.Hosp., Bedford, MA 01730.

Electrochemical oxidation of serotonin has yielded a partially oxidized compound (5HTe-) which is similar chromatographically to one found in Alzheimer patient cerebrospinal fluid. To assess its toxicity, 5HTe- (80ug in 20ul buffer) was injected into the lateral ventricles of rats. Animals were sacrificed at 6 days post-injection and stained with the Fink-Heimer method for nerve terminal degeneration. Cell death and terminal degeneration was evident in superficial layers of prefrontal, insular, and posterior cingulate cortices, and in CA1, CA3, and dentate gyrus sectors of hippocampus. 5HTe-injection (5ug in 5ul buffer) into cingulate and hippocampal cortices resulted in cell death and terminal degeneration. In hippocampus, degeneration was prominent in CA1 and dentate gyrus. Projection areas of cingulate cortex such as anterodorsal thalamic nucleus and contralateral cingulate cortex also showed terminal degeneration. Thus, 5HTe- is neurotoxic and may contribute to the pathogenesis of Alzheimer's disease. (Supported by USPHS AG06419 and the Veterans Administration).

313.2

ENDOGENOUS NEUROTOXIN FORMATION IS DUE TO NON-ENZYMATIC OXIDATION OF MONOAMINES. K.J. Axt and L.S. Seiden. Dept. Pharm/Phys. Sci., Univ. of Chicago, Chicago, IL 60637.

We have previously reported the formation of endogenous neurotoxins in the rat brain shortly after a single high dose of methamphetamine (MA) and p-chloroamphetamine (pCA). We have speculated that MA and pCA neurotoxicity are mediated by these neurotoxins (Seiden & Vosmer, 1984; Commins et al., 1987a,b). We now report that the endogenous formation of 6HDA and 5,6-DHT likely result from autoxidation of monoamines and not through the action of a hydroxylase. Evidence includes 1) the inability of hydroxylase inhibitors, other than a-methyl-p-tyrosine (AMT), to prevent the MA-induced long-term depletions of DA and 5HT; 2) the ability of aminotriazole, a catalase inhibitor, to enhance MA-induced DA depletions and 6HDA formation (in vivo and ex vivo). Aminotriazole pretreatment does not enhance MA-induced 5HT depletions nor formation of 5,6-DHT from 5HT ex vivo using a Fenton reaction system. This latter result may be explained by in vitro studies using a Fenton reaction system in which excess hydrogen peroxide markedly enhances degradation of 5,6-DHT. This research was supported by PHS MH-14274 Training Grant; RSA MH-10562 (L. Seiden) and NIDA DA-00250.

313.3

TOXICITY OF 6-OHDA IN THE HUMAN NEUROBLASTOMA CELL LINE SH5Y5Y IS INDEPENDENT OF DOPAMINE UPTAKE. D.E. Decker*[1], S.E. Buxser*[1], J.S. Althaus*[2] and P.F. VonVoigtlander[2] (SPON: K. Lookingland). [1]Cell Biology and [2]CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Toxicity of 6-hydroxydopamine (6-OHDA) in vivo has been shown to be dependent on the competitive binding of 6-OHDA to norepinephrine (NE) and dopamine (DA) uptake systems in both peripheral and central catecholamine (CA) containing neurons. The human neuroblastoma cell line SK-NSH-SY5Y (SY5Y) also shows toxicity to 6-OHDA (Castiglioni et al. 1982, Biochem. Pharm. 31:181). This toxicity could be neutralized by catalase and was thought to be dependent upon the generation of H_2O_2. We examined 6-OHDA toxicity in the SY5Y to determine if specific toxicity occurring through a DA dependent uptake mechanism could be demonstrated. As a measure of 6-OHDA toxicity, uptake of the nonmetabolizable amino acid alpha amino isobutyric acid (AIB) was measured after treatment with 6-OHDA for 1 hr at 37°. The IC_{50} value for 6-OHDA in inhibiting AIB uptake in SY5Y cells was 50 µM. Toxicity of 6-OHDA was prevented in the presence of 3 U/ml of catalase. Since catalase alone could prevent toxicity of 6-OHDA, toxicity occurring through a CA uptake system under these conditions was doubtful. HPLC analysis using electrochemical detection showed that less than 10% of the 6-OHDA was detectable after incubation at 37° for 1 hr. However, inclusion of 1 mM ascorbate (ASC) under these conditions stabilized the 6-OHDA. When 1 mM ASC was included in the incubation medium and AIB uptake measured, an increase in the toxic potency of 6-OHDA was observed, and this could not be completely blocked with 3 units/ml of catalase. Initially it was presumed that the increased toxicity of 6-OHDA in the presence of 1 mM ASC reflected the stabilization of 6-OHDA and subsequent toxicity through a CA specific uptake system. However, when 1 mM DA was included in the incubation medium to block 6-OHDA uptake, toxicity still occurred. Alternatively the increased toxic potency of 6-OHDA in the presence of 1 mM ASC may result from an increased concentration of H_2O_2 liberated by 6-OHDA through a redox-recycling mechanism in the presence of ASC. This hypothesis was supported by the result that the toxicity of 6-OHDA in the presence of 1 mM ASC could be blocked by increasing the catalase concentration to 30 units/ml. Although we showed that SY5Y cells possess a DA uptake system that was specifically blocked by 1 µm nomifensine, toxicity of 6-OHDA did not appear to occur as the result of 6-OHDA uptake into cells via the DA uptake system.

313.4

MECHANISM OF METHAMPHETAMINE-INDUCED NEURONAL DAMAGE: A POSSIBLE ROLE FOR FREE RADICALS. G.C. Wagner and M.J. DeVito* Depts. of Psychology and Toxicology, Rutgers Univ. New Brunswick, NJ 08903

The hypothesis that methamphetamine-induced neuronal damage is mediated by free radical production was evaluated by pretreating rats with either antioxidants or a superoxide dismutase inhibitor. Methamphetamine (6.25 - 25mg/kg delivered four times at 2 h intervals) or saline was administered to rats pretreated with ascorbic acid (10 - 1000 mg/kg), ethanol (1 g/kg), vitamine E (2 g/kg), mannitol (2 g/kg) or diethyldithiocarbamate (200 - 800 mg/kg). Rats were sacrificed two weeks later, striatal tissue was removed and dopamine and serotonin levels determined.

Administration of methamphetamine resulted in significant depletions of striatal dopamine (to 50% of control) and serotonin (to 50% of control). Pretreatment with any of the antioxidants significantly attenuated or completely eliminated the long-lasting monoaminergic depletions while pretreatment with DDC increased the magnitude of these depletions by approximately 20%.

These observations indicate that the repeated administration of methamphetamine results in long-lasting depletions of striatal dopamine and serotonin. The attenuation of this toxicity by three different antioxidants indicates that oxygen free radicals may have a role in the methamphetamine-induced neuronal damage.

313.5

TYRAMINE-INDUCED ALTERATIONS IN STRIATAL MONOAMINES. S.L. Walsh and G.C. Wagner. Department of Psychology, Rutgers, The State University, New Brunswick, NJ 08903

Tyramine, a potent releaser of catecholamines, is found endogenously in the central nervous system as well as exogenously in several food products. In an effort to determine if exposure to high doses of tyramine would result in altered striatal metabolism and/or cause long-lasting monoaminergic depletions, the following studies were conducted.

Swiss-Webster male mice were treated (s.c.) with 100 mg/kg tyramine HCl, sacrificed and striata dissected 20, 40, or 80 minutes later. It was observed that there were significant increases in the DOPAC/DA ratio at forty minutes which returned to baseline by 80 minutes, as well as increases in serotonin (50%) and 5HIAA (86%) at each time tested.

Swiss-Webster male mice were treated (s.c.) with 20, 40 or 80 mg/kg of tyramine HCl four times at two hour intervals then sacrificed one week later. It was observed that there was a significant decrease in striatal dopamine concentration at each dose with the greatest loss observed (35%) at the highest dose.

These observations indicate that peripheral administration of tyramine at high doses may result in both acute and long-lasting alterations in striatal neurotransmitter systems.

313.6

TRIADIMEFON INDUCES SEX-DEPENDENT CHANGES IN STEREOTYPED BEHAVIOR AND BIOGENIC AMINE ACTIVITY. O.D. Walker*, M.H. Lewis, K.C. Crofton, and R.B. Mailman (SPON: R.C. MacPhail). Curriculum in Toxicology and Biological Sciences Research Center, UNC, Chapel Hill, NC, and US-EPA, RTP, NC.

Triadimefon, [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone], is a triazole fungicide that has been observed to induce hyperactivity in mice and rats, and stereotyped behavior in female rats. The present study contrasted the behavioral effects produced by triadimefon in male and female Sprague-Dawley rats using a computer-supported observational method. Triadimefon was administered i.p. (0, 50, 100, or 200 mg/kg) in corn oil (2 ml/kg) four hours prior to behavioral assessment (n=10). Results indicated a highly significant dose-dependent change in stereotyped behavior in both sexes, with the highest dose producing increases in head weaving, circling, and a six-fold increase for females in backward locomotion. Sex differences were also observed in gnawing, rearing, locomotion, and inactivity. Concentrations of dopamine (DA), serotonin (5-HT) and their metabolites were determined by HPLC in nigrostriatal and mesolimbic terminal areas. In corpus striatum, triadimefon produced decreases in DOPAC at all three doses, but increases in 5-HIAA at the two highest doses. At the highest dose, DA concentrations were decreased while HVA was doubled. Dopamine utilization in the olfactory tubercles was much less altered by the fungicide with an increase in HVA observed only at the highest dose. Sex differences in monoaminergic activity were also examined and related to behavioral effects. (Supported in part by ES07126)

313.7

THE NE DEPLETING EFFECTS OF DSP-4 ARE NOT MAO B DEPENDENT AND DO NOT INCREASE WITH AGE. K. T. Finnegan*, L. E. DeLanney, I. Irwin*, G. A. Ricaurte, and J. W. Langston. *Institute for Medical Research, San Jose, CA 95128; Johns Hopkins Univ Sch of Med, Baltimore, MD 21205.* Age-related increases in MAO B activity may play an important role in the age-dependent effects of toxins that employ this enzyme for their bioactivation. The toxicity of the noradrenergic toxin, DSP-4, is reported to be prevented by the MAO inhibitors pargyline and deprenyl, suggesting that DSP-4 is metabolized by MAO B to a toxic metabolite (Gibson, 1987). Therefore, we examined the age-dependent hippocampal NE depleting effects of DSP-4 in 2 month and 10 month old C57BL/6 mice 1 week after DSP-4 administration. DSP-4 produced equivalent, dose-related depletions of hippocampal NE in the 2 age groups, suggesting that either: 1) other factors besides MAO B dependent activation are necessary for a toxin to produce age-related toxic effects; or 2) the ability of deprenyl to prevent NE toxicity may not be related to MAO B inhibition, but rather its ability to block NE reuptake. To test the latter possibility, we pretreated mice with deprenyl either 1 hr or 24 hrs before DSP-4. Deprenyl protected NE neurons when given 1 hr before DSP-4, but at the 24 hr pretreatment interval protection diminished significantly. Since MAO B is still completely inhibited 24 hrs after deprenyl, it seems unlikely that the protective effects of deprenyl can be solely attributable to MAO B inhibition. Thus the protection afforded by the 1 hr pretreatment interval may, at least in part, be due to NE reuptake blockade, mediated by the transient presence of deprenyl or its metabolites meth-amphetamine and amphetamine. These results may explain the inability of DSP-4 to cause increased toxicity with age.

313.8

ACUTE THIAMINE DEFICIENCY IN THE RAT: BRAIN LESIONS, AMINO ACID CHANGES AND MK-801 PRETREATMENT. P.J. LANGLAIS, R.G. MAIR, & W.J. MCENTEE, Research Service 151C, VA Med. Ctr., Brockton, MA 02401.

We have observed permanent tissue loss and amino acid reductions within thalamus, and learning-memory deficits in a rat model of Korsakoff's disease produced by an acute bout of pyrithiamine-induced thiamine deficiency (PTD). In the present study, rats were sacrificed at two stages of acute PTD to determine the progression and extent of pathological and amino acid changes. In an early acute group, a selective and marked loss of neurons was observed within intralaminar and posterior thalamic nuclei. At a later stage, severe neuronal and tissue loss and gliosis were present throughout the entire thalamus and to a lesser degree within hypothalamus, central and periacqueductal grey. In the late acute group, hemorrhagic lesions were present within the mammillary body (MB), geniculate (MG-LG) nuclei and thalamus. Affected areas were marked by large reductions of glutamate and aspartate and a 2-3 fold increase of glycine. Pretreatment with the NMDA antagonist MK-801 markedly attenuated the extent of cell loss, hemorrhages, and amino acid changes. These observations suggest that in PTD: i) a neurotoxic process originates within intralaminar nuclei and spreads into remaining thalamic and surrounding tissue; ii) damage to MB and DG-LG is primarily hemorrhagic and a later event; and iii) NMDA receptors play a role in the pathophysiological processes.

Supported by Veterans Administration research grant.

313.9

DETECTING CIRCUMVENTRICULAR ORGAN (CVO) TOXICITY. A. C. Scallet, J. W. Bryan, IV*, R. Rountree* and A. Andrews*. National Center for Toxicological Research, Jefferson, AR 72079.

The CVOs (arcuate nucleus, area postrema, and others) are preferentially exposed to blood-borne neurotoxicants since they lack the protection of a blood-brain barrier. Various chemicals produce necrosis of CVO cells, but monosodium glutamate (MSG) has most often been used experimentally. To compare methods to detect altered CVO function, male rats (n=17-23) received subcutaneous MSG on postnatal days 2, 4, 6, 8, and 10 (4 mg/g/day, group SC4) or orally by gavage (4 mg/g, group PO4 or 2 mg/g, group PO2) according to the same schedule. Controls (Cs) received SC or PO saline. PO2 and PO4 rats showed a dose-related decrease in body-weight through 3 mo of age. SC4 rats weighed the least until 13-14 wk, when their weight-gain accelerated markedly. At 8 mo, the nose-to-anus length of PO4s was less than Cs ($p < 0.01$), and SC4s were shorter than either PO4s or Cs ($p < 0.01$). On a hotplate analgesia test, SC4s and, to a lesser extent, PO4s had longer baseline latencies and greater response to morphine than Cs. The results signify that oral dosing of neonates with MSG may have prolonged effects on their growth and development. Immunohistochemical evaluation of the CVOs of these animals is underway.

313.10

EXCITOTOXIC MEDIATED SPINAL CORD DAMAGE: POSSIBLE ROLE OF THE NMDA RECEPTOR. V.Q. Gardner, V.J. Caiozzo, S. Munden, and R.J. Bridges. (SPON: S. van den Noort). Div. Orthop. and Dept. of Neurol., Univ. California, Irvine, CA 92717

Glutamate has been identified as a major excitatory transmitter in the mammalian CNS and, when present in excessive amounts, a potent neurotoxin. Recent studies have implicated NMDA (N-methyl-D-aspartate) receptor activation and increased intracellular Ca^{++} concentrations as mechanisms involved in this excitatory mediated cell death. In the present study, we have examined the effects of Ca^{++}, glutamate, and NMDA receptor antagonists on protein degradation in the spinal cord.

Spinal cords were rapidly removed from male Sprague-Dawley rats and divided into 4 segments. These segments were then either frozen immediately (time 0 control) or allowed to incubate for 3 hours at 23° C in physiological buffer with or without: 1) Ca^{++}, 2) glutamate, or 3) a noncompetitive antagonist of the NMDA receptor. The tissue samples were homogenized and the proteins were separated by gradient SDS-polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue R-250.

Spinal cords incubated in buffer containing Ca^{++} or Ca^{++} plus glutamate consistently exhibited a dramatic decrease in band density at molecular weights of 200, 150, and 68 kDa. These bands correspond to the known molecular weights of proteins that make up the neurofilament triplet. Importantly, this degradation could be attentuated by the inclusion of ketamine (i.e., a noncompetitive inhibitor of the NMDA receptor). These findings demonstrate increased protein degradation in spinal cord in response to high levels of glutamate and suggest that the NMDA receptor may be involved in excitatory mediated spinal cord injury.

Supported in part by a grant from the American Paralysis Association and the Irvine Community Foundation.

313.11

SOMAN IN MULTIPLE LOW DOSES: DAMAGE TO SELECTED POPULATIONS OF NEURONS IN RAT BRAIN. R.C.SWITZER III[+], M.R.MURPHY^ S.K.CAMPBELL*, S.Z.KERENYI[#*], S.A.MILLER[#*], & S.L.HARTGRAVES[#]. [+]University of Tennessee, Med. Ctr., Knoxville TN 37920; ^Systems Research Lab., & [#]Radiat. Sci. Div., USAF Sch. Aerosp. Med., Brooks AFB, TX.

Soman is a fast acting, irreversible anticholinesterase organophosphate neurotoxin present in the chemical arsenals of a number of countries. Until recently sensitive methods had not been applied to show that single, severe symptom-producing doses cause CNS degeneration. We sought to determine if multiple exposures to single doses, each of which produce no anatomical or severe clinical symptoms, could result in CNS degeneration. Rats were given 54 μg/kg/day of soman for 5 days. Rats surviving the initial effects were allowed to survive from 7 to 35 days. Degeneration in 40μ freeze-cut sections was assessed using the cupric-silver staining method of de Olmos. Rats recovering rapidly from loss of weight showed no brain degeneration, but rats requiring extra care to recover from severe dehydration and weight loss displayed extensive degeneration in specific areas, including specific regions and layers of cortex, select thalamic nuclei, septum, amygdala, hippocampus and olfactory bulb. Hypothalamus, brain stem and cerebellum were devoid of degeneration.

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313.12

SOMAN TOXIC SYNDROME: PERFORMANCE, PHYSIOLOGY, BIOCHEMISTRY, AND NEUROPATHOLOGY. M. R. Murphy[+], S. Z. Kerenyi*†, S. A. Miller*†, R. C. Switzer III‡, D. W. Blick*†, & S. L. Hartgraves* *Systems Research Laboratories, ‡U. Tenn., & †Radiation Sci. Div., USAF School of Aerospace Medicine, Brooks AFB, TX 78235-5301.

Rats exposed for 5 days to 54 μg/kg/day of soman, an organophosphate that irreversibly inactivates cholinesterase (ChE), showed brain and blood ChE inhibition, weight loss, and variable motor symptoms (including convulsions). After soman administration ceased, surviving rats fell into dichotomous groups: 1) those recovering lost weight rapidly and showing no brain degeneration or abnormal behavior; and 2) those requiring extra care to recover from profound dehydration and weight loss, and, after recovery, displaying spontaneous, recurrent convulsions, hyperreactivity, changes (not necessarily deficits) in learning and performance of two-way shuttle avoidance, and extensive but localized brain degeneration.

This research was performed under USAF Contract F33615-85-D-0659, with funding from the Naval Medical Research and Development Command, project MF4561.001.

313.13

PROTECTIVE EFFECT OF BOTULINUM TOXIN ON MUSCLE TOXICITY OF SOMAN. M.E. Clinton, K.E. Misulis, W-D. Dettbarn. Neurology & Pharmacology Depts. Vanderbilt Univ., Nashville, TN 37212.

Acetylcholinesterase (AChE) inhibitors induce fasciculations and necrosis of skeletal muscle, which is presumed to be due to excess of acetylcholine (ACh), although this has not been proven. Botulinum toxin (BTX) a presynaptic inhibitor of acetylcholine release, was injected directly into the hindlimb of rats. Two days later the rats were given soman. One group was studied by electromyography (EMG) during the acute intoxication and a second group histologically with muscle taken 24 hours later.

Motor unit firings were recorded and digitized. In the acute group, within 60 min of receiving soman, controls had 584/min motor unit firings compared with 44.7/min in those with soman plus BTX. In the second group rats were sacrificed after 24 hours, and the left extensor digitorum longus was taken for histological staining. Those with soman alone had 35.3 lesioned muscle fibers per cross section compared with 3.8 for the BTX group.

Inhibition of ACh presynaptically prevents both fasciculations and the myopathy due to soman poisoning, further evidence that ACh excess is the responsible factor in soman induced myopathy and likely similar myopathies seen with other AChE inhibitors.

313.14

INFLUENCE OF PRETREATMENT AND THERAPEUTIC COMPOUNDS ON THE MORPHOLOGIC EFFECTS OF ACUTE SOMAN INTOXICATION IN THE RHESUS MONKEY. I. J. Hayward*, H.G. Wall*, N.K. Jaax*, J.V. Wade, J.B. Nold*, and D.D. Marlow*. US Army Medical Research Institute of Chemical Defense, APG, MD 21010-5425

The potential of anticonvulsant treatment to ameliorate brain lesions induced by the irreversible organophosphorus cholinesterase inhibitor soman (pinacolyl methylphosphonofluoridate) was examined in rhesus monkeys. Monkeys were pretreated with pyridostigmine (1.2 mg/kg, q8hr x 4, p.o.), injected 4.5-5 hr later with soman (76.5 ug/kg, i.m.), and then treated 1 min post-soman with atropine (0.4 mg/kg, i.m.), pralidoxime chloride (2-PAM, 25.71 mg/kg, i.m.), and diazepam, midazolam (1 mg/kg, i.m.), or sterile water. Treatment with diazepam or midazolam hastened recovery following soman challenge, when compared to animals receiving atropine and 2-PAM without an anticonvulsant. At 48 hr, tissues were prepared for routine histopathology with emphasis on brain, in particular frontal and entorhinal cortex, amygdala, caudate, and hippocampus. Neuronal lesions were generally minimal to mild regardless of treatment, except for occasional moderate lesions in animals that did not receive an anticonvulsant. Neuronal degeneration and necrosis were decreased significantly in the highly vulnerable entorhinal cortex, caudate nucleus, and hippocampus of anticonvulsant-treated animals. Thus, anticonvulsant therapy ameliorated brain lesion development in some areas and hastened the time to recovery post-soman challenge.

313.15

ORGANOPHOSPHATE INTOXICATION ALTERS DISTRIBUTION OF ELEMENTS IN CHICKEN PERIPHERAL AXONS. R. M. LoPachin, D. M. Lapadula* and M. B. Abou-Donia. Dept. of Anesthesiology, SUNY, Stony Brook, NY 11794 and Dept. of Pharmacology, Duke Medical School, Durham, NC 27710

Alterations in subcellular distribution of Na, Ca and other elements appear to play an important role in mediating cellular damage induced by a variety of injurious processes. We have used x-ray microprobe analysis to investigate the possibility that delayed nerve damage caused by organophosphates involves a perturbation of axonal elemental homeostasis. Leghorn Chickens were given a single oral dose of either tri-o-cresyl phosphate (TOCP, 750 mg/kg) or control solution. Fourteen days later control and treated chickens were sacrificed and mid-thigh sections of sciatic nerve were frozen in situ and removed for analysis. Concentrations (mmol element/kg dry wt) of Na, P, Cl, K and Ca were determined in frozen, unfixed, ultrathin (<200 nm) sections of nerve. Four sciatic nerve compartments were examined; axoplasm, mitochondria, myelin and ECF. In both axoplasm and mitochondria from control chickens K was the most abundant element whereas Na and Ca were present in very low concentrations. In myelin, P was most prominent with other elements being present at concentrations <200 mmol/kg dry wt. The ECF exhibited relatively high concentration of Na, Cl and Ca. These results are quantitatively similar to those from rat sciatic nerve (LoPachin et al., *J. Neurochem.*, 1988). TOCP intoxication was associated with a marked derangement of intracellular elemental distribution. Sodium levels increased 2-3 fold in both mitochondria and axoplasm whereas K concentrations decreased by one-third. In axoplasm, Ca levels increased only slightly, while mitochondrial levels of this element arose substantially. Changes in myelin or ECF were not observed. These results suggest that changes in intra-axonal levels of Na, K and Ca are involved in the mechanism of organophosphorous compound-induced delayed neurotoxicity. Supported by NIH Grants ES03810, OH02003 and ES02717.

313.16

NEUROBEHAVIORAL EFFECTS OF TRICHLORFON IN B6C3F1 MICE DURING A SUBCHRONIC DOSED FEED STUDY. G.B. Freeman, J. Killinger*, R. Trejo*, P.C. Chan*, A. C. Peters*. Battelle Columbus Division, Columbus, OH and NIEHS, Research Triangle Park, NC.

Trichlorfon is an organophosphate used commercially as an insecticide. In this 13-week subchronic dosed feed study, male and female B6C3F1 mice were administered trichlorfon in the diet for 13 weeks at dose levels of 0, 62, 185, 555, 1666 and 5000 ppm. Neurobehavioral evaluations were performed after 6 and 12 weeks of dosing. Total motor activity was reduced compared to control in both sexes at both study intervals in the 1666 and 5000 ppm dose groups. Female mice in the 1666 ppm group showed a 17% decline at 6 weeks and a 16% decrease at 12 weeks. The 5000 ppm female group demonstrated a 24% and 30% reduction at 6 and 12 weeks, respectively. Similar declines were reported for male mice; however, decrements became more severe at 12 weeks (1666 ppm at 6 weeks = 17%, 1666 ppm at 12 weeks = 27%, 5000 ppm at 6 weeks = 22%, 5000 ppm at 12 weeks = 39%). While the two sexes showed similar changes in motor activity, significant differences in grip strength were reported only for the 5000 ppm female group at 12 weeks. The lack of body weight reductions in mice suggested that grip strength deficits were not related to body weight. The results of the subchronic study indicated potential neurotoxic effects of trichlorfon, and that 5000 ppm was the apparent Maximum Tolerated Dose (MTD) in mice.

313.17

DISTINCT TOXICITIES OF INSECTICIDE PARATHION AND PARAOXON. K.-P. Shaw and J.C. Liu.* Dept. Biol. Anat., Natl. Def. Med. Ctr. Taipei, Taiwan, ROC

The purpose of these experiments was to characterize the mechanism of protection and therapy of organophosphate poisoning produced by insecticide parathion (IP) and its metabolite paraoxon (PO) which are widely used in many countries. PO (3-8 mg%) can potentiate indirect nerve twitch of sartorius muscle of frog, Rana catesbeiana to 126% of control value. PO (10 mg%) can completely abolish the direct muscle twitch and indirect twitch without potentiation. After washing for 1 hour, recovery of indirect twitch to 48% was observed. The commercial insecticide IP (8 mg%; contains organic solvent 3.5% xylene) induces depolarization and blocks indirect twitch completely without potentiation. After washing for 3 hours an 8% recovery of the indirect twitch was recorded. 3.5% xylene alone depolarizes and depresses the direct and indirect muscle twitch within 20 minutes. Pretreatment with atropine sulfate (1 mg/kg) and physostigmine salicylate (0.1 mg/kg) 10 minutes prior to PO (but not IP) exposure increases LD_{50} by 8 times in Balb/C mice. In conclusion, the distinct toxicities may relate more to the solvent than to the cholinergic effect of the insecticide. (Grant NSC 77-0412-B016-32, ROC)

313.18

NTE INHIBITION IS NOT FOLLOWED BY RETROGRADE AXONAL TRANSPORT DEFICIT AND POLYNEUROPATHY IN CHICKS. A. Moretto*, M. Lotti*, P.S. Spencer and M.I. Sabri. Institute of Neurotoxicology, Albert Einstein College of Medicine, Bronx, NY and Istituto di Medicina del Lavoro, Universita' di Padova, Italy.

Some organophosphorus esters induce central-peripheral distal axonopathy in sensitive species. The initiation mechanism is related to the inhibition and aging of more than 70% of axonal neuropathy target esterase (NTE) within hours of dosing. A progressive deficit of retrograde transport (RT) of I125-tetanus toxin along sciatic nerve follows with a peak inhibition 7 days after treatment. Clinical and morphological signs of neuropathy appear 10-14 days later. Morphological feature of this neuropathy is the degeneration of the distal, non terminal parts of the long and large-diameter axons in both spinal cord and peripheral nerves. Young animals are resistent to this toxicity despite the fact that nervous system NTE is inhibited by organophosphates. We treated 19-day old chicks with di-n-butyl-dichlorovinyl phosphate (DBDCVP;1mg/kg,s.c.), a neurotoxic dose for hens. Twenty four hours after dosing, NTE was inhibited more than 70% in chick brain, spinal cord and peripheral nerve. NTE in the nervous system of chicks was higher than the activity in hen nervous system. Seven days after DBDCVP treatment, accumulation in dorsal root ganglia and ventral spinal cord of retrogradely transported I125-tetanus toxin in treated animals did not significantly differ from that of vehicle-treated animals. Morphological examination on day 7, 14 and 21 after dosing showed no damage to either spinal cord or peripheral nerve axons. These data show that DBDCVP does not inhibit RT and cause neuropathy in chicks. Supported by NS19611.

313.19

TREMOR AND GRAIN PESTICIDES. L.J. Chapman*, Sauter S.L.*, Henning R.A.*, Matthews C.G.*, Levine R.L.*, Peters H.A.* (SPON: R. Daly). Speech Motor Control Labs., Neurology Department, University of Wisconsin, Madison, WI 53705-2280.

Although most tremor diagnoses are personal judgements based on visual observations, the unassisted clinician's eye can detect only gross differences in tremor amplitude and frequency. In the present study, computer-monitored strain gauge measurement of index finger tremor was able to demonstrate significant tremor amplitude and frequency differences between 19 grain industry employees and 19 controls. All the grainworkers were exposed to fumigant pesticides containing carbon disulfide, a known parkinsonian neurotoxicant and chronic tremorogen. Discriminant function analyses determined that computerized tremor measurement was more sensitive than conventional clinical tremor examination and did not misclassify any of the normal controls. Tremor power spectra in the grainworker group were abnormally shifted toward the lower frequencies and compressed into sharp frequency peaks. These results suggest that computerized finger tremor measurement may provide an indication of adverse neurotoxic effects prior to clinical diagnoses of a functional parkinsonian syndrome or a manifest overt disease state.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS III

314.1

AUTORADIOGRAPHIC EVIDENCE THAT SPINOTHALAMIC TRACT NEURONS POSSESS ADENOSINE RECEPTORS. J. I. Choca, R. D. Green*, and H. K. Proudfit. Dept. of Pharmacology, University of Illinois at Chicago, College of Medicine, Chicago, IL. 60612.

The purine nucleoside, adenosine, modulates pain transmission at the spinal cord level by interacting with adenosine A_1 and A_2 receptors, which are located predominantly on intrinsic neurons. We investigated the possibility that some of these spinal cord adenosine receptors may be localized on neurons of ascending somatosensory pathways. The spinothalamic tracts were lesioned by unilateral injections of the fluorescent, retrogradely-transported neurotoxin, doxorubicin, into the thalamic nuclei of rats. Autoradiographic studies were performed on paired spinal cord sections using the A_1-selective agonist, [^{3}H]R-PIA, and [^{3}H]NECA, an adenosine agonist that binds with equal affinity to A_1 and A_2 receptors. These lesions of the spinothalamic tract differentially decreased the [^{3}H]R-PIA and [^{3}H]NECA binding densities in the substantia gelatinosa. The results suggest that some spinal cord adenosine receptors are located on the cell bodies or dendritic branches of the spinothalamic tract neurons. Analgesia, which is produced by adenosine, may result from an adenosine-mediated inhibition of the spinothalamic tract neuron's response to painful stimuli. (Supported by USPHS Grant DA 03980)

314.2

MULTIPLE GRADIENTS OF ADENOSINE A_1 RECEPTORS IN THE CA1 REGION OF THE HIPPOCAMPUS. K.S. Lee. Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA 19107

Adenosine A_1 receptors are highly concentrated in the CA1 region of the hippocampus and exhibit a gradient of density along the longitudinal or septo-temporal axis of the hippocampus. Receptor localization studies utilizing autoradiographic techniques were undertaken to study the possibility of an additional gradient within the CA1 region along the transverse axis of the hippocampus. ^{3}H-cyclohexyladenosine (CHA) was utilized as a ligand for labeling A_1 receptors in cryostat sections as previously described (Lee and Reddington, Neurosci. 19: 1986). Quantitative densitometric measurements of CHA binding to coronal sections of rat and gerbil brains were performed using an Analytical Imaging Concepts system. The strata radiatum and oriens were measured in areas CA1a, CA1b and CA1c at a point approximately one-third of the distance from the septal pole along the longitudinal axis of the hippocampus. The relative densities of CHA binding can be summarized as follows: CA1a > CA1b > CA1c. Thus, two gradients of adenosine A_1 receptors are observed within the CA1 region, one oriented along the longitudinal axis of the hippocampus and a second along the transverse axis. The physiological impact of the differential distribution of A_1 receptors will be discussed as will the potential relevance of this pattern of distribution for the selective vulnerability of CA1 subpopulations.

314.3

AUTORADIOGRAPHIC LOCALIZATION OF ADENOSINE A-2 RECEPTORS IN THE RAT BRAIN. M.F. Jarvis, R.H. Jackson* and M. Williams Research Dept. Pharmaceuticals Div. CIBA-GEIGY Corp. Summit, NJ 07901

Purinergic neuromodulation appears to be mediated through pharmacological activity at two cell surface receptors, adenosine A-1 and A-2. While A-1 receptors have been well characterized, the study of A-2 receptors has been limited by the lack of appropriate ligands. In the present study, quantitative receptor autoradiography was used to localize binding sites for the nonselective agonist [^{3}H]NECA in the rat brain. The regional distribution of [^{3}H]NECA binding was similar to that for [^{3}H]CHA binding to A-1 receptors with the exception that the greatest density of binding was found in the striatum. When 50 nM CPA was included in the assay to block binding to A-1 receptors (Bruns et al., 1986; Mol. Pharmacol. 29: 331), [^{3}H]NECA binding sites (Kd = 9 nM and Bmax = 230 fmol/mg tissue) were exclusively concentrated in the striatum and olfactory tubercle. Pharmacological analysis of [^{3}H]NECA binding (+ 50 nM CPA) in striatum revealed a selective labeling of A-2 receptors (i.e. NECA showed the greatest activity in inhibiting [^{3}H]NECA binding (IC_{50} = 4 nM) followed by 2-CADO > R-PIA > CPA > S-PIA). These results indicate that some of the neuromodulary actions of adenosine may be mediated, at least in part, by A-2 receptors in the basal ganglia.

314.4

DOPAMINE D-2 RECEPTOR AUTORADIOGRAPHY IN THE FOREBRAIN OF BALB/cJ AND CBA/J MICE. K. Ozsvath* and D.C. German. Depts. of Psychiat. and Physiol., UT Southwestern Med. Cntr., Dallas, TX 75235-9070.

BALB/cJ mice exhibit greater spontaneous locomotor activity, possess 20% more midbrain dopaminergic neurons and 33% more D-2 receptor sites in the striatum than CBA/J mice (Baker et al. PNAS, 77:4369-4373, 1980; Boehme & Ciaranello PNAS, 78:3255-3259, 1981). Because of the important role of the nucleus accumbens in locomotion, we sought to measure the densities of D-2 receptors in this structure using receptor autoradiography techniques with ^{3}H-raclopride. In preliminary studies using scintillation counting of coronal sections through the forebrain, the specific binding of ^{3}H-spiperone was saturable with a Bmax = 148 ± 8 fmole/mg protein for the BALB/cJ mice and 194 ± 15 for the CBA/J mice (mean ± S.E.M., n = 6, $p < 0.05$). The Kd values were the same for both strains (1 nM). The autoradiographic experiments will reveal which forebrain nuclei (striatum, nucleus accumbens, etc.) are responsible for the overall greater number of binding sites in the CBA/J strain. Research supported by MH-30546.

314.5

ANATOMICAL AND ELECTROPHYSIOLOGICAL EXAMINATION OF A10 DOPAMINERGIC NEURONS IN INBRED MOUSE STRAINS. G.L. Bernardini, K.F. Manaye and D.C. German. Depts. of Physiol. and Psychiat., UT Southwestern Med. Cntr., Dallas, TX 75235.

The A10 dopamine (DA)-containing neurons, of the ventral tegmental area, play a role in locomotion and motivation. BALB/cJ and CBA/J mice have been shown to differ in A10 cell numbers and DA-related behaviors. The purpose of the present experiment was to determine whether the number of cells influences the firing properties of the cells. The locations and sizes of tyrosine hydroxylase immunohistochemically stained A10 neurons were entered into a computer from BALB/c, CBA, C3H/He and DBA/2 mouse strains (n = 5/strain). Single cell recordings from A10 neurons were made in the in vitro slice preparation (n > 15/strain). Although there were 25% more cells in the BALB/c mouse than in mice with the smallest number of cells, there was no significant difference in the baseline firing rates of cells among the four strains (3.0 ± 0.3 impulses/sec./strain). These data suggest that the baseline firing rate is not dependent upon total cell number in the nucleus. Research supported by MH-30546.

314.6

ANATOMICAL AND NEUROCHEMICAL DIFFERENCES IN THE MESOTELENCEPHALIC DOPAMINERGIC SYSTEMS OF INBRED MOUSE STRAINS. S.G. Speciale, B.E. Gonzalez*, K.F. Manaye and D.C. German. Depts. of Psychiat. and Physiol., UT Southwestern Med. Cntr., Dallas, TX 75235-9070.

Inbred mouse strains possess different numbers of midbrain dopamine (DA)-containing neurons and exhibit different motor behaviors (Fink & Reis, Brain Res. 222:335-349, 1981). We examined the relationship between the number of midbrain DA neurons and the concentrations of DA and its metabolites (DOPAC and HVA) in the cell body (nuclei A8, A9 and A10) and axon terminal (striatum and n. accumbens) regions of BALB/c, CBA, C3H/He and DBA/2 mice. The locations and sizes of tyrosine hydroxylase-positive neurons were entered into a computer, and DA and DA metabolite concentrations were measured with HPLC from micropunches from the mesotelencephalic regions. BALB/c mice had 19% more midbrain DA neurons than the strain with the fewest cells (C3H/He), but there was no correlation between the number of neurons and the biochemical measures in the midbrain or forebrain. These data suggest that differences in these neurochemical indices depend upon factors other than cell number. Research supported by MH-30546.

314.7

LOCALIZATION OF OPIOID RECEPTOR SUBTYPES IN THE DOPAMINE MESOCORTICOLIMBIC SYSTEM R.P. Dilts and P.W. Kalivas Dept. of VCAPP, Washington State Univ., Pullman, WA 99164

It has been demonstrated that opioids produce both a dopamine dependent and dopamine independent increase in locomotor behavior (Kalivas et al., J. Pharm. Exp. Ther. 227:229, 1983). In an effort to discern if opioid peptides modulate the mesocorticolimbic system through one or more receptor subtypes we have employed receptor autoradiography using selective ligands. DAGO, DPEN^{2,5}-enkephalin, and D-Pro^{10} dynorphin 1-11 were iodinated using the lactoperoxidase method to label mu, delta, and kappa receptors, respectively. Additionally, localization to dopaminergic or non-dopaminergic neurons has been studied utilizing the selective neurotoxins 6-hydroxydopamine and quinolinic acid administered within the ventral mesencephalon. Preliminary observations suggest that iodinated DPEN selectively labels delta receptors which are present in very low density within the mesolimbic projections. The binding of DPEN was resistant to either neurotoxin suggesting these receptors are not on neurons emanating within the A10 dopamine region. Kappa receptors labeled with the dynorphin analog are present within the mesolimbic projections but their anatomical locus is still under study. These results suggest a differential distribution of opioid receptor subtypes within dopaminergic systems. Furthermore, they provide a functional significance to differential processing of the pre-pro parent peptides.

314.8

VARIATIONS IN THE RATIO INDEX MEASUREMENT OF [^{18}F]-N-METHYLSPIROPERIDOL BINDING IN HUMAN BRAIN USING PET. S.L. Dewey, D.J. Schlyer*, A.P. Wolf*, N. Volkow, J. Brodie*, J.S. Fowler, C.-Y. Shiue*, D.R. Christman*, Brookhaven National Laboratory, Upton, NY 11973.

Quantitative measurements of Dopamine (D2) receptor binding can be obtained in the living human brain with Positron Emission Tomography (PET) by utilizing the selective D2 receptor ligand [^{18}F]-N-methylspiroperidol (NMSP). The ratio index (defined as the slope of the least squares fit through the ratio of Striatal activity/Cerebellar activity versus time, Wong, et al., 1986) in a group of normal male volunteers (n=12) was examined in order to determine the biological and methodological variability. In the first group (n=5) the ratio index was obtained by selecting the striatal region of interest as the four contiguous pixels with the highest activity. This method produced a ratio index of 3.65 ± 0.68. In the second group of subjects (n=7) the striatal region of interest was selected based upon the known anatomical structure of the human striatum. In this case, the ratio index was 3.52 ± 0.81. Thus the ratio index approach to receptor availability in linear studies is relatively insensitive to the method of striatal analysis. Slope variability using the subject as his own control is negligible (<2.0%). We conclude that the ratio index method is a valid approach to probing the effect of pharmacological intervention. Research supported by USDOE, OHER, NIH NS-15638.

314.9

CNS RECEPTOR TOPOGRAPHY IN THREE STRAINS OF RAT AND THE DOMESTIC CAT.

B.S. Neal, M.S. Bauer and J.N. Joyce. Dept. of Psychiatry and Pharmacology, Univ. Pennsylvania School of Medicine, Philadelphia, PA

We have utilized quantitative autoradiography to map receptor systems in 3 strains of rat and the cat. Long-Evans (LE), Sprague-Dawley (SD) and Wistar (W) rats were housed in Wahmann running wheels and entrained to a 12:12 hr light:dark cycle. Total daily wheel revolutions were recorded for 2 weeks. LE rats ran more than SD (25% of LE) or W (59% of LE) rats. There is evidence that the striatum (an area rich in DA) is functionally and anatomically organized into a lateral motor and a medial nonmotor region. Thus, we first chose to examine the topography of DA D1 and D2 receptors, and DA uptake sites in this region. We compared the striatal organization of rats with the cat, as the cat has a differentiated caudate and putamen made up uniformly of gray matter, while the rat caudatoputamen (CPU) is a single structure widely dispersed with white matter. DA receptors, D1 (labeled with [^{3}H]SCH23390) and D2 (labeled with [^{3}H]spiperone), show lateral-to-medial gradients in rat CPU, as do DA uptake sites (labeled with [^{3}H]mazindol). CPU from LE rats had more pronounced gradients (1.2-1.5-fold greater) compared to SD and W rats, due to fewer binding sites medially. The cat striatum does not show lateral-to-medial gradients, but has highly compartmentalized binding. High-density zones of DA D2 receptors and DA uptake sites were found in the dorsolateral head of the caudate, while DA D1 zones were more dispersed. Further studies are currently underway to examine other brain regions and receptor populations. **Supported in part by AFAR and Scottish Rite Foundation grants to JNJ.**

314.10

RESPONSE OF STRIATAL DOPAMINERGIC AND CHOLINERGIC RECEPTORS TO DOPAMINERGIC DENERVATION: EVIDENCE FOR A ROLE FOR A NEUROTROPHIC SUBSTANCE

D. L. Schambron, P.B. Molinoff and J. N. Joyce. Departments of Pharmacology and Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA.

Striatal dopamine (DA) D1 and D2 receptor subtypes appear to be regulated independently. In order to examine this furter, various methods of DA denervation were compared in their ability to modulate dopaminergic and cholinergic systems. Rats were prepared with a unilateral 6-OHDA lesion of the substantia nigra (SN), chronically treated with reserpine or saline, or injected with colchicine in the SN. Two weeks later the brains were removed and processed for quantitative autoradiography. The loss of DA and DA uptake sites (visualized with [^{3}H]mazindol) in the striatum was virtually complete on the side of the 6-OHDA lesion and resulted in an increase in the density of DA D2 receptors ([^{3}H]spiroperidol) in the lateral portion of the caudate-putamen. The increase in D2 receptors was topograph-ically correlated with an increase in choline uptake sites (visualized with [^{3}H]hemicholinium-3). There was an apparent reduction of D1 receptors ([^{3}H]SCH-23390) and both M1 ([^{3}H]pirenzepine) and M2 ([^{3}H]N-methylscopolamine) muscarinic receptors. In contrast to the 6-OHDA effect, reserpine treatment produced an up-regulation of both D2 receptors and D1 receptors. There was a down-regulation of muscarinic receptors. Injection of colchicine in the SN produced no significant up-regulation of striatal D2 receptors, but did produce both a loss of D1 and muscarinic receptors. **Supported by a grant from the American Federation for Aging Research to JNJ and GM 34781 to PBM.**

314.11

A METHOD FOR THE SIMULTANEOUS AUTORADIOGRAPHIC LOCALIZATION OF DOPAMINE RECEPTORS AND DEGENERATING FIBERS. L.D. Loopuijt*, L.Rotgers* and J. Korf* (SPON: ENA). Dept. Biol. Psychiatry, Univ. Groningen, Groningen, the Netherlands.

In order to compare the heterogeneous distributions of corticostriatal afferents and striatal dopamine receptors in detail, a convenient method for simultaneous localization of degenerating fibers and dopamine receptors was developed.

Solid kainic acid was apposed to the dura of somatosensory cortex of rats. After 5 or 6 days the rats were injected i.v. with 100 uCi (^{45}Ca)CaCl$_2$ and 20 h later with 100 uCi (^{3}H)N-n-propylnorapomorphine ((^{3}H)NPA, a dopamine agonist). They were sacrificed 1 h later. For the detection of ^{45}Ca, sections were covered with emusion (Ilford G5) coated coverslips, exposed, developed and counterstained. For the detection of (^{3}H)NPA, sections were rinsed in distilled water, defatted in xylene, dipped in nuclear emulsion (Ilford G5), exposed, developed and counterstained. This procedure results in a detailed localization of ^{45}Ca accumulating degenerating fibers at exposure times of 1-2 weeks, and, in adjacent sections, detailed visualization of in vivo labeled dopamine receptors at exposure times of 6-8 weeks.

314.12

NEUROLEPTICS HAVE HIGH AFFINITY FOR BOTH SEROTONIN AND DOPAMINE SITES LABELED BY ^{3}H-SCH-23390 IN HUMAN CHOROID PLEXUS. S.J. Boyson and L. O'Keefe*. Departments of Neurology & Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

A high density of binding sites in the choroid plexus was found in studies using the related isomers ^{3}H-SKF-83566 in rats (Boyson *et al.*, *J. Neurosci.* 6:3177-3188, 1986) and ^{3}H-SCH-23390 in humans (Boyson & O'Keefe, *Soc. Neurosci. Abs.* 13(1):711, 1987). Subsequent studies in pig and dog showed displacement of this binding by the 5HT$_{1c}$-selective ligand mesulergine (Nicklaus *et al.* *Soc. Neurosci. Abs.* 13(2):1197, 1987).

The pharmacological profile of neuroreceptor binding sites labeled by ^{3}H-SCH-23390 in human choroid plexus was investigated in membranes prepared from tissue collected postmortem; binding in human caudate/putamen, which contains a high density of D_1 and D_2 receptors, was compared. When choroid plexus membranes were incubated in 2 nM ^{3}H-SCH-23390, 2 sites ($p<.001$) were identified with virtually all ligands studied, including the neuroleptics α-flupenthixol, fluphenazine, SKF-83566, and SCH-23390; mesulergine; and the agonists serotonin and dopamine; these sites were split approximately 70:30. The larger site appears to be the 5HT$_{1c}$ receptor, because this is the site for which 5-HT has the higher affinity. All of the neuroleptics examined also showed higher affinity for this site. Mesulergine showed nanomolar affinity for this site. The smaller site, for which mesulergine shows an even higher affinity, is a dopamine receptor, as evidenced by sub-micromolar affinity for this site of dopamine. Supported by USPHS NS01195.

314.13

REGIONAL AND LAMINAR DENSITY OF THE NORADRENALINE INNERVATION IN ADULT RAT HIPPOCAMPUS. Sharon Oleskevich and Laurent Descarries, Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Québec, Canada H3C 3J7.

We used a recently developed radioautographic technique, based on the uptake labeling of monoamine terminals (axonal varicosities) in vitro, to quantify the noradrenaline (NA) innervation in adult rat hippocampus. After incubation of brain slices with 1µM [^{3}H]NA, the labeled varicosities were visualized as small aggregates of silver grains, in light microscope radioautographs prepared at 3 equidistant horizontal levels across the ventral 2/3 of the hippocampus. Using a computer-assisted image analyzer, counts were obtained from the subiculum (SUB), 3 sectors of Ammon's horn (CA1, CA3a, CA3b) and 3 sectors of the dentate gyrus (DG-medial blade, crest and lateral blade), every lamina being sampled in each of these regions. Based on initial results expressed in number of varicosities per mm^2 of section, the NA innervation of the hippocampus appeared generally denser and less uniform than that of the cerebral cortex. Its average density was 20% higher ventrally than dorsally. Both SUB and DG were more strongly innervated than Ammon's horn, in which CA1 showed the lowest overall density. In terms of laminar distribution, there was a clear predilection of the NA innervation for the stratum moleculare in SUB and CA1. In CA3, there was also a narrow band of even stronger innervation in the stratum radiatum, near the apical border of the stratum pyramidale, contrasting with the 3 times lower density in this cell layer and the stratum oriens. In DG, the innervation was again the weakest in the cell layer (granule), but exhibited an almost threefold greater density in the polymorph layer, the highest of all hippocampus. After correction for incomplete detection and measurement of varicosity diameter in EM radioautographs, it will be possible to express these quantitative results in number of terminals per volumetric unit of tissue. (Supported by MRC grant MT-3544).

314.14

THE PROJECTION OF NORADRENERGIC NEURONS IN THE KÖLLIKER-FUSE NUCLEUS (A7) TO THE SPINAL CORD DORSAL HORN. F.M. Clark and H.K. Proudfit. Dept. of Pharmacol., Univ. of Ill. at Chicago, Chicago, Il 60680.

Catecholamine neurons in the Kölliker-Fuse (A7) nucleus project to the spinal cord. However, the specific areas of termination are not known. The following two studies were done to determine the projection of A7 catecholamine neurons to the spinal cord dorsal horn. The first experiment involved unilateral lesions of the A7 nucleus. Cervical and lumbar spinal cord sections were processed for dopamine beta hydroxylase immunocytochemistry. We observed a marked (50-70%) decrease of DBH positive terminals in the ipsilateral spinal cord dorsal horn in both cervical and lumbar spinal segments. To further investigate the A7 spinal projection, a unilateral iontophoretic injection of the anterograde tracer, phaseolus vulgaris leucoagglutinin, was made into the A7 region. A large ipsilateral projection was observed in laminae 1 and 2 in both the cervical and lumbar spinal cord. There was a moderate projection to the contralateral dorsal horn comprising 10-20% of the total spinal projection. These results indicate that there is a substantial spinal projection of the A7 catecholamine cell group to the ipsilateral superficial dorsal horn and a moderate contralateral projection. (This work supported by USPHS DA 03980.)

314.15

HETEROGENEOUS DISTRIBUTION OF 3H-DPAT AND 3H-CYANOIMIPRAMINE (CN-IMI) BINDING SITES IN RAT DORSAL RAPHE NUCLEUS (DRN) J. Hensler, G. Kovachich, C.E. Aronson* & A. Frazer; VA Med. Ctr. & Univ. of Pennsylvania Sch. of Med., Philadelphia PA 19104.

The distribution of 5HT-1A receptors within the rat DRN as measured by quantitative autoradiography using 3H-DPAT (1.5 nM+2uM serotonin) was compared with the distribution of serotonin (5-HT) uptake sites in adjacent sections labeled with 3H-CN-IMI (03.nM+ 1uM sertraline). At the level of plate 50 of the atlas of Paxinos and Watson (1986), specific binding of 3H-DPAT in the DRN was 378 +23 fmol/mg protein and in DRN subregions was: ventromedial, 468+ 32; dorsomedial, 407+35; lateral, 274+42. A heterogeneous distribution of 3H-CN-IMI binding was also seen (fmol/mg protein): ventromedial, 3640+106, dorsomedial, 1855+86; lateral, 2473+214. 3H-CN-IMI binding in the entire DRN was 2565+97. In rats sacrificed 3 weeks after i.c.v. injections of 5,7-dihydroxytryptamine, the binding of 3H-DPAT (62+5 fmol/mg protein) or 3H-CN-IMI (357+44 fmol/mg protein) in the DRN was reduced. There was an essentially uniform reduction in 3H-DPAT binding in the different DRN subregions. 3H-CN-IMI binding was reduced significantly less in the ventromedial area (68+4%) than in either the lateral or dorsomedial areas, 87-93%. 3H-DPAT binding sites were not reduced in entorhinal cortex whereas 3H-CN-IMI sites were reduced markedly in this terminal field area. It may be inferred that 5HT-1A receptors and 5-HT uptake sites are located on serotonergic cell bodies and/or dendrites. The hetergeneous distribution of 5HT-1A sites in the DRN should be considered when investigating regulatory influences on 5HT-1A receptors in the DRN. Supported by Research Funds from the Vet. Admin. and USPHS GM34781.

314.16

AUTORADIOGRAPHIC LOCALIZATION OF DISTINCT SEROTONERGIC AND NONSEROTONERGIC [^{3}H]KETANSERIN BINDING SITES. S.J. O'Dell and J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA. 92717.

Since its introduction as a radioligand for labeling 5-HT2 recognition sites, [^{3}H]ketanserin has been used extensively to define binding characteristics, quantify, and map the distribution of this serotonin receptor. Autoradiography of [^{3}H]ketanserin binding in rat brain shows high densities of binding sites in distinct cortical layers and in the striatum. Formerly, this binding was assumed to reflect the distribution of a pharmacologically uniform population of 5-HT2 receptors.

Recently, homogenate binding studies have shown that [^{3}H]ketanserin binds to two distinct populations of sites in rat brain. We have confirmed that one site has a high affinity (ca. 1 nM) for [^{3}H]ketanserin and binding to this site can be displaced by a variety of 5-HT2 antagonists. The second site has a lower affinity (ca. 20 nM) for [^{3}H]ketanserin and radioligand binding is displaced by the catecholamine-depleting drug tetrabenazine (TBZ), but not by serotonergic agents. Autoradiographic localization of this TBZ-displaceable site shows high binding in areas with significant dopaminergic innervation: caudate-putamen, nucleus accumbens, olfactory tubercle, lateral septum, etc. Lesions of the mesostriatal dopamine fibers with 6-hydroxydopamine cause massive decreases in TBZ-displaceable [^{3}H]ketanserin binding, confirming that the site is associated with dopaminergic nerve terminals.